INTERNATIONAL CONFERENCE ON BIOLOGICAL SCIENCE

ADVANCES IN BIOLOGICAL SCIENCE:
Respect to Biodiversity from Molecular to Ecosystem
for Better Human Prosperity

PROCEEDINGS

Organized By

Faculty of Biology Universitas Gadjah Mada
Yogyakarta, Indonesia
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This publication reports papers presented at the International Conference on Biological Science Faculty of Biology Universitas Gadjah Mada 2009 (ICBS BIO-UGM 2009), Advances in Biological Science: Respect to Biodiversity from Molecular to Ecosystem for Better Human Prosperity, organized by and held at the Faculty of Biology, Universitas Gadjah Mada, Yogyakarta, Indonesia on October 16-17, 2009. The conference addressed a range of important research from various fields in biological science likely to play role in the improvement of human prosperity. Three kinds of session were held at the conference: plenary session featuring keynote and invited papers, oral presentation session, and poster presentation session. This proceeding features a number of papers presented in these sessions, which represent 5 themes covered in the conference, i.e. genetics and molecular biology, ecology and conservation, systematics and evolution, physiology and developmental biology, and biomedics.

I wish to thank my fellow Organizing Committee for their efforts towards the success of the conference. On behalf of the Organizing Committee, I wish also to thank keynote speaker, all invited speakers, paper presenters, academic reviewers, participants, and sponsors who have made the conference a success. Last but not least, I hope that the conference leaves us and all participants with memorable and fruitful experience.

Maryani
Chair of the Organizing Commitee
WELCOMING SPEECH FROM CHAIR PERSON OF THE ORGANIZING COMMITTEE

Distinguish guests

- Rector Universitas Gadjah Mada, Prof. Ir. Sudjarwadi, M.Eng., Ph.D
- Keynote Speaker, invited speakers, participants, sponsorships, ladies and gentlemen

Good morning and may God be with us

It is my great privilege to greet you all to the International Conference on Biological Science Faculty of Biology Universitas Gadjah Mada 2009 (ICBS BIO-UGM 2009), Advances in Biological Science: Respect to Biodiversity from Molecular to Ecosystem for Better Human Prosperity, held in Faculty of Biology, Universitas Gadjah Mada, Yogyakarta, Indonesia. I realize that you are all fully dedicated to the sessions that will follow, but I do hope that you all will also take time to enjoy our fascinating Yogyakarta, with its education trade mark, city of culture, batik, as well as its multicultural people.

Ladies and gentlemen, I recognize that this conference is principally designed to enhance the contribution of biological science to the development of other applied sciences related towards a more sustainable use of biological resources. With this fast development of studies and researches on biological topics, we realize that biology highly contribute to applied sciences and sectors, including medicine, pharmacy, agriculture, veterinary, and food as well as health industries. In this case, I am very alert to the increasing needs to understand biology in respect to biodiversity from molecular to ecosystem beneficial in the improvements of human prosperity. Therefore, I wish that this event will be a great opportunity and a wonderful venue for us to lay down a cooperative framework and to establish scientific collaboration between scientists internationally. An impressive roster of distinguish speakers and attendants from Japan, Malaysia, Egypt, and Indonesia has been gathered in this conference.

Hereby, on behalf of the Organizing Committee, I acknowledge Prof. Dra. Sukarti Moeljopawiro, M.App. Sc., Ph.D. (Universitas Gadjah Mada) as a keynote speaker, and also to these following invited speakers, Prof. Dr. Yasunori Machida (Nagoya University, Japan), Chiyoko Machida, Ph.D. (Chubu University, Japan), Prof. Dr. Hitoshi Sakakibara (RIKEN Plant Science Center, Japan), Prof. Ir. Bambang Sugiharto, M.Agr.Sc., Ph.D. (Universitas Jember, Indonesia), Prof. Dr. Campbell O.Webb (Harvard University, USA), and Dr. Richard Noske (Charles Darwin University, Australia), for delivering their valuable scientific information.
To make this program happen, I would like to gratefully acknowledge to the valuable contributions from personal and institutional sponsorship and funding including Institute for Research and Community Services Universitas Gadjah Mada, PT. Fajar Mas Murni Semarang, ILLUMINA PT. Pandu Anugerah Analitika, Drs. H. Maryadi Broto Suwandi, M.Kes Yogyakarta, Prima Grafika Yogyakarta, Argus Optical Yogyakarta, and BTKL (Balai Teknik Kesehatan Lingkungan) Yogyakarta.

I also gratefully thank to the Dean and Vices Dean of Biology Faculty, Universitas Gadjah Mada for giving us opportunity and support to organize this conference. Heartfelt thank is delivered to the Steering Committee, the Academic Reviewers, members of the Organizing Committee for their strong support, active participation, cooperation and hard works throughout this year in preparing and organizing this meaningful meeting and to those who have contributed their untiring effort in making this conference success.

Despite our best efforts, it is inevitable that there is a lack in organizing this conference and I profoundly apologize to all invited speakers, oral and poster presenters, attendants, donators and committee members.

Finally, I would like to offer my best wishes for a highly enjoyable, successful, productive and fruitful conference.

Thank you

Maryani
Chair person of the Organizing Committee
OPENING REMARKS FROM THE DEAN OF FACULTY OF BIOLOGY UGM

Distinguish guests,

- Rector Universitas Gadjah Mada, Prof. Ir. Sudjarwadi, M.Eng., Ph.D.
- keynote speaker, invited speakers, and dear participants,

Assalamualaikum Wr.Wb. May God give us health and happiness

Welcome to Yogyakarta, the city of youth, education, and culture. It has been an honor for me to be here standing in front of you to speak in the prestigious International Conference on Biological Science, Faculty of Biology Universitas Gadjah Mada (ICBS BIO-UGM) 2009 with special theme of “ADVANCES IN BIOLOGICAL SCIENCE: Respect to Biodiversity from Molecular to Ecosystem for Better Human Prosperity”, that invited 9 honorable speakers mostly from foreign countries including Japan, Australia, United States of America. My special gratitude to the speakers who have spent their time travelling to Indonesia in your busy activity. This international conference also attracts more than 200 scholars and students mostly come from Indonesia, and some participants come from Egypt, Japan, and Malaysia. This occasion is such a good opportunity for us to share our experiences in research and good practices done that could inspire students and other researchers. Furthermore, it is also a chance for creating research collaboration among participants.

In line with vission of the Faculty of Biology UGM that the institution will be one of the center of excellence for higher education nationally that generate biologists who respect to tropical biodiversity researches. Therefore, national and international conferences will be held regularly in order to support local researchers and students in mastering their research communication competencies. New paradigm of Education for All (EFA) born by UNESCO nowadays is focusing on Education for Sustainable Development (EfSD). It promotes quality of education based on values, principles, and practices necessary to respond effectively to current and future challenges that includes all people. My deep appreciation goes to the Steering Committee, Academic Reviewers and the Organizing Committee that spend almost their valuable time to review articles and also to manage and organize this conference effectively. I also acknowledge our sponsors either institutional or individual, without their contribution this conference may not happen.
I wish this two days conference will enlighten tropical biological researches and researchers in Indonesia and give benefit to all of us. Thank you

Yogyakarta, October 16th, 2009

Dr. Retno Peni Sancayaningsih, M.Sc.
OPENING REMARKS FROM THE RECTOR UNIVERSITAS GADJAH MADA

Distinguished guests, ladies and gentlemen

On behalf of the Gadjah Mada University, I wish to congratulate and express my gratitude to the Faculty of Biology UGM and to the Organizing Committee of the International Conference on Biological Science (ICBS) 2009: Advances in Biological Science: Respect to Biodiversity from Molecular to Ecosystem for Better Human Prosperity for succeeding this conference. My sincere thanks are also addressed keynote speaker and all invited speakers to support this conference.

Biology is a core of fundamental science and the contribution of applied biology sector on the national economic development for Indonesia needs to be strengthened through the effort of developing prospective domestic and export of potential biodiversity and biotechnology products as mentioned in this conference theme. We still have some problems in biodiversity and biotechnology sector and that is why, this conference is now being conducted.

I wish, the meeting will be successfully bring the audience to exchange and brainstorm the scientific knowledge in order to provide valuable results for supporting the national biodiversity and biotechnology development. I also strongly hope that some ideas produced in this conference will be applied for practical application of biology in Indonesia in the near future.

Thank you and have a nice conference

Prof. Dr. Ir. Sujarwadi, M.Eng.
The Rector of Gadjah Mada University
Yogyakarta, Indonesia
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• Giyarto
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• Darsono
• Nahrowi
ACKNOWLEDGMENT

The Following personal and Organization are gratefully acknowledged for Supporting this International Conference on Biological Science (ICBS 2009 BIO-UGM)

ADVANCES IN BIOLOGICAL SCIENCE:
Respect to Biodiversity from Molecular to Ecosystem for Better Human Prosperity

Institute for Research and Community Services
Universitas Gadjah Mada, Yogyakarta, Indonesia

PT. Fajar Mas Murni Semarang, Indonesia

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BTKL (Balai Teknik Kesehatan Lingkungan) Yogyakarta, Indonesia

BNI UGM Branch, Yogyakarta
Thematic Oral Presentation

Topic 1: Molecular Biology, Genetics and Bioinformatics

1. Leaky and Linear Scanning Translation of Chicken Anemia Virus Leader Sequence
   Afiono Agung Prasetyo, Toshio Kamahora and Seiji Kageyama

2. Efficient Translation Initiation Directed by Cap-dependent Chicken Anemia Virus Leader Sequence in Stress Condition
   Afiono Agung Prasetyo, Toshio Kamahora and Seiji Kageyama

3. Metagenomic Sequence Analysis Reveals Diverse 16S rRNA Gene In Termite Intestine Bacteria
   Irvan Faizal, Retno Lestari, Agung Heru Karsono, Abdul Latif, Dudi Hadianto, Nila Kusumawati, Indra Rachmawati, Bambang Marwoto, Nadirman Haska and Wahyu Purbowasito

4. Genomic and Proteomic Characterization of Insulin Receptor (hINSR) of DM Patients
   Fatchiyah, S. Widyarti, W. Fajriani, M.N.Y. Putri, L. Firdausi and DW. Soeatmaji

5. Application of Random Amplified Polimorphic DNA (RAPD) Markers for Detection of Powdery Mildew Resistance Gene in Diverse Melons
   Budi Setiadi Daryono

6. The Study of PIT1 Gene Polymorphism in the Najdi Cattle Using PCR-RFLP Method

7. Detection of Pib (Pyricularia oryzae resistance-b) Gene in Indonesian Local Rices (Oryza sativa L.)
   Annisah Isnaini and Budi Setiadi Daryono

8. Study of Sugarcane SucroseTransporter cDNA by Functional Expression in Yeast
   Slameto, Bambang Sugiharto, Kim Kyung Min and Ryza Aditya Priatama

9. Cloning and Transient Expression of Promoter from Elongation Factor 1 Alpha Gene (MeEF2) from Cassava (Manihot esculenta Carnitz.)
   Sony Suhandono, Armelia Aprilianti Melkias, Lidya, Tati Kristianti and Nadia Hanum

10. Cloning and Sequence Analysis of Stearoyl-acyl Carrier Protein Desaturase (sad) Gene from Oil Palm (Elaeis guineensis Jacq.)
    Sony Suhandono and Dewi Yustika Sofia

11. In silico Docking and Molecular Dynamics Simulation of New Alkaloids from Rauvolfia caffra Stem Bark
    A. Pamuji, H. Sudrajat and U. Lathifah

12. Expression of Parthenocarpic Gene, DefH9-iaaM On Transgenic Tomato Lines
    Saptowo J.Pardal, R.Purnamaningsih, E. G.Lestari and Slamet

Topic 2: Ecology and Conservation

1. Density and Biomass of the Macrobenthic Fauna of the Intertidal Area in
2. Low Biomass of Macrobenthic Faunat at a Tropical Mudflat: An effect of Latitude?
   Agus Purwoko and Wim J. Wolff

3. The Distribution Pattern and Abundance of Asteroid and Echinoid at Ringgung Waters South Lampung
   Arwinsyah Arka, Agus Purwoko and Oktavia

4. Effort for ex-situ conservation of Some Cultivars of Clove Syzygium aromaticum (L.) Merrill et. Perry and Its Rhizosphere Microorganism from Temate and Saparua, North Maluku
   R.P. Sancayaningsih, A. Indriyanto and E.H. Poentyanti

5. Reintroduction and Survivorship of the Threatened Palm Pinaga Javana Blume in the Gunung Halimun National Park, West Java
   Didik Widyatmoko

6. Toward The Indonesian Redlist Book: Species Priority Setting for Conservation of Indonesian Threatened Plants
   Didik Widyatmoko and Rosniati A. Risna

7. Phytoremediation Potential of Salvinia molesta and Eichornia crassipes in the Water that Contaminated by Sidoarjo Mudflow
   Dewi Hidayati, Aunurohim, Irmina Kris Murwani and Atika Ayu Permatasarai

8. Invasion of Waterhyacinth (Eichornia crassipes) and Golden Apple Snail (Pomacea canaliculata) in Mangrove Ecosystem Segara Anakan, Central Java
   Tjut Sugandawaty Djohnan

   Joeni S. Rahajoe and Alhamd L.

10. Soil enzimatic activities of natural forest in permanent plot of low land national park “Gunung Gede Pangrango”
    Antonius Sarjiya, Fauzi Rachmat, and Joeni Setijo Rahajoe

11. Soil of Gunung Salak National Park as Source for Methanotrophic and Phosphate Solubilizing Bacteria
    I Made Sudiana and Maman Rahmansyah

**Topic 3: Systematics and Evolution**

1. Chloroplast DNA Sequences to Reveal Phylogenetic Relationship of Indonesia Banana Cultivars
   Amin Retnoningsih and Y. Ulung Anggraito

2. Genetic Diversity Analysis of Indigenous Bacillus thuringiensis Isolates Pathogenic to Crocidoloma binotalis by Using Molecular Phylogenetic Approach Based on 16S rRNA Gene Sequences
   Langkah Sembiring, Christina L. Salaki, Jesmandt Situmorang and Niken S.N. Handayani

3. The Comparison of Morphological and Molecular Characters of Vanda tricolor Lindl. Merapi, Bali, East Java and West Java Forms
   Endang Semiarti, Aziz Purwantoro, Rindang Dwiyan, Tantri Swandari, and Esti Sri Lestari
4. The Diversity of Foliose Lichens in the Forest of Tahura R Soeryo, Batu, East Java
   Miftahul Jannah, Dwi Anggorowati Rahayu, Devi Arifianti Mahadi, Murni Saptasari and Ludmilla Fitri Untari

5. A Preliminary Study of Moss Flora of Mount Lumaku, Sabah, Malaysia
   Fadzilah Awang Kanak, Monica Suleiman

6. Phylogenetic relationship of the Genus Trichotosia species in Sabah, Malaysia
   Norhaslinda Malekal and Monica Suleiman

7. Morphology of White Oyster Mushroom (Pleurotus ostreatus Jacq. ex. Fr. Kummer) on Modification Media of Mushroom Cultivation
   Rina Sri Kasiamdari and Armesi Sugara

8. Morphological Variation of Cibotium barometz from West Sumatra
   Rugayah, Titien Ng. Praptosuwiryo and D.M. Pustaningtyas

9. The Distribution of West Malesian Fern Genus Diplazium (Woodsiaceae) Inside and Outside Malesia
   Titien Ngatine Praptosuwiryo, Edi Guhardja and Dedy Darnaedi

10. Species Diversity of Rattans in the Genus Calamus in Muna Regency, Southeast Sulawesi, Indonesia
    Ratna Susandarini, Andilombo, Purnomo

11. Tuber Morphology Variation and Classification of Yogyakarta Water Yam (Dioscorea alata L.) Cultivars
    Purnomo and Ratna Susandarini

**Topic 4: Physiology and Developmental Biology**

1. 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) Increase Desmin Expression In Mouse Fetuses Palatal Tissue
   Salomo Hutahaean, S. Mangkoewidjojo, M. Sagi, dan W. Asmara

2. Dimethyl Sulfoxide’s Effect on Sperm Quality of Goramy Fish, Osphronemus goramy Lacepede, 1801 twenty four hours post-cryptopreservation
   Abinawanto, Mariana D, Bayu I and Ade Sunarma

3. Molecular Effects of Methoxyacetic Acid on Human Skin Fibroblast Cells in vitro
   Didik Priyandoko, Nashi Widodo, Tetsuro Ishii, Renu Wadhwa and Sunil C. Kaul

4. Stress Induced Microspore Embryogenesis in Mimulus
   Ari Indrianto

5. Mechanistic Studies Exploring the Effect of Absisic Acid on Gibberelin Contents
   K. Dewi and P.M. Chandler

6. The Effect of Storage Periode and Gibberellin on Dormancy Breakage and Amylase Activity of Rice Seeds (Oryza sativa L. var C64)
   K. Dewi and R.R. Aisyandari

7. The Growth of Chlorella pyrenoidosa in wastewater of molasses ethanol fermentation (vinase)
   Theresia Tri Suharni and Ludmilla Fitri Untari

8. How do Plants Respond to Nitrogen-shortage by Regulating Nitrate Uptake?
   Takatoshi Kiba and Hitoshi Sakakibara
9. Fungal Diversity Inside The Semuluh Cave, Semanu, Gunung Kidul, Yogyakarta  
   **Endah Retnaningrum, Anastasia Wahyu Widayati and Ratih Aryasari**

10. Tiller Number Comparison among Three Hybrid Rice in Seedling Methods  
    **Setiarti Sukotjo**

11. Classical Quantitative Structure-Activity Relationship Studies of Flavylium Salts as Xanthine Oxidase Inhibitors  
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| 2. Molecular Genotyping of HBV by using Nested PCR and RFLP among Hepatitis B Patients in Yogyakarta and Surrounding Area  
   **Aris Haryanto, Nenny Sri Mulyani, Titis Widowati and Nastiti Wijayanti** |
| 3. Antioxidant properties of two genus of Zingiberaceae (Etlingera spp. and Zingiber spp.) from Sabah, Malaysia  
   **Farrawati Sabli, Maryati Mohamed, Asmah Rahmat and Mhd Fadzelly Abu Bakar** |
| 4. Papuan DNA Mitochondrial Database, a Genomics Resource Supporting Population Genetics Studies and Biomedical Research  
   **Richardo Ubyaan, Irwandi Y. Suaka, Epiphani I. Y. Palit, Semuel Unwakoly and Yohanis Ngili** |
| 5. Detection and Identification of Influenza Viruses by Polymerase Chain Reaction (PCR)  
   **Akhirta Atikana, Ungke Anton Jaya and Herman Kosasih** |
| 6. Cloning of Large Hepatitis B Surface Antigens (L-HBsAg) from Indonesian Isolates: Development of the Third Generation Vaccine  
   **Dina Rachmi Ramdhani and Ernawati Arifin Giri Rachman** |
| 7. Essential Oil Profile of Temu Lawak (Curcuma xanthorrhiza Roxb.) Callus After Having Photoperiod and Sucrose Treatments  
   **L. Hartanto Nurogroho, I. Sumardi, M. Wisnu and M. Permanawati** |
| 8. Phytochemicals, Antioxidant and Anticancer Properties of Boesenbergia Species (Zingiberaceae) Endemic to Borneo  
   **Ling Jing Jing, Maryati Mohamed, Asmah Rahmat and Mohd Fadzelly Abu Bakar** |
| 9. Antifertility Activity of Flagellaria indica L. Fruit Extract on Female Albino Mice (mus musculus L.) Swiss Webster  
   **Yohanes E. Gunawan, Meda, G. SARAHAYU, A. HARYONO, and SUATMA** |
| 10. Molecular Effects of Methoxyacetic Acid on Human Skin Fibroblast Cells in vitro  
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| 2. Study on the Interaction between OsKANADI1 and a florigen Hd3a in Rice  
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| 3. Genetic Polymorphism of mt-DNA Cytochrome b (cyt b) in Indonesian Domestic Cattle  
  Muhammad Cahyadi, Wayan T. Artama and Tety Hartatik |
| 4. Genetic Relationship between Ongole Hybrid Cattle and *Bos javanicus* in Indonesia based on Partial D-loop mtDNA  
  Tety Hartatik, Tri Satya Mastuti Widi, Muhammad Cahyadi and Muhammad Affan Mu'in |
| 5. Diagnostic Sensitivity Comparison between Polymerase Chain Reaction-High Resolution Melting (PCR_HRM) and Sequencing Techniques to Detect KRAS Mutation  
  Farid Sastra Negara |
| 6. Mutations Analysis in D-loop Region of Mitochondrial DNA in Human Gastric Tissues and Its Influence on the ROS and Cell Cycle  
  Samuel Unwakoly, Felicia M. Lekatompessy, Epiphani I. Y. Palit, Richardo Ubyaan and Yohanis Ngili |
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  Tuty Arisuryanti, Herlianti Annisa, Idha Nur Chasanah and Gerhana Yuhu Rohyana |
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  Rina Sri Kasiamdari, Ganiez Riza Aristya and Budi Setiadi Daryono |
| 10. Insertion of Activation Tag into *Batutegi* and *Kasalath* Rice Cultivar  
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  Tigor Nauli and Zalinar Udin |

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| 2. Potential of Mosses as Atmospheric Heavy Metal Pollution Indicator at Mount Kinabalu, Sabah, Malaysia.  
  Khairul Nizam Yakob, Suhaimi Md. Yassir and Monica Suleiman |
| 3. The Utilization of the Space Field by Sundanese at RPH Cirangsad, BKPH Jasinga, KPH Bogor, West Java  
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4. Stand Structure in Permanent Plot Mt. Gede Pangrango National Park, Bogor, West Java
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5. Habitat Manipulation in the Rice Field Using Aquatic Weeds for Increasing the Agroecosystem Stability
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6. The Capacity of Ceratophyllum demersum L. and Scirpus grossus L. in Accumulating Chromium from Waste Water
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International Conference on Biological Science

ADVANCES IN BIOLOGICAL SCIENCE:

Respect to Biodiversity from Molecular to Ecosystem

for Better Human Prosperity

Plenary Sessions
BUAH MERAH (Pandanus conoidues Lamk.): POTENCY AGAINST CANCER CELLS

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INTRODUCTION

A number of traditions came to dominate the practice of herbal medicine in the Western world at the end of the twentieth century. In developed countries, the practice of herbal medicine could be connected with the trend favourable to the so-called “sustainable development” and to some extent with the observed decline of patent applications in organic chemistry paralleled by the rise of life science applications. In developing countries, this is sustained by the search for biologically active compounds obtainable from locally available plants particularly with a view to reducing public health cost which have significantly been raised due to acquisition of synthetic drugs from industrialized countries.

Plants have an almost limitless ability to synthesize bioactive substances. Most are secondary metabolites, of which at least 12,000 have been isolated, a number estimated to be less than 10% of the total. Only a small percentage of the 400,000 to 500,000 species in the plant kingdom has been phytochemically investigated.

BUAH MERAH

Buah merah (Pandanus conoideus Lamk.) grows naturally in Papua, Indonesia at 1,200–2,000 m above sea level, at 23–33 °C and light intensity at 1000–3000 lux. Buah merah can also be found in Papua New Guinea and Maluku. The plant grows in secondary forest, with moist and acidic conditions. Reproduction of buah merah happens on vegetative reproduction by budding from stem or root and generative reproduction with seed (Budi and Paimin, 2005).

There are four local varieties of buah merah; oblongus red, rectangular oblongus red, brown, and yellow. They vary in size and weight from 27 to 102 cm in long and 2 to 10 kg in weight. They vary also in diameter from 6.9 to 20 cm. Seed position forms irregular rows on the axis.

According to Budi and Paimin (2005), buah merah contains high antioxidant, vitamin and mineral. According to Palupi et al. (2007), total carotenoid, carotene, tocopherol, α-
tocopherol and content of *buah merah* extract depend on extraction methods. *Buah merah* content unsaturated fatty acid that dominated by Oleic acid and Palmitic acid.

One of factors which influence secondary metabolites production is variety. Different variety has different content of micronutrient. As studied by Budi (2005, unpublished), showed that, total caroten of red *buah merah* was 12,233.34 ppm, yellow *buah merah* was 11,719.46 ppm, while the brown colour was only 10,235.12 ppm.

Heavy metals (copper, silver, nickel, gold, plumbum, arsenic) were not detected in *buah merah* in both red and yellow *buah merah* (Setyorini, 2007). Calcium in yellow cultivar was not significantly different from red cultivar, Fe and Mn in yellow were higher than in red, while Mg in yellow was lower than in red cultivar (Damayanti, 2007).

Traditionally *buah merah* are used as a food, natural dye, craft, tribal ceremony, meat and sagu preservative, and medicine. *Buah merah* is multi advantageous natural medicine. Active compounds of *buah merah* functioning as medicine originally introduced by Drs. I Made Budi, M.Si.. In the beginning, as he found that the local people who consumed *buah merah* hardly suffered from degenerative disease such as cancer, hypertension, and diabetes. Recently, *buah merah* has been extensively used to treat HIV/AIDS, diabetic mellitus, uric acid, osteoporosis, and cancer.

**POTENCY OF BUAH MERAH AGAINST CANCER CELLS**

The scientific studies of anticancer activity of *buah merah* on the growth of cancer cells are relatively very few. According to Moeljopawiro et al. (2007), *buah merah* oil produced by I Made Budi has ability to inhibit breast, cervix, and colon cancer cells proliferation. The activities of *buah merah* to inhibit the growth of cancer cells were determined by direct counting using trypan blue staining. The cell proliferation kinetics profile was observed by doubling time assay. An apoptosis study was done by DNA staining using acridine orange-ethidium bromide combination, for observing the cell morphology. The results showed that *buah merah* oil had higher cytotoxic potency on colorectal cancer than breast cancer and cervical cancers. It was shown that LC$_{50}$ of *buah merah* oil on breast cancer and cervical cancers were 600 ppm and 1000 ppm respectively, while only 200 ppm for colorectal cancer. Doubling time assay showed an inhibition of cell growth, it was confirmed that *buah merah* oil had an antiproliferative effect.

The toxicity of *buah merah* against cancer cells were also studied on three local varieties of *buah merah*, as well as the bioactive compounds. Moeljopawiro et al. (2008) studied the toxic compound on breast and cervix cancer cells of those 3 local varieties of
buah merah, using MTT assay to determine IC_{50}. The results showed that the lowest IC_{50} was methanol extract of Barugum both on breast cancer (IC_{50} 132,830 μg/ml) and cervix cancer (IC_{50} 153,083 μg/ml), followed by chloroform extract of Maler that was good for breast cancer (IC_{50} 139,720 μg/ml). The worst was Yanggiru (yellow colour) that did not have any anticancer activity. Detection of potential fraction showed that fraction of methanol extract of Barugum had IC_{50} 65.29 μg/ml and IC_{50} of fraction of chloroform extract of Maler was IC_{50} 45.15 μg/ml. Those values were better than Doxorubicin that has IC_{50} 66.69 μg/ml.

The bioactive compounds of chloroform extract of Maler and methanol extract of Barugum that has toxicity on breast and cervix cancer cells was the same, that was terpene (Moeljopawiro et al. 2009). It was consistent with finding by Astri that the major active compound of buah merah that was extracted using n-hexane was terpene (Astri, 2008).

Terpenoids are the most abundant secondary metabolites that have various molecular structures. More than 23,000 structures of terpenes have been identified. The functions of terpenes are as phytoalexins in plant as a direct defence, or as a signal in indirect defense responses which involves herbivores and their natural enemies. Therefore, the most researchers think that the terpenes have more ecological than physiological functions. Moreover diterpenes have a physiological function as a plant hormone and gibberellin. Some of diterpenes have a cytotoxic activity against tumour (Harbone, 1987; Cheng et al., 2007).

According to Candrasari (2009), bioactive compounds in buah merah was not only terpenoid but also alkaloids, terpenoids and saponins. Alkaloids have activity as anticancer that induce apoptosis (Huang et al., 2004, Bennouna et al., 2003 and Padmanabha, 2006) and saponins could inhibit the proliferation of colorectal cancer (Ruth, 2005, Ellingthon et al., 2005).

Moeljopawiro et al. (2009) showed further that results of MTT assay of isolates obtained from preparative TLC among 9 fractions, the most toxic fraction had IC_{50} value 41.64 μg/ml. It was lower than that of Doxorubicin (45.32 μg/ml). Whereas the most toxic fraction on HeLa did not show any anti cancer activity (IC_{50} value more than 100 μg/ml). The structure of toxic compounds on T47D cells were elucidated further using spectroscopy method (UV, IR and MS). The structure of these compounds on T47D cells by spectroscopy UV showed that the compounds did not have chromophore groups and conjugated bonds. Spectra IR revealed that the compounds were long chain compounds with carboxylic bond. Spectra MS showed that the toxic compounds were hexadecanoic acid and 9-Octadecenoic acid (Z). These findings are consistent with Yanti (2009) that using different method of
isolation revealed that toxic fraction on T47D cells were hexadecanoic acid, 9-Octadecenoic acid (Z) and 1-nonadecene.

According to Widaryanti (2008), the IC$_{50}$ of red fruit extract was 488 ppm at 24 hours incubation, while at 48 hours incubation the IC$_{50}$ of its water fraction was 163 ppm. Red fruit extract and its water fraction had no effect on cell cycle progression using flowcytometri. The extract was able to induce apoptosis through caspase-3 activation by western blot assay. This finding suggests that red fruit extract is more suitable for cancer chemoprevention.

**IN VIVO STUDIES OF BUAH MERAH AGAINST CANCER**

A few invivo studies have been carried on the effect of *buah merah* to cancer growth. Pujasari (2006), studied the effect of *sari buah merah* on proliferaton and apoptotic activities. Apoptotic activity was detected using tunnel assay, while proliferaton activity was measured by histochemical technique, AgNOR (silver-staining nucleolar organizer regions). Statistical significance of Apoptosis Index (AI) and AgNOR value in control and groups treated with varied doses of RPO were calculated. Variant analysis indicated that there was no significant difference of volume, weight and AI among control group and treated groups (p>0.05). However, there was a tendency to an increased apoptotic activity on the treated groups. On the contrary, AgNOR values were significantly different (p<0.05) among groups. Therefore, it could be concluded that there was an inhibition of proliferation activity found on RPO-treated mice. Dose 2 ml was the optimal dose to reduce proliferation activity.

Mun‘im et al. (2007), obtained that extract of *buah merah* could inhibit carcinogenesis. The experiment was done using 7.12-dimethylbenz[a]anthrasene (DMBA)-induced rat lungs cancer model in female Sprague-Dawley rats. Treatments were done at 0.21 ml/200 g bw; 0.43 ml/200 g bw and 0.88 ml/200 g bw, and terminated at day 120. Lung histology was used to evaluate carcinogenesis inhibition. The result showed that the extract at 0.21 ml/200g bw had higher lung carcinogenesis inhibition than other doses.

**CONCLUSION**

Most of bioactive compounds isolated from plants do not have cytotoxic activity, so they called chemopreventive compounds. Eventhough, according to Moeljopawiro et al. (2008), extract of *buah merah* have cytotoxic effects on breast, cervix, and colon cancer cells. Chemopreventive compounds are more selective than cytotoxic compounds on cancer cells because chemopreventive compounds have specific molecular targets. Their targets were to inhibit carcinogenesis, cell cycle, and angiogenesis. Chemoprevention could be
described as a compound that prevent and inhibit carcinogenesis or prevent invasive cancer development (Meiyanto et al., 2005).

Chemopreventive compounds could not be applied solely for cancer therapy. They can only be used as a supplement to prevent cancer development (Middleton and Kandaswami, 1993). In addition, the chemopreventive compounds also lowering cancer incidence and risk caused by cancer. Therefore, therapy using combination of cytostatic and chemopreventive compounds gives more effective and efficient results.

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PLANT BIODIVERSITY INVENTORY IN INDONESIA: PARATAXONOMISTS, BARCODING AND THE SEMANTIC WEB

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Rapidly changing landuse in the tropics threatens plant diversity, and reduces the time left to document the many plant species that are yet to be discovered. Traditional methods of collecting and describing species are probably insufficient for the pragmatic task of recording this diversity before it is gone. Solutions to this biodiversity information crisis i) could use the abundant resource of enthusiastic, networked, national biology students, ii) will employ increasingly low-cost molecular methods and high resolution photographs, and iii) could employ biodiversity informatics and social networking tools to efficiently distribute information, and engage specialists and the wider community. I will describe ongoing plant biodiversity inventory and informatics work at the gunung palung national park in west kalimantan. I believe that a new golden age of biodiversity exploration may be dawning, just as biodiversity itself is most threatened, and am hopeful that increasing knowledge of biodiversity will be a positive force to slow its loss.
CYTOKinin BIOSYnthEsis PATHwAY AND ITS REGULAtion: HOW DO PLANTS REGULATE THEIR GROWTH AND DEVELOPMENT BY CYTOKININ ACTIONS?

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Abstract

Cytokinins play a crucial role in various aspects of plant growth and development. Spatiotemporal distribution of bioactive cytokinins is finely controlled by the metabolic enzymes. Cytokinins, such as $N^6$-(△2-isopentenyl)adenine (iP) and trans-zeatin (tZ), in higher plants are synthesized by adenosine phosphate-isopentenyltransferase (IPT), cytokinin $trans$-hydroxylase CYP735A, and cytokinin-specific phosphoribohydrolase LOG. Our recent studies have demonstrated that IPTs, CYP735As, and LOGs are expressed in various parts during growth and development, and differentially regulate the synthesis of iP- and tZ-type cytokinins. Multiple mutants of Arabidopsis LOGs showed pleiotropic morphological phenotypes, suggesting that the activation step via LOG play a pivotal role for regulating cytokinin activity in normal growth and development. We will outline recent progress in cytokinin biosynthesis and its regulation.

Biological Actions of Cytokinins and the structural variations

Cytokinins are a group of phytohormones that are involved in a wide variety of plant growth and development processes, such as delay of senescence (1), root proliferation (2, 3), apical dominance (4), and nutritional signaling (5) (Fig. 1). Major natural cytokinins are adenine derivatives that carry either an isoprene-derived side chain at the $N^6$-terminus, such as iP and tZ (Fig. 1) (6, 7). They are distinguished by characteristics of the side chain, namely the presence or the absence of a hydroxyl group at the end of the prenyl chain. Although there is no widely accepted explanation for the physiological relevance of these structural variants at present, differences have been found in their activity and stability. In Arabidopsis thaliana, three cytokinin receptors, AHK2, AHK3, and AHK4/WOL/CRE1, were identified (8, 9). In vitro studies with AHK3 and AHK4/WOL/CRE1 showed that AHK3 has higher affinity to tZ than iP, whereas AHK4/WOL/CRE1 has similar one (10). This difference might be involved in specialization of each cytokinin receptor to perceive different cytokinin species.
Biosynthesis Pathway of Cytokinins

The first step of isoprenoid cytokinin biosynthesis in higher plants is catalyzed by IPT, and the products are iP-ribotides (11, 12) (Fig. 2). The iP-ribotides are hydroxylated to tZ-ribotides by CYP735A, a cytochrome P450 monoxygenase (13). The iP- and tZ-riboside 5’-monophosphates are converted to active forms by two pathways: the LOG pathway and the two-step pathway. In the former pathway, cytokinin riboside 5’-monophosphates are directly converted to free-base cytokinins by LOG (14). In the latter pathway, the ribotides are dephosphorylated to the ribosides and subsequently converted to free-base cytokinins, but the corresponding genes have not been identified yet.
Spatially Differential Distribution of \textit{IPTs} and \textit{CYP735As} regulates the synthesis of iP- and tZ-type cytokinins

When we measure cytokinin contents in various organs of \textit{Arabidopsis}, similar amounts of iP and tZ-type cytokinins could be detected (Fig. 3). \textit{IPT} genes are expressed in numerous organs including roots, leaves, stems, flowers, and siliques (15, 16), whereas the \textit{CYP735A} genes are expressed predominantly in roots (13). Superimposition of the expression patterns of \textit{IPT} and \textit{CYP735A} genes in \textit{Arabidopsis} reveals differential distribution of \textit{de novo} synthesis pathways for iP and tZ because the expression sites of \textit{IPTs} and \textit{CYP735As} do not always overlap (Fig. 4). For instance, \textit{AtIPT3} is expressed in rosette leaves, but expression of \textit{CYP735As} in rosettes is scarcely detected. On the other hand, both \textit{IPTs} and \textit{CYP735As} are expressed in roots. Such differential distribution of cytokinin biosynthesis genes might be important to produce different cytokinin species in underground and aboveground organs.
LOG-dependent activation pathway plays a pivotal role for regulating cytokinin activity during normal growth and development

LOG gene was first identified as the causal gene of the rice lonely guy mutant, which has defects in shoot apical meristem function. LOG is encoded by a small multigene family. Recent studies on LOG family genes in rice and Arabidopsis suggest that activation of cytokinin occurs in nearly all parts of plant body (14, 17). In Arabidopsis, atlog3 4 7 triple mutant shows a semi-dwarf phenotype and formed fewer flowers than the wild type, suggesting a reduced activity of the inflorescence meristem (17). Since multiple mutants of Arabidopsis genes related to cytokinin signaling also show a reduction in meristem activity in shoots (18-21), the triple mutant phenotype supports the conclusions from these previous findings that cytokinins positively regulate shoot meristem activity in Arabidopsis.

Concluding remarks

Cytokinins are produced by the coordinative expression of IPT, CYP735A, and LOG. Spatial distribution and combination between IPT and CYP735A are important for differential production of iP and tZ in plant body. At present, biological importance of side chain structural variation of cytokinins has not been elucidated. However, addressing this question
is one of the important clue to fully understand the biological function of cytokinins in plant growth and development.

References

INDONESIA’S WORSENING BIODIVERSITY CRISIS
AND POSSIBLE SOLUTIONS

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The biodiversity of Indonesia rivals that of the world-famous Amazon and East African plains, yet it has been stated that Indonesia has the world’s worst track record for nature conservation, and is at risk of losing one of its most precious assets through unsustainable levels of exploitation.

Straddling two of the world’s six major terrestrial biogeographic regions, Indonesia largely owes its extraordinarily rich wildlife to (1) colonisation of its large islands by animals from two strikingly different continents, mainland Asia and Australia-New Guinea, and (2) in situ evolution of a distinctive fauna in the intervening area of Sulawesi, Maluku and Nusa Tenggara. This latter area is known as Wallacea, named after the British naturalist (Sir Alfred Russell Wallace) who brought scientific attention to it, and in so doing, shaped the ideas of Charles Darwin, who in turn, developed the most important theory in contemporary biology.

Despite covering a mere 1.3% of the world’s land area, Indonesia hosts 12% of the world’s mammals, 16-17% of its birds, reptiles and amphibians, and 20% of its fish species. This faunal richness is partly due to extraordinarily high levels of endemism, which are partly a consequence of massive fluctuations in sea level, particularly during the Pleistocene. About 36% of Indonesia’s mammals and 24% of its birds are endemic. Indonesia has almost twice as many bird species with a restricted-range (less than 50,000 square kilometres) as any other nation on the globe. With approximately 10% of the world’s bird species threatened with extinction by human activities, it is hardly surprising that Indonesia tops the bill with 117 threatened species – many more than any other nation on Earth.

The primary cause of the biodiversity crisis is the destruction of forests through logging, both legal and illegal, reducing the available habitat for forest-dependent wildlife, many of which require large areas to live (e.g. Sumatran Tiger and Rhinoceros, Orang-utan,
raptors and hornbills). Already Sumatra and Borneo have lost most of their primary forest, and the situation is worsening in the face of increasing investment in the oil palm industry. With little unprotected rainforest remaining in that region to exploit or clear, the attention of the timber and oil palm industries has now turned to one of the world’s last rainforest wildernesses, Papua.

Another significant threat to biodiversity is illegal wildlife trade, which not only threatens iconic species like the tiger, but has also reduced populations of many cage birds to perilously low levels (e.g. Bali Starling, Straw-headed Bulbul). Some of this trade is monitored under the international convention of CITES, but a large volume of domestic trade goes unchecked due to inadequate law enforcement.

The biodiversity of Indonesia now faces a more insidious threat that has been largely fuelled by industrialisation and the excesses of more affluent nations. The effects of climate change on fauna in the world’s temperate regions have been well documented, but its present and likely future impact on tropical animals is very poorly known. There is much concern over the effects of sea-level rise and warmer sea temperatures on corals and coral-dependent marine animals, and given that the richest coral region on Earth – the Coral Triangle – falls within its waters, Indonesia has the most to lose. Yet outside the few marine parks, coral ecosystems in Indonesia have little protection from the many who seek to exploit its riches in a non-sustainable manner.

The terrestrial animals and plants that are most likely to suffer from global warming are high altitude taxa, adapted to cold montane environments. The majority of birds endemic to the Greater Sundas (Sumatra, Borneo and Java) are montane, due to speciation that took place during Pleistocene glacial periods. There is some non-empirical evidence that these birds are already being affected by climate change, with altitudinal ranges expanding or contracting upwards for lowland and montane birds, respectively. This is of concern as the montane biota has until now been somewhat shielded from anthropogenic activities, since logging and wildlife trade are concentrated in the more bio-diverse lowlands. Empirical research on distributional patterns is required to determine whether this pattern is real or not.

Another group of birds that may suffer from global warming are the migrants that make Indonesia their home for up to six months of each year. About 25% of the avifauna of the Greater Sundas is comprised of species that migrate from temperate Asia along the Eastern Flyway to Indonesia. The Eastern Flyway is one of three of the world’s major migratory bird routes. Research in Europe and North America has shown that many migratory species using the other two flyways are leaving their winter quarters (in Africa and
the neotropics, respectively) earlier than normal to take advantage of early warm temperatures on their breeding grounds. Whether such changes are occurring among avian migrants to Indonesia, where arrival and departure dates are little studied, is unknown; nor is the effect that such changes might have on resident birds with whom they temporarily share their resources.

Clearly there is no one solution to Indonesia’s worsening biodiversity crisis, but education, research and poverty alleviation must be key elements of any national strategy designed to halt or reverse current trends. The remainder of this presentation will discuss some proposed solutions. Despite its rich assets, the nation’s eco-tourism industry is underdeveloped and even in Java, some national parks in receive few visitors due to poor infrastructure and management. Yet even villagers in remote parts of Papua are able to earn a living well beyond subsistence through ecotourism.

Indonesia has relatively few active conservation biologists, and a large proportion of them are employed by international NGOs. Recent incentives to reward research effort in universities are a promising sign.

In concluding, the author proposes one initiative to increase awareness about biodiversity and the need for its conservation, while collecting empirical data that may help to answer some of the questions raised earlier in this presentation.
MOLECULAR MECHANISMS OF LEAF DEVELOPMENT
IN Arabidopsis thaliana

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Leaves of angiosperms exhibit remarkable diversity in terms of both shape and complexity. Nonetheless, the basic structure of each leaf generally develops along three axes, namely the proximal–distal, medial–lateral and adaxial–abaxial axes. Thus plants appear to exploit common mechanisms that are responsible for the establishment of these axes during leaf development. Leaves develop as lateral organs from the shoot apical meristem. Various mutants have been isolated with alterations in leaf morphology that are related to the development of leaf shape along each of the three axes, adaxial–abaxial identity and the overall shape of the leaves. The loss-of-function mutations in both the ASYMMETRIC LEAVES2 (AS2) and ASYMMETRIC LEAVES1 (AS1) genes of Arabidopsis thaliana cause pleiotropic phenotypes in leaves such as downward curling of leaves, reduced complexity of leaf venation pattern, generation of lobes, and slightly shorter petiole. We conclude that the AS2 and AS1 genes of Arabidopsis thaliana are required for symmetrical and flat lamina expansion. AS2 encodes a plant specific protein that contains AS2/LOB domain, and AS1 encodes a myb (SANT) domain protein. AS2 and AS1 are thought to act as a transcriptional regulator of certain genes including class 1 KNOX genes, BP, KNAT2 and KNAT6. However it was not known that AS2 and AS1 regulate genes other than class 1 KNOX genes, and its molecular mechanism regulating leaf development is unclear. To know the genes regulated by AS1 and AS2, we performed microarray analysis and quantitative RT-PCR. Results showed that ETT/ARF3, KAN2, YAB5, which are abaxial determinants, were upregulated in as1 and as2 and downregulated in over expressor of AS2, as well as class 1 KNOX genes. To assess whether AS2 acts directly regulates ETT, KAN2, YABBY5, and BP, we demonstrated chromatin immunoprecipitation assay. We report how AS2 and AS1 regulate expression of the abaxial determinants in the process of leaf development. Furthermore we will discuss the mechanisms of formation of symmetrical and flat leaf lamina.
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CREATION OF HIGH SUCROSE YIELD OF SUGARCANE CULTIVARS THROUGH GENETIC ENGINEERING

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Sugarcane is a major plant for sucrose production cultivated in tropical region. The sugarcane is photosynthetically grouped into a C4 plant that has a high photosynthetic efficiency compared to other plants. Depend on the plant species, some plants accumulate sucrose as end products of photosynthetic carbon assimilation. With regard to sucrose synthesis, sucrose-phosphate synthase (SPS; EC 2.4.1.14) is believed to be a key enzyme in plant. Characterization of SPS activity revealed that the enzyme determines sucrose synthesis in sugarcane as well as other plants. Thus, cDNA encoding for SPS were cloned from sugarcane and overexpression of the SoSPS1 gene resulted in elevation of SPS activity followed by sucrose accumulation in transgenic tobacco and sugarcane. Although, the SPS activity was increased almost doubled in transgenic sugarcane, but the sucrose content and yield in sugarcane stem were not increased corresponding to the SPS activity. This luck discrepancy between sucrose synthesis and its accumulation in sugarcane stem raised a question that sucrose transport may have a pivotal role for sucrose accumulation. Sucrose is a carbon mobile that synthesized in source tissue (leaves) and exported to sink tissue such as root, stem, and others non-photosynthetic tissue. The transportation of sucrose from source to sink tissue is facilitated by a protein called sucrose-transporter protein (SUT). Elevation of SUT protein by overexpression of SUT gene increase sucrose translocation and accumulation in transgenic plants. Cloning of cDNA encoding for SUT proteins found presence of SUT gene family that the expression were confirmed in all organ of sugarcane. At least two SUT-cDNAs were isolated from sugarcane, named SoSUT1- and SoSUT2-cDNA. Functional studies by their expression in yeast demonstrated that both cDNAs seem to have their activity as sucrose transporters. However, the expression of SoSUT1 cDNA produced a higher affinity for sucrose loading than SoSUT2 cDNA. Collectively, it is important to postulate here that the creation for high sucrose yield of sugarcane cultivars could be achieved by double over-expression of the genes for SoSPS1 and SoSUT1 to increase the synthesis as well as translocation of sucrose.

Keywords : Sugarcane, genetic engineering, sucrose-phosphate synthase, sucrose transporter protein, transformation.
International Conference on Biological Science

ADVANCES IN BIOLOGICAL SCIENCE:

Respect to Biodiversity from Molecular to Ecosystem

for Better Human Prosperity

Oral Sessions
## TOPIC 1: MOLECULAR BIOLOGY, GENETIC AND BIOINFORMATIC

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**IN SILICO DOCKING AND MOLECULAR DYNAMICS SIMULATION OF NEW ALKALOIDS FROM Rauvolfia caffra STEM BARK**

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Alkaloids isolated from *Rauvolfia caffra* stem bark significantly inhibit acetyl- and butyrylcholinesterase enzyme, suggesting discovery of inhibitors for nervous-system disorders. Studying interactions with the active site of the acetylcholinesterase enzyme, we have identified hydrophobic interactions inside the aromatic gorge area as the major stabilizing factor in enzyme-inhibitor/protein-ligand complexes of these alkaloids. All compounds exhibit a similar binding mode, and therefore they are an ideal target for a systematic variation of the substituents. Because of these docking results, we were able to understand the inhibition type of the ligands which is mainly of a noncompetitive nature. The subsequent Molecular Dynamics simulation of a predicted complex indicates that ligand binding does not extensively alter enzyme structure, but reduces flexibility at the gorge. The establishment of any quantitative relationship between inhibitory activity and molecular properties will require a detailed analysis of the electronic structure by means of quantum chemical methods and/or further docking experiments. It will take into account explicitly the influence of hydration on complex formation between enzyme and substrate.

**Keywords:** *In silico* docking, molecular dynamics simulations, alkaloids, *Rauvolfia caffra*, acetylcholinesterase

*not presented*
O-MB02

LEAKY AND LINEAR SCANNING TRANSLATION OF CHICKEN ANEMIA VIRUS LEADER SEQUENCE

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ABSTRACT

The Chicken Anemia Virus (CAV) major transcript is an unspliced polycistronic mRNA. The 5\textsuperscript{\prime}-proximal ORF (ORF-VP2) and the second ORF (ORF-VP3) overlap each other and are initiated by AUGs codon in a suboptimal context. The third ORF (ORF-VP1) overlaps partially with the ORF-VP2 and has a strong context for translation initiation. The start codon for the VP1 occur 499 nucleotides downstream of the 5\textsuperscript{\prime} terminal transcription start point. It is unclear how translation of the tricistronic mRNA is regulated.

To study the translational features of the CAV leader sequence (CLS), the CLS was fused to the reporter gene FLuc (Firefly Luciferase) driven by a CMV promoter. In order to find out the influence of the upstream AUGs (uAUGs) to the downstream AUG, the VP2 or and VP3 ORFs in CLS were optimized and or knocked out by mutagenesis. These constructs were co-transfected with Renilla Luciferase plasmid into COS-1 cells and assayed by dual luciferase assay, western blotting, and real-time PCR.

The levels of transcripts were very similar among all construct, however, FLuc activities was differentially affected by the mutations of these uAUGs. FLuc and VP3 expression was decreased and increased significantly in VP2 strong and knocked out situation, respectively. Interestingly, no significant differentiations of FLuc activities were found in VP3 strong or knocked out situation.

The present data indicated that the ribosomes reached the ORF-VP1 initiation start by leaky scanning from VP2 and continued by linear scanning from VP3.

Keywords: CAV, Chicken Anemia Virus, leaky scanning, linear scanning, leader sequence
INTRODUCTION

Chicken anemia virus (CAV) is an immunosuppressive pathogen for newborn chickens and was first isolated in Japan (1, 2). CAV is the only member of genus Gyrovirus within family Circoviridae (3). CAV is a small non-enveloped virus with a single-stranded circular genome in the size of approximately 2.3 kb and replicates via a circular double stranded replicative form (4). The major transcript of CAV is an unspliced polycistronic mRNA with possesses three overlapping ORFs encoding for VP1 (52 kDa), VP2 (24 kDa) and VP3 (14 kDa). The 5’-proximal ORF which is initiated by AUG codon in a suboptimal context, encode the VP2, a non-structural protein with dual specificity phosphatase activity. The VP3 (named as apoptin) encoded by the second ORF overlap with ORF-VP2 is important for DNA replication and virion formation of CAV, also initiated by AUG codons in a suboptimal context. With an A residue at position −3, and a G at position +4, the third ORF overlap partially with ORF-VP2, encode for VP1 a major viral capsid protein, has a strong context for translation initiation (5-11). However, the start codon for the VP1 occur 499 nucleotides downstream of the 5’- terminal transcription start point. This long leader sequence contains two AUG codons which are functionally and well conserved among CAV isolated worldwide reported (98%-100%). Recently, it was found that this leader sequence translation mechanism is in cap-dependent manner (Prasetyo, et al, submitted for publication). However, it is unclear how translation of this tricistronic mRNA is regulated. This study was conducted to determine the mechanism of CAV-VP1 translation initiation at the CAV major polycistronic mRNA.

MATERIALS AND METHODS

Cells

African green monkey kidney cell expressing SV40 T antigen, COS-1 cells, were subcultured twice weekly at 37 °C in Dulbecco’s modified Eagle medium supplemented with 10% fetal bovine serum (FBS, Immuno-Biological Laboratories, Fujioka, Japan), 2mM glutamine and 0.01% each of penicillin and streptomycin.

Plasmid pCLS-Fluc

A 499 nucleotides of CAV 5’-leader sequence (named as Chicken Anemia Leader Sequence or CLS in this report) was propagated by PCR using plasmid pCAV1.3G (12) as a template with primers CAV331s (5’-GTAGGTATACGCAAGGCAGG-3’) and CAV770as (5’-CTTACAGTCTTA TACACCTTCTTGC-3’). The PCR product was then subcloned into
pBK-CMV (Stratagene, La Jolla, CA) Smal site to construct the plasmid pBK#722fk. A KpnI/BglII fragment from the plasmid pBK#722fk was inserted into the same restriction digested region of plasmid pPCR#410 which contains Firefly (Photinus pyralis) Luciferase gene cloned from a plasmid pGL3 (Promega, Madison, Wisconsin) to construct pPCR#410ffk. A 3.1 kb KpnI/NotI digested fragment from the plasmid pPCR#410ffk was then subcloned into the same restriction site of expression vector pHM6 (Roche Diagnostics, Mannheim, Germany). Two steps of inverse PCR was done to give a proper transcription from CMV promoter and to trim a sequence between CAV 5'-leader sequence and a Firefly luciferase gene. The resultant plasmid, pCLS-Fluc (or pCLS-VP2w,VP3w-FLuc), can start transcription at a CAV mRNA 5'-exact end. The sequences around Firefly Luciferase start codon are exactly same as that of CAV-VP1.

**Mutagenesis on the initiation codon of VP2 and or VP3 open reading frame**

In order to eliminate the influence of VP2 and or VP3 in this study and to find out their role in VP1 translation, we knocked out the VP2 and or VP3 open reading frame in CLS, by introduced a point mutation in the initiation codon. To knock out the VP2, we introduced a point mutation A<sup>359</sup>TG to CTG based on CAV A2C15 sequence (GenBank AB031296)). Briefly, a reversed long PCR was performed by QuickChange Site-directed Mutagenesis Kit (Stratagene) using pCLS-Fluc as a template and a pair of primers, VP2nf (5'- CCG GGT GGC<sup>359</sup> TGC ACG GGAA-3') and VP2nb (5'- TTCC GTG GCA GCC ACC CGG-3'). The mutant plasmid was named as pCLS-VP2N,VP3w-Fluc. To knock out the VP3, we introduced a point mutation A<sup>465</sup>TG to ACG by mutagenesis. The mutation was designed to be silent to VP2 using mApM-s (5'-GCA AGT AAT TTC AAA<sup>465</sup> CGC TCT CCA AG-3') and mApM-as (5'-CT TGG AGA GCC TTT CAA ATT ACT TGC-3') (8). The mutant plasmid obtained was named as pCLS-VP2w,VP3N-Fluc. Using this plasmid as template, pCLS-VP2N,VP3N-Fluc, which VP2 and VP3 knocked out, was constructed using the primer same as above.

CAV-VP2 initiation codon (UGGAUGC) has a weak by means of the Kozak’s rule (9-11) and VP3 also has a weak type of initiation codon (CAGAUGA). To find out the influence of these weak initiation codon in VP3 or VP1 translation mechanism, pCLS-VP2s,VP3w-Fluc (VP2 strong initiation codon) and pCLS-VP2w,VP3s-Fluc (VP3 strong initiation codon), were produced by introducing point mutation. Briefly, to construct pCLS-VP2s,VP3w-Fluc, a pair or primers VP2sf (5'-CCG GGA GGA TGG AGG GGAA-3') and VP2sb (5'-TTCC GTG CCA TCC TCC GG-3') were used using pCLS-Fluc as template. To construct pCLS-VP2w,VP3s-Fluc, a pair of primers, VP3sf (5'-GCA AGT AAT TTG AAG TGG TCT CCA AG-3')...
3') and VP3sb (5'-CT TGG AGA CCA TTT CAA ATT ACT TGC-3') were used using pCLS-Fluc as template.

**Tagged VP2 or VP3 in an expression vector, pHM6**

Molecular clones to complement VP2-defective CLS, pCLS-VP2N, VP3w-Fluc and pCLS-VP2N, VP3N-Fluc, were constructed. A complete open reading frame of the VP2 gene was amplified by PCR using primer VP2f (5'-CGG GTA CCC\textsuperscript{382} ACG GGA ACG GCG GAC-3') and VP2b (5'-CCG AAT TCT\textsuperscript{1010} CAC ACT ATA CGT ACC GG-3). For cloning, each primer was attached with a KpnI or EcoRI recognition site (indicated in italics). The PCR product digested with KpnI/EcoRI was subcloned into the respective sites of pHM6 vector. The clone thus obtained, pVP2, expressed a wild type VP2 with HA-epitope at its N-terminus.

To complement VP3-defective CLS, pCLS-VP2w, VP3N-Fluc and pCLS-VP2N, VP3N-Fluc, pApWT was constructed as described elsewhere (8). A complete open reading frame of the VP3 gene was amplified by PCR using primer 475s (5'-CGG GTA CCA\textsuperscript{488} ACG CTC TCC AAG AAG ATA CT-3') and 830-as (5'-CCG AAT TCT\textsuperscript{630} TAC AGT CTT ATA CAC CTT CT-3'). For cloning, each primer was attached with a KpnI or EcoRI recognition site (indicated in italics). The PCR product digested with KpnI/EcoRI was subcloned into the respective sites of pHM6 vector (Roche Diagnostics, Mannheim, Germany). The clone thus obtained, pVP3, expressed a wild type VP3 with HA-epitope at its N-terminus. All constructs were analyzed by PCR, restriction enzyme digestion, and DNA sequencing. The nucleotide sequence was analyzed by Genetyx version 7.0 (Genetyx, Tokyo, Japan).

**Dual Luciferase assay**

COS-1 cells in amount of 2x10\textsuperscript{5} cells of per 1.5 ml were delivered into 35 mm dish 12h prior of transfection in Dulbecco’s modified Eagle medium free from antibiotics. Relative Luciferase activity was determined by co-transfection of 1 x 10\textsuperscript{11} copies of CLS-Fluc plasmid and 5 x 10\textsuperscript{10} copies of Renilla luciferase plasmid (pRLuc) using FuGENE HD (Roche Diagnostics) according to the manufacturer's instruction. The cells at 12 h posttransfection were washed with dication-free phosphate buffered saline [PBS(−)] and assayed using Dual Luciferase Reporter Assay System (Promega) following the manufacturer’s protocol and quantitated using Gene Light 55. Assays were performed in duplicate and results represent the averages of three independent experiments after normalization to Renilla Luciferase activities.

**Western blot analysis**
Transfected cells were collected in Eppendorf tubes, washed with PBS and lysed in RIPA buffer (1% NP40, 1% SDS, 0.5% DOC, 50 mM Tris-HCl pH 8, 2 mM EDTA) supplemented with N-ethylmaleimide (NEM, Sigma) and protease inhibitor cocktail (Roche Diagnostic). After measuring concentrations using the Bradford method, equal amounts of protein were loaded onto precast gradient gels (NuPage, Invitrogen) and subjected to SDS-PAGE. Samples were then transferred to nitrocellulose membranes (Schleicher & Schuell). The membranes were blocked for 1 hour with casein blocking buffer (Roche Diagnostic) which was 1:10 diluted in PBS, and incubated with primary antibodies for 1 hour. After three washes in PBS-Tween (0.5%), secondary antibodies were applied for 30 minutes. After three washes with PBS supplemented with 0.5% Tween-20, the membranes were incubated with ECL Western Blotting detection reagent (Amersham) and exposed to films (Kodak). A mouse anti-Firefly Luciferase monoclonal antibody (anti-luciferase) (Novus Biologicals) was used to detect Firefly Luciferase expression. All experiments were done three times.

RNA isolation and quantitative real-time PCR (qPCR)

Total RNA was extracted using TRizol reagent (Invitrogen), followed by DNase treatment and first strand cDNA synthesis using Superscript II reverse transcriptase (Invitrogen). qPCR was performed on 50 ng cDNA on an ABI Prism 7000 using SYBR Green PCR mastermix (Applied Biosystems). We used two different primer sets: Renilla luciferase: 5’-GCT TGT TTG GCA TTT CAT TAT AGC T-3’ and 5’- TTC ACG ACG AGG CCA TGA TAA TGTT-3’; Firefly luciferase: 5’-GCA AAA AAA GCT CCC AAT CATC-3’ and 5’-AAG GAC TCT GGC ACA AAA TCGT-3’ (14).

RESULTS AND DISCUSSION

CAV Leader Sequence (CLS)

CAV has a leader sequence from nt 333 – 831 (nt 333 will be refered to as position +1 in this report). The initiation codon of VP1 is in strong context (AAGA831UGG) but both of start codon of VP2 and VP3 are in not high favorable sequence context (9-11); UGGA359UGC for ORF-2 (VP2) and CAAA465UGA for ORF-3 (VP3). In order to examine the effect of the CLS on VP1 translation, we generated a construct with the CLS arranged upstream of the Firefly Luciferase reporter gene that is driven by a CMV promoter. Plasmids were co-transfected into the cells and assayed for the relative Firefly Luciferase activity.

The VP3 protein reading frame terminates before the VP1-AUG on the mRNA, therefore it is also interesting to examine whether the VP3 proteins would have any effect on VP1 translation initiation. The VP3 was eliminated in pCLS-VP2w,VP3N-Fluc and pCLS-
VP2N, VP3N-Fluc. The influence of VP2 on the VP1 translation initiation also checked. Intriguingly, no significant differentiations of Firefly Luciferase activities were found in VP3 knocked out situation. However, Firefly Luciferase activities was increased significantly in VP2 knocked out situation. It suggested that VP1 was translated by leaky scanning from VP2 and continued by linear scanning from VP3. Complementary experiment using VP3 and or VP2 expression plasmids did not influenced these results indicated that the VP3 and or VP2 protein had little or no effect on downstream AUG utilization (data not shown).

**VP3 translated via leaky scanning mechanism**

How of ribosome reach VP3 also checked in this study. The initiation codon for VP3 located 107 nucleotides downstream of the VP2 start codon. In the most cases, translation initiation at two start sites is regulated by the rules of leaky scanning. This requires that the first ATG is in an unfavorable sequence context (9-11). In pCLS-VP2N, VP3w-Fluc, the initiation codon of VP2 was removed by site-directed mutagenesis. Quantitative analysis by western blotting from three independent experiments indicated significant enhancement in VP3 expression levels from this plasmid relative to VP3 translation from the wild type. The results suggested that the mechanism responsible for VP3 translation is in leaky scanning and in a manner that is relatively affected by the presence of the upper ORF (Open Reading Frame) to provide ribosome access to the internal VP3 start codon. To confirm this result, we optimize the initiation codon of VP2 by site directed mutagenesis. These optimization resulted significant lower of VP3 expression level. These data indicated that VP3 was translated via leaky scanning mechanism from VP2.

**Mutation of first methionin of uAUGs increased the expression of Free luciferase/VP1**

The most important question is how ribosome reach VP1 initiation site? The VP1 initiation site is in optimal context while both of the uAUGs are in suboptimal context relative to the consensus sequence, therefore it is highly possible that the initiation of VP1 is via the leaky scanning mechanism, in which some of the 40S ribosomal subunits scan past the VP2 and VP3 initiation codon and initiate translation further downstream at the VP1 AUG. In general, uORFs exerts a negative effect on translation initiation at downstream ORFs (10, 14, 15). In this study, the mechanism of VP1 translation initiation was investigated by measuring the varied expression rates of a reporter gene (Firefly Luciferase) at VP1 position caused by upstream genetic manipulation. To test this prediction experimentally and to obtain an estimate of how efficiently ribosome access the internal Firefly Luciferase/VP1 start site, the two AUG codons that occur upstream of the VP1 ORF were manipulated by site-directed mutagenesis to generate monocistronic plasmid with the Firefly Luciferase/VP1
start site representing the 5’-proximal start codon. We constructed a series of mutants, containing corresponding point mutations for each uAUG (i.e. ATG changed to ACG), which removed and or strengthen the initiation codons for VP2 and or VP3, respectively. The plasmids were named as pCLS-VP2s,VP3w-FLuc (VP2 strong + VP3 weak), pCLS-VP2w,VP3s-FLuc (VP2 weak + VP3 strong), pCLS-VP2s,VP3s-FLuc (VP2 strong + VP3 strong), pCLS-VP2N,VP3w-FLuc (VP2 knocked out + VP3 weak), pCLS-VP2w,VP3N-FLuc (VP2 weak + VP3 knocked out), and pCLS-VP2N,VP3N (VP2 knocked out + VP3 knocked out).

Firstly, the effect of the mutation on the first methionin of VP2 and or VP3 open reading frame was checked. The mutation of their start codon resulted of the absence of VP2 and or VP3 in SDS-PAGE confirmed with Western blotting (data not shown). The mutation of these start codon does abolish its expression completely. Next, we checked the mutation to strengthen of the initiation context of VP2 and or VP3 open reading frame. As expected, the expression of VP2 and or VP3 increased significantly when the VP2 and or VP3 initiation codon were strengthened.

Next, these wild-type and mutant construct were transiently co-transfected with Renilla Luciferase plasmid into COS-1 cells. After transfection, cellular extracts were prepared and assayed for translational (luciferase assay) and transcriptional (real-time RT-PCR, Firefly/Renilla mRNA) levels. The levels of transcripts were very similar among all constructs, indicating that the point mutations did not alter transcription levels (data not shown). In contrast, Firefly Luciferase activity was differentially affected by the mutations of VP2 AUG but not by the VP3 AUG.

In VP2 weak + VP3 strong condition, the Firefly Luciferase activities was no significant different with that of wild type (VP2 weak + VP3 weak). In VP2 knocked out + VP3 weak and in VP2 knocked out + VP3 knocked out, the Firefly Luciferase activity was 4 fold significant higher than that of wild type. In VP2 strong + VP3 weak and in VP2 weak + VP3 knocked out the Firefly Luciferase activities were significantly depressed (10^5 and 10 times lower than that of wild type, respectively). Intriguingly, in VP3 strong + VP2 weak or VP3 strong + VP2 strong no significant different of the Firefly Luciferase expression were found with that of wild type. Moreover, in VP2 knocked out + VP3 weak, the Firefly Luciferase expression was 4 fold higher than that of the wild type. Mutation knocked out the VP2 initiation codon caused 4-fold increase in Firefly Luciferase activity and mutation of the initiation start of VP3 had no significant effect. These results suggest that the repression of the Firefly Luciferase activity is due to the presence of the CLS occurring at a translational level, and that the VP2 AUG is involved in this repressive effect synergistically. Taken
together, it suggested that VP1 was translated by leaky scanning from VP2 continued by linear scanning from VP3.

To determine whether the decrease in luciferase activity was caused by the 5'UTR's inhibitory effect on transcription or translation, real-time PCR and Western blots were performed to measure the reporter gene luciferase protein and mRNA levels. Consistent with the luciferase activity data, the luciferase protein levels in cells transfected with VP2 weak + VP3 strong plasmid was no significant different with that of wild type (VP2 weak + VP3 weak). In cells transfected with VP2 knocked out + VP3 weak and in VP2 knocked out + VP3 knocked out plasmid, the luciferase protein levels was significant higher than that of wild type. In cells transfected with VP2 strong + VP3 weak plasmid and in cells transfected with VP2 weak + VP3 knocked out plasmid the luciferase protein levels were significantly depressed. In cells transfected with VP3 strong + VP2 weak or VP3 strong + VP2 strong plasmid, no significant different of the luciferase protein levels were found with that of wild type. Moreover, in VP2 knocked out + VP3 weak, the luciferase protein level was higher than that of the wild type. However, the luciferase mRNA levels in all samples were similar. These data suggest that the down regulation of reporter Firefly Luciferase gene expression was a result not of its effect on gene transcription, but of its inhibitory effect on protein translation.

This study was conducted to determine the mechanism of CAV-VP1 translation initiation at CAV major polycistronic mRNA. Our observation that the removal of the upper ORF-VP2 not the upper ORF-VP3, resulted in elevated Firefly Luciferase expression and the optimization of the upper ORF-VP2 not the upper ORF-VP3 silenced the Firefly Luciferase expression was consistent with synthesis of VP1 via a leaky scanning mechanism from VP2 continued by linear scanning from VP3.

In conclusion, we provide evidence that CAV-VP1 is inhibited at the translational level by the presence of the upper ORF-VP2. Leaky scanning is involved in inhibition of physiological AUG-initiated VP1 translation, resulting in weak expression of VP1 under normal conditions (6).

The molecular mechanism by which the 5'UTR regulates VP1 gene expression and its interaction with the CAV promotor remains elusive.
REFERENCES


O-MB03

EFFICIENT TRANSLATION INITIATION DIRECTED BY CAP-DEPENDENT CHICKEN ANEMIA VIRUS LEADER SEQUENCE IN STRESS CONDITION

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ABSTRACT

IRES is believed responsible for efficient translation in stress condition. The major transcript of Chicken Anemia Virus (CAV) is an unspliced polycistronic mRNA possesses three overlapping ORFs encoding for VP1, VP2 and VP3 with a leader sequence upstream of the ORF-VP1 initiation codon. The translation initiation feature and the efficient translation of this leader sequence in stress conditions have been investigated.

The CAV leader sequence (CLS) was fused to the reporter gene FLuc (Firefly Luciferase) and assayed in a classical mono- and bicistronic assays with ECMV-IRES mono- and bicistronic constructs for control. To find out the initiation translation features of CLS, cap and uncapped CLS-Fluc mRNAs were assayed in in-vitro and in-vivo translation assay. To analyze the efficient translation of the CLS-dependent translation in stress condition, pCLS-FLuc was co-transfected with pRLuc (Renilla Luciferase) into Vero, COS-1, HEP-2, BEAS-2B, and RD cells. The cells were exposure to heat and oxidative stress and assayed by the dual luciferase assay, western blotting, northern blotting and real-time PCR.

In mono- and bicistronic assays, it was found that the CLS does not contain an IRES and the translation initiation of the CLS is in cap-dependent manner. Interestingly, in heat and oxidative stress conditions the CLS-dependent translation was as efficient as ECMV-IRES-dependent translation in all cells tested compare to significant reduction of the non-CLS-dependent translation constructs.

The present data provides the novelty of CLS, a leader sequence in a cap-dependent manner, could drive an efficient translation initiation in stress condition.

**Keywords:** CAV, Chicken Anemia Virus, leader sequence, cap-dependent, stress
INTRODUCTION

Chicken anemia virus (CAV) was first isolated in Japan (1). The virus is immunosuppressive in newborn chickens, and induces generalized lymphoid atrophy, severe anemia and increased mortality (2). CAV is a small non-enveloped virus with a single-stranded circular DNA genome in the size of approximately 2.3 kb (3, 4). CAV is the only member of genus Gyrovirus within family Circoviridae. CAV has open reading frames (ORFs) only on the antigenomic strand, in contrast to the other members of circoviruses that have ambisense genome (3, 5, 6).

CAV replicates via a circular double-stranded replicative form (RF) (3). The major transcript of CAV is an unspliced polycistronic mRNA which possesses three overlapping ORFs encoding VP1 (52 kDa), VP2 (24 kDa) and VP3 (14 kDa) (3, 7). VP1 is the major viral capsid protein. VP2 is a non-structural protein with phosphatase activity of dual specificities and has been shown to interact with VP1 (8, 9). VP3, also named apoptin, is a non-structural protein made of 121 amino acids found to be important for CAV DNA replication and virion formation (10).

The arrangement of ORFs of CAV is quite similar to that of torque teno virus (TTV) (5, 11). These two viruses have a >80% similarity in a 36-nt stretch near the replication origin, but nucleotide sequences of the remaining genome show no apparent similarities (11). Their transcription patterns to produce three or more spliced mRNAs are also similar to each other (12-14). Recently, it was reported that TTV-VP3 could complement the DNA replication and virion formation of CAV VP3-knocked out (10). The common features of CAV-VP3 and TTV-VP3 presents another evidence of the close relation between CAV and TTV. TTV is currently classified into genus Anellovirus in family Circoviridae (5, 10, 11, 15).

The start codon for the VP1 occur 499 nucleotides downstream of the 5′-terminal transcription start point. This long leader sequence contains two AUG codons which are functionally and well conserved among CAV isolated worldwide reported (98%-100%). How the translation features of this CAV leader sequence is still unclear.

MATERIALS AND METHODS

Cells

MDCC-MSB1 cells were subcultured twice weekly at 39 °C in RPMI1640 medium (Sigma, Stenheim, Germany) supplemented with 10% fetal bovine serum (FBS, Immuno-
Biological Laboratories, Fujioka, Japan), 2 mM glutamine, and 0.01% each of penicillin and streptomycin. Vero, COS-1, RD, HEP-2, and BEAS-2B cells were subcultured twice weekly at 37 °C in Dulbecco’s modified Eagle medium supplemented with 10% FBS, 2mM glutamine and 0.01% each of penicillin and streptomycin.

**Mono- and bicistronic plasmids**

The 499 nucleotides of CAV 5'-leader sequence (named as Chicken Anemia Leader Sequence or CLS in this report) was fused to the reporter gene FLuc (Firefly Luciferase) and assayed in a classical mono- and bicistronic assays. For monocistronic experiments the CLS was fused to the reporter gene Fluc driven by a CMV promoter to construct the pCLS-Fluc. For cap dependent scanning translation control, a cap dependent scanning plasmid named as pRLuc was prepared. The plasmid is a Renilla Luciferase expression plasmid driven by the CMV promoter. For IRES (Internal Ribosome Entry Site) translation control, the pIREFluc, an ECMV-IRES leader sequence fused with the reporter gene Fluc, were used as control. The plasmid has ECMV-IRES upstream of the Firefly Luciferase gene. For bicistronic experiments, pRLuc-Fluc, pRLuc-CLS-Fluc, pRLuc-IRES-Fluc were involved in the experiments. All constructs were made as described elsewhere (Prasetyo, et al, submitted for publication).

**In vitro transcription**

Prior to in vitro transcription, the plasmid were linearized. In vitro transcription was performed using mScript mRNA Production System according to manufacturer’s instruction (EPICENTRE, Madison, Wisconsin). Capped transcript were synthesized including m7GpppG cap structure analogues (New England Biolabs) in the reaction mixtures to monitor cap-independent translation. The cap structure analogue will compete for cap binding proteins required for cap-dependent translation in the in vitro translation system. The integrity of the RNAs was checked by electrophoresis on agarose gels, and their concentration was measured by NanoDrop (NanoDrop Technologies, San Diego, CA). The capped and uncapped RNAs were self ligated using T4 DNA ligase (Promega).

**In vivo translation**

Firstly, the in vivo translation was done in COS-1 cells. The cells at 2x10^5 cells of per 1.5 ml were delivered into 35 mm dish 18h prior of transfection in Dulbecco's modified Eagle medium free from antibiotics. The capped and uncapped RNAs in amount of 1 μg were transfected using FugeneHD (Roche Diagnostics) according to manufacturer's instruction. The cells at 12 h post-transfection were washed with PBS (-) and assayed.
In vivo translation also checked in MDCC-MSB1 cells, the nature of host cells culture for CAV. MDCC-MSB1 cells (1 x 10^6 cells/2mL) in RPMI medium free from antibiotics were delivered to 35 mm dish. The capped and uncapped RNAs in amount of 1 g were transfected using FugeneHD. The cells at 24 h post-transfection were washed with PBS (-) and assayed. Assays were performed in duplicate and results represent the averages of three independent experiments.

**Heat Shock and Oxidative Stress**

In this study, the role of heat shock and oxidative stress in CLS elements were examined in mammalian cells. Vero, COS-1, RD, HEP-2, and BEAS-2B cells in amount of 2x10^5 cells per 1.5 ml were delivered into 35 mm dish 18h prior of transfection in Dulbecco’s modified Eagle medium free from antibiotics. The cells were transiently transfected with monocistronic plasmid by FugeneHD. Cells were co-transfected with 1x10^11 copies Fluc plasmid and 5x10^10 copies of pRLuc. Three hours after transfection, cells were washed with PBS (-) and transferred into an incubator preset at 42 °C for 1 h, while control cell monolayers were maintained at 37 °C. After heat shock exposure for 1 h, the cells were returned into 37 °C incubator for two hours to recover. For oxidative stress, the cells were exposure to H_2O_2 0.1 mM and 1 mM for 1 h three hour post transfection. The cells were washed with PBS (-), fresh medium was added, and the cells were continued incubated for two hours. After washed by PBS (-),cells were lysed by freeze-thawing and both Renilla and Firefly Luciferase activities were measured to monitor the expression levels of the reporter genes. Assays were performed in duplicate and results represent the averages of three independent experiments after normalization to Renilla Luciferase activities.

**Dual Luciferase assay**

The Firefly and Renilla Luciferase activities were assayed using Dual Luciferase Reporter Assay System (Promega) following the manufacturer’s protocol and quantitated using Gene Light 55. Assays were performed in duplicate and results represent the averages of three independent experiments.

**Western blot analysis**

Transfected cells were collected in Eppendorf tubes, washed with PBS and lysed in RIPA buffer (1% NP40, 1% SDS, 0.5% DOC, 50 mM Tris-HCl pH 8, 2 mM EDTA) supplemented with N-ethylmaleimide (NEM, Sigma) and protease inhibitor cocktail (Roche). After measuring concentrations using the Bradford method, equal amounts of protein were loaded onto precast gradient gels (NuPage, Invitrogen) and subjected to SDS-PAGE.
Samples were then transferred to nitrocellulose membranes (Schleicher & Schuell). The membranes were blocked for 1 hour with casein blocking buffer (Roche) which was 1:10 diluted in PBS, and incubated with primary antibodies for 1 hour. After three washes in PBS-Tween (0.5%), secondary antibodies were applied for 30 minutes. After three washes with PBS supplemented with 0.5% Tween-20, the membranes were incubated with ECL Western Blotting detection reagent (Amersham) and exposed to films (Kodak). A mouse anti-Firefly luciferase monoclonal antibody (anti-luciferase) (Novus Biologicals) was used to detect Firefly luciferase expression. All experiments were done three times.

**RNA isolation, Northern blotting and quantitative real-time PCR (qPCR)**

Total RNA was extracted using TRIzol reagent (Invitrogen), followed by DNase treatment and first strand cDNA synthesis using Superscript II reverse transcriptase (Invitrogen). RNA was then transferred onto a Hybond-N+ membrane (Amersham Pharmacia Biotech), fixed by UV irradiation, labeled with digoxigenin (DIG) using a DIG RNA labeling kit (Roche Diagnostics). The filter was prehybridized in DIG Easy hybridization buffer (Roche Diagnostics) and subsequently hybridized in prehybridization solution. The filter was then washed and incubated with anti-DIG antibody labeled with alkaline phosphatase. The mRNA bands were visualized using a DIG luminescent detection kit (Roche).

Quantitative real-time PCR was performed on 50 ng cDNA by an ABI Prism 7000 using SYBR Green PCR mastermix (Applied Biosystems). We used two different primer sets: Renilla luciferase: 5'-GCT TGT TTG GCA TTT CAT TAT AGC T-3' and 5'- TTC ACG AGG CCA TGA TAA TGGT-3'; Firefly luciferase: 5'-GCA AAA AAA GCT CCC AAT CATC-3' and 5'-AAG GAC TCT GGC ACA AAA TCGT-3' (16).

**RESULTS AND DISCUSSION**

CAV has leader sequence from nt 333 – 831 (nt 333 will be refered to as position +1 in this report). This 499 nt has 57.22 % GC content. Neither IRES pattern was found after analyzed the leader sequence using UTRscan (17) nor similar known patterns. A cap site was determined to lie at position –3 to +2 (3). However there is no direct evidence yet about the translation initiation feature of CLS for the best of our knowledge.

**Cap-dependent manner of CLS translation initiation**

The CLS, from position nt 333 – 831 was inserted upstream of the Firefly Luciferase gene in classical mono- and bicistronic systems. First, the expression of Firefly Luciferase was checked on transient transfection using COS-1 cells in monocistronic systems. After in
vitro transcription, the resulting mRNAs (capped and uncapped) were translated in vivo in COS-1 cells. Interestingly, there was a 2600-3000 times difference in the yield of Firefly Luciferase activity arising from translation of the CLS-capped transcript comparing with that of CLS-uncapped transcript. This high cap dependence of translation prompted us to look for the cap-dependency translation feature within the CLS. Similar results were obtained using MDCC-MSC1 cells.

Addition of the cap-analogue to the translation mixture drastically reduced the translation level showing the reaction was cap dependent. Furthermore, a comparison between in vitro transcribed capped and uncapped RNAs in vitro and in vivo showed that the translation efficiency of uncapped RNA of pCLS-Fluc was only 0.01 % and 10 % respectively of its capped counterpart. As a control, mRNAs transcripted from IRES-capped only 5-30 times higher than IRES-uncapped transcripts. Northern blotting did not reveal any small transcripts that might have resulted from a cryptic splice site in the CLS. Also the possibility of splicing was ruled out by using an RNA transfection assay.

**Translation of Firefly Luciferase/VP1 is IRES-independent**

The CLS was also screened for the presence of an internal initiation element using the classical bicistronic assay (18). The bicistronic assay has been widely used to detect the activity of sequence elements involved in IRES-mediated translation. The CLS was inserted into the intercistronic spacer of a bicistronic construct coding for Renilla Luciferase (5'-cistron) and Firefly Luciferase (3'-cistron). While the upstream Renilla Luciferase reporter is translated via cap-dependent scanning, the downstream Firefly Luciferase reporter is translated dependent on the upstream CLS. A similar bicistronic vector carrying the encephalomyocarditis virus (ECMV) 5'-UTR provided a positive control for IRES-mediated translation (pRLuc-ECMV-FLuc), and an empty bicistronic plasmid was used as a negative control (pRLuc-FLuc). These vectors were each transfected into the cells, and the ratios of Firefly:Renilla Luciferase activities were evaluated. Transfection of the bicistronic construct into cells should result in the transcription of a single mRNA that encodes both the upstream and downstream cistrons. Transcription of this message is dependent on the promoter sequence upstream of the first cistron. However, if the putative IRES harbor a sequence that can function as a promoter, then a monocistronic mRNA encoding only the downstream cistron will be produced. As expected, in dual luciferase assays, the expression of the Renilla Luciferase was significantly higher than that of Firefly luciferase. These data further analyzed by individual evaluation of Renilla and Firefly luciferase activity. Our result indicated that Firefly Luciferase/VP1 is not dependent on a sequence- or structure-based cis element that facilitates direct internal ribosome entry.
We also did directly transfected bicistronic mRNAs into COS-1 cells. In this way, the processes of transcription and RNA processing are bypassed and there is no possibility of cryptic promoter activity or cryptic splicing through the putative IRES encoding region. Two bicistronic mRNAs were produced by in vitro transcription. One mRNA included the CLS in the forward direction and the other mRNA included the ECMV-IRES. In addition, a 7-methylguanosine cap and a polyA tail of 30 nt was incorporated into both mRNAs. The mRNAs were transfected into COS-1 cells. In CLS construct the expression of the downstream cistron (Firefly Luciferase) was not enhanced by the CLS while in the ECMV-IRES construct the expression of the downstream cistron was enhanced by the ECMV-IRES supporting the conclusion that CLS is not able to promote cap-independent translation. Recently we also found that the ribosomes reached the VP1 initiation start by leaky scanning from VP2 and continued by linear scanning from VP3 (Prasetyo, et al, submitted for publication). Taken together, we concluded that CLS translation initiation mechanism is in cap-1 dependent manner.

**Efficient translation initiation directed by CLS in heat shock and oxidative stress**

It is believed that an internal ribosome entry site (IRES) within the 5’-UTR of the transcript, may have evolved to allow continued expression of key proteins involved in cell survival during cellular stress when conventional cap-mediated translation is reduced. Many cellular mRNAs contain IRES that become functional under conditions of cellular stress, when general cap-dependent translation is inhibited (19). We tried to use heat shock and oxidative stress experiment to get additional evidence to prove the cap-dependency of the VP1/Firefly Luciferase translation initiation. However, intriguingly, we found that heat shock and oxidative stress enhances expression of the Firefly luciferase at the level of translation as efficient as the IRES-dependent translation of encephalomyocarditis virus.

In Vero, COS-1, RD, HEP-2, and BEAS-2B cells, the expression of Firefly Luciferase activities were comparable, however, in all cases the expression of Firefly Luciferase downstream of the CLS were 3-4 times higher and 5-15 times higher than that of control cells after heat shock and oxidative stress, respectively. For comparison, the ECMV-IRES increased the expression of Firefly Luciferase 4-5 times higher after heat shock and 8-15 times higher after oxidative stress. Therefore, the CLS could drive translation initiation as efficient as ECMV-IRES in stress condition. To determine whether the enhancement in luciferase activity was caused by the 5’UTR’s effect on transcription or translation, real-time PCR and Western blots were performed to measure the reporter gene luciferase protein and mRNA levels. Consistent with the luciferase activity data, the luciferase protein levels in cells transfected with CLS plasmid were significantly higher in the treatment cells than in the
control cells. However, the luciferase mRNA levels in all samples were similar. These data suggest that the up regulation of reporter Firefly Luciferase gene expression was a result not of its effect on gene transcription, but of its effect on protein translation.

CAV has similar genomic organization with TTV. Configurations of ORFs in CAV and TTV were similar to each other (11, 20). The major transcript of TTV is an unspliced polycistronic mRNA which possesses three overlapping ORFs in similar configurations with that of the major transcript of CAV. The start codon for the TTV-VP1 occur 471 nucleotides downstream of the 5' terminal transcription start point. At present TTV was found ubiquitous in human but TTV has indistinct pathogenicity. It would be interesting to find out the TTV leader sequence translation initiation mechanism and its possible role in its pathogenesis in human diseases.

Finally, we provided the first direct evidence of cap-dependent translation initiation of CLS. Also, we showed for the first time that a cap-dependent leader sequence, not only IRES, could drive efficient translation initiation of the downstream gene in the stress condition.

REFERENCES


O-MB04

DETECTION of Pib (Pyricularia oryzae resistance-b) GENE IN INDONESIAN LOCAL RICES (Oryza sativa L.)

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ABSTRACT

Rice is a staple food for Indonesian people. It is consumed by more than half Indonesian people. Quality and result of rice can be decrease due to infection by several diseases. Rice blast is one of the serious diseases caused by infection of fungi, Pyricularia oryzae cav. The fungi infect all growth stages of the plant and all parts of the plant (leaf, neck, and node). The characters of the disease are spindle shaped spots on leaves and neck region develops a black color. An impact of this disease is the empty paddy seeds. Rice resistance researches to the disease have done by some scientist, but for Indonesian local rices have not. The objective of this study was to detect the disease on Pib gene in Indonesian local rice (Oryza sativa L.). This research was conducted by using four varieties of rices, Rojolele, Pandanwangi (Cianjur), Supertoy, and Kumah Wangi. Isolation was conducted by using Nucleon-Phytopure kit. Amplification and detections were conducted by using Pib Primer and can be known if the length of this gene is 389 bp. It could revealed that all rice varieties (Rojolele, Pandanwangi (Cianjur), Supertoy, and Kumah Wangi) are resistant against the rice blast disease.

Keywords : Oryza sativa L., Blast, Pyricularia oryzae cav., Resistance

INTRODUCTION

Rice is the most important staple food for world’s society. Production and consumption are concentrated in Asia, 90% is produced and consummated include Indonesia. The production’s result double in 25 years later from 257 ton each year to 520 ton in 1990 (David, 1991).
Indonesia is an agrarian country; key agricultural result is rice, staple food for Indonesian. There are many cultivated Indonesian local rice and Indonesian farmers be fond of those because good taste, aromatic, and good quality (Widodo, 2005).

Failure is the most important factor that must be concentrated. Much failure can be happened. It cause of insects pest, wereng; caterpillar; rat; and weevil (Tat, 1980), and some disease cause of fungi, bacteria, virus/micoplasm, and nematode (Mukelar and Kardin, 1991). \textit{Pyricularia oryzae} is one of microscopic fungi can make paddy grain become empty seed or half seed (Tat, 1980).

Characters of this disease are leaves spot, stem node, neck panicle, branch panicle, and grain (Mukelar dan Kardin, 1991). These fungi infect leaves and can cause leave’s \textit{necrosis}. \textit{Pyricularia oryzae} can attack paddy in all of growth stages, since growth stage until harvest stage (Chaudhary, 2001).

\textit{Pib} (\textit{Pyricularia} resistance-b) is an endurance gene to blast disease. \textit{Pib} gene (\textit{Pyricularia oryzae} resistance-b) has been successfully isolated in 1999 with cloning map-based strategy. This gene placed in long arm chromosome number 2. \textit{Pib} gene has 4 introns. As long as known there are not other genes that resistance to blast disease. This success is the first success for blast resistant gene in molecular level (Wang \textit{et al.}, 1999). And today, six endurance gene to blast disease include \textit{Pib} (\textit{Pib}, \textit{Pi-ta}, \textit{Pi2}, \textit{Pi9}, \textit{Piz-t}, and \textit{Pi-d2}) have been successfully cloned (Dai \textit{et al.}, 2007).

The consequence of this disease is serious, but still a few researches about this problem in Indonesia. The aim of this research was to detect \textit{Pib} gene in four Indonesian local rice cultivars such as Pandanwangi (Cianjur), Kumah wangi, Rojolele and Super Toy.

\section*{MATERIAL AND METHOD}

\subsection*{Plant Materials}

Materials for this research are comes from seeds. Kumah wangi seeds from Lombok and the other seed sample (Pandanwangi (Cianjur), Rojolele and Super Toy) are from Genetic Laboratory, Faculty of Biology, Gadjah Mada University.

\section*{METHOD}

1. DNA isolation

Plant’s leaves were cut used sterile scalpel, then leaves were weighted between 0.1-0.5 g. Then brayed with pestle in cold temperature, then added first Phytopure reagent 400
μl, brayed smoothly. After that, the sample was entered to 1.5 μl and added second Phytopure reagent 200 μl and shake with hands smoothly. The sample was incubated for 10 minutes 65°C in water bath and placed in ice for 20 minutes. The sample was then added with cold chloroform. Resin was added 50 μl vertically tube and centrifuged at 1300 Rcf for 10 minutes. The supernatant was moved carefully in new 1.5 ml tube and added with cold isopropanol that the volume was the same as supernatant’s volume and shake carefully. Centrifugation was done with 4000 Rcf in 5 minutes. The supernatant was loosed up, the DNA pellet was white color in based of the tube. The DNA pellet was washed with added 70 % Ethanol 100 μl, and centrifuged with 4000 Rcf in 5 minutes. The ethanol's residues were discarded and then the DNA pellet was waited until dry. Finally, the DNA pellet was added with 1XTE buffer.

2. Total DNA analysis

Agarose was made with 1% concentration. Mini-Moped Electrophoresis was set and added 1XTAE/TBE buffer. Agarose gel was put dawn in mini moped. Isolation’s DNA was took 5 μl and added 1 μl blue juice \textit{(loading dye)}, then was entered in gel. After that was ran in 100 voltages for 15-20 minutes. Ethidium bromide was prepared and then entered the gel there for 23 minutes. For the last, the DNA band was seeing in illuminator UV and was took a picture.

3. Amplification Gene (PCR)

Used master Mix PCR. Mixed the PCR component 25 μl of total volume in 200 μl. Tube was turned in centrifuge with 8000 rpm for 30 second -1 minute. PCR machine was set with annealing temperature was 64 °C and 34 cycles. Finally, the PCR product was kept in freezer (20°C).

4. Gel electrophoresis analysis

Agarose’s gel was made in 2% concentration. Mini-moped electrophoresis was set and was added 1XTAE/TBE buffer. Agarose gel was located in mini-mupid electrophoresis. PCR product was took 5 μl dan was added 1 μl blue juice \textit{(loading dye)}, then was entered in agarose gel. After that was run in electrophoresis 100 voltage for 15-20 minutes. Ethidium bromide was prepared and then entered the gel there for 23 minutes. For the last, the DNA band was seeing in illuminator UV and was took a picture.

RESULTS AND DISCUSSION
1. DNA isolation

Total DNA isolation from four cultivars of O. sativa’s leaves was conducted with Nucleon-Phytopure Kit (Amersham, USA) and The DNA quality was determined with agarose gel electrophoresis. The gel electrophoresis result of total DNA of four rice cultivars shown in Figure 4. The total DNA was collected from nucleus, chloroplast and mitochondria.

The method that used to this DNA isolation was Nucleon Phytopure Kit (there are three reagents: first reagent, second reagent, and resin). The first step in DNA isolation was paddy’s leaves were brayed with mortar. This process was broke the plant tissue. This process was done in cold temperature to inactive DNAsse because DNAsase was active in room temperature. After that was added the first Phytopure reagent to lyses cell wall. The process could happen because of there were detergent and cellulose. Detergent could dissolve lipid of cell membrane and could make protein be denaturized, and cellulose could broke cellulose. The second Phytopure reagent was then added. This reagent has function to lyses nuclear membrane. Incubation of sample at 65°C to lyses nuclear membrane. Sample was then placed in ice box for 20 minutes to stop lytic enzyme, hence DNA could be isolated from nucleus, chloroplast, and mitochondria.

The next step is added cold chloroform into the sample. The aim of this step was to clean cell wall’s residues. Resin was added to separate between cell debris and DNA total. The sample was then centrifuged in 1300 Rcf. The centrifugation’s principal is separate chemical based on the molecule’s weight. The result of this step shown in Figure 1.

![Figure 1](image)

Figure 1. Centrifugation product supernatant: cell debris: chloroform

The up layer (1) is DNA total (chromosomal DNA and extra chromosomal DNA), while the second layer is cell’s debris and all parts of cell except DNA and the last layer is chloroform.

The next step is addition of cold Isophropanol into the sample and centrifuged in 4000 Rcf. The aim of this step was to precipitate DNA, hence the pellet can be shown on the based of the tube (Figure 2)
Then pellet is cleaned with 70% ethanol to maximal precipitate DNA and the pellet is weighted.

Table 1. Pellet weight and added with TE

<table>
<thead>
<tr>
<th>Kutivar</th>
<th>Berat pellet (g)</th>
<th>Vol. TE (µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Supertoy</td>
<td>0.0015</td>
<td>100</td>
</tr>
<tr>
<td>Rojolele</td>
<td>0.0039</td>
<td>100</td>
</tr>
<tr>
<td>Kumah</td>
<td>0.0028</td>
<td>100</td>
</tr>
<tr>
<td>Pandan wangi</td>
<td>0.0016</td>
<td>100</td>
</tr>
</tbody>
</table>

TE is added based on pellet’s weight. In this research, 100 µL TE is added to all pellets. The aim of this step is dissolved the DNA. Then, the DNA is placed in freezer -20°C to keep the DNA stable.

2. DNA Total Gel Electroporation

Total DNA is checked with electrophoresis, and the result showed in Figure 3.

Figure 4. Gel agarose electrophoresis of total DNA from rice leaves. A: Supertoy; B: Pandanwangi (Cianjur); C: Rojolele; D: Kumah wangi.
DNA total is checked with 1% agarose gel because DNA total is weight so it needs a media that make easy when separated.

3. Amplification and Pib Gene Detection

The aim of Polymerase Chain Reaction (PCR) is to clone DNA so easy to detect. Annealing temperature that used into this research is the same as TM from Pib primer, 64°C. TM is temperature where 50% of DNA is denaturized, the double stranded of DNA separate to be a single stranded. In this research is using 35 cycle, so the DNA template that reach from this research is $2^{35}$

\[34.359.738.370\]

![Pib Gene Detection](image)

Figure 3. Pib gene detection from four cultivars rice leaves. M: Marker, A: Supertoy, B: Pandanwangi (Cianjur), C: Rojolele, D: Kumah wangi, E: Aquadest (negative control)

The figure above showed that all sample of this research (A: Supertoy, B: Pandanwangi (Cianjur), C: Rojolele, D: Kumah wangi) has Pib gene, it means that all plant's sample are endurance to blast disease. It can be known by length of detected DNA band was 389 bp.

Other research gave evidence that rice cultivation of other places of the world also endurance to blast disease. Some of the research are Eizenga et al. (2006) in her written explain that rice comes from Bangladesh, Bhujon Kolpo, Bogra, Khoja; Brazil, IRGA409; rice from China-CD, Fu No83, Sheng10, Zhang 32; rice from Ivory Coast, Ita 406 and Let 3137; rice from USA, Saber have endurance from blast disease.

The other research is Bustamam et al., (2004) from their research explain that Asahan variety, B8503E, Muncul, Krueng Aceh, Way Rarem, Sentani, and O. ilanos have endurance to blast disease. Sobrizal et al., (2002) from their research explain that Asahan and Laka variety have endurance to blast disease.
It explains that four Indonesian local rice has resistance gene against blast diseases and it could be useful for cultivation and breeding program of local Indonesian rice. This results showed that four Indonesian local rice have \textit{Pib} gene and it would be an important thing to increase Indonesian local rice production and its quality.

\textbf{Acknowledgement}

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O-MB05

THE STUDY OF PIT1 GENE POLYMORPHISM IN THE NAJD CATTLE USING PCR-RFLP METHOD

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ABSTRACT

The part of the bovine genome which shows a superior action and explains the major part of variation of the economical production traits were known as QTL.J. PIT1 gene, which is also termed hormone factor-1, is a pituitary-specific transcription factor which has responsible for pituitary development and hormone expression in mammals. The main function of PIT1 is for binding and trans- activity promoters of both growth hormone (GH) and prolactin (PRL) gene. It was subjected to different molecular studies as a key role for understanding genetic variation in dairy cattle. This study was aimed to detect the polymorphism in a part of PIT1 gene, exon 6, in Najdi Cattle. DNA was extracted from blood sample collected from 84 Najdi Cattle and submitted for polymerase chain reaction (PCR) followed by digestion with HinfI restriction enzyme. Base on PCR-RFLP method, we found that the frequency of the A and B alleles of this gene for Najdi Cattle were 18.45% and 81.55% respectively. The genotypic frequencies of AA, AB and BB were 3.57%, 29.76% and 66.66% respectively. X\textsuperscript{2} test indicated that these two populations were at Hardy-Weinberg equilibrium. Thus, the selection base on these loci in the population is possible to be done. The results of this study also indicated that there was no any significant effect of the obtained genotypes on milk traits.

Key words: PIT1 gene, Polymorphism, allele, Najdi Cattle,
INTRODUCTION

The growth hormone factor-1/pituitary-specifictranscription factor, Pit-1, a member of the POU family of homeo-domain transcription factors, activates gene expression for thyrotropin and prolactin (PRL) and growth hormone (GH) but also has a role in pituitary cell differentiation and proliferation. Renaville et al (1997). The inhibition of PIT1 synthesis leads to a marked decrease in expression of PRL and GH to a dramatic decrease in proliferation of cell lines producing PRL and GH. In humans, different mutations of the PIT1 gene also have been reported in patient with familial pituitary hypoplasia or with sporadic combined pituitary hormone deficiency Scully et al (2000). Finally, mutations in the PIT1 gene are responsible for the dwarf phenotypes of the Snell and Jackson mice and lead to anterior pituitary hypoplasia. During development, PIT1 gene expression precedes GH and PRL gene expression in somatotrophic and lactotrophic cells, respectively, and in the major cell specific activator of hormone expression from this cell types. The PIT1 gene is controlled by several factors that interact with its 5 regulatory the PIT1 gene itself also occurs as there are two PIT1 binding sites in the 5 flanking region. Zwierzchowski et al.(1993) explored the molecular mechanism responsible for activation of the PIT1 gene. They demonstrated that an enhancer element, located more than 10 kb upstream of the transcriptional start site, was essential for pituitary specific expression of the PIT1 gene in transgenic mice. Rajas et al (1998) characterized 12 kb of genomic DNA upstream of the PIT1 promoter. They identified a distal region that decreased the basal transcriptional activity of the pit1 minimal promoter, indicating that this region behaves as a silencer. This distal regulatory region contains 3 PIT1 autoregulatory elements. Oprzadek et al(2003).

MATERIALS AND METHODS

Samples and Bleeding Locations: A total of 84 individuals were used in this study. Whole blood samples were collected from the following populations: Shoushtar (n= 32), Shadegan (n=39), Mahshahr (n=13). DNA extraction was carried out by the method of Boom et al. (1989) as follows: Briefly, to an aliquot of 100 µl blood (after thawing), 400 µl of lysis buffer (Guanidin Thiocyanate, 20 mM; EDTA, 20mM; Tris-HCl, 10 mM; Triton X100, 40 g/l; DTT, 10g/l) was added, the mixture was vortexed and incubated at 65°C for 5 min. The cells were resuspended in20 µl of nuclease solution (Silica gel: 4g, Guanidine solution: 100 ml) and spun for 10 sec at 12,000 ×g. The pellet was resuspended in 200 µl of lysis buffer again. The suspended white blood cell suspension was then added to 400 µl of saline buffer (NaCl, 1M; Tris-HCL, 10 mM; KCl, 1M and EDTA, 20 mM), the mixture was vortexed and then spun for 10 sec at 5,000 ×g. The DNA was precipitated with 45-55 µl of extra gene solution (Ion Exchange Resin): 10%, Orange G color:0.02%, Triton X100: 0.01%) and was incubated in
65°C for 3-5 min. Then protein was precipitated by centrifugation (3 min at 1000 × g) and the upper layer containing the DNA was transferred to another tube. The relative purity of DNA was determined using a spectrophotometer based on absorbances at 260 and 280 nm, respectively. PCR–RFLP Analysis: The sequences of the forward and reverse primers for the amplification of the Pit-1 gene were:

PIT1F: 5′- AAACCAT-CATCTCCTTCTT-3′
PIT1R: 5′- AATGTACAATGTGCC-TTCTGAG-3′

The polymerase chain reaction for the Pit-1 gene was performed in a 25 μl reaction mixture containing 1.5 mM MgCl2, 200 μM of each dNTPs, 15 pmol of each primers, 2 μl 10X PCR buffer, 1U Taq polymerase and 100 ng of genomic DNA template. The reaction mixture was placed in a DNA thermal cycler. Thermal cycling conditions included: an initial denaturation step at 94.5°C for 5 min, followed by 30 cycles of 94°C for 40 sec, primer annealing at 56°C for 40 sec, PCR products synthesis at 72°C for 40 sec, and final synthesis at 72°C for 4 min using a DNA thermal cycler. Amplified DNA was digested with HinfI enzyme. The digestion product was separated by horizontal electrophoresis through 2% agarose gels in 1X TBE and 1 μM ethidium bromide.

RESULTS AND DISCUSSION

The following DNA restriction fragments were obtained for the PIT1-HinfI polymorphism: 244 and 207 bp for the BB genotype, 451, 244 and 207 for the AB and 451 for the AA (see figure). The BB genotype was the most frequent in all the studied herds 0.6666, followed by the heterozygotic AB 0.2976, whereas the AA was the least frequent 0.0357. The frequency of the PIT1A ranged was 0.1845 and PIT1B 0.8155. The genetic equilibrium in the studied population was not detected.

A main goal of the animal breeder is to select superior animals for breeding. Screening favorable alleles for selection at the DNA level provides an ideal tool for marker-assisted selection. RFLP polymorphism within the bovine Pit-1 gene was first detected with HinfI nuclease by Woolard et al. (1994). Sabour et al. (1996) showed that allele A in Pit-1 locus positively affected milk production traits in Friesian cattle. This allele (frequency of 0.18) showed a significant superiority over allele B for milk and milk protein yields and body conformation traits within Italian Holstein Friesian cattle.
The allele and genotype frequencies are variable among different studied populations and also the favorable allele and genotype frequencies in Iranian populations (*Bos indicus*) were comparable to published results, especially on *Bos taurus*. The PIT1 A allele frequencies have been estimated to be 0.45 in Angus; 0.26 in Holstein; 0.21 in Herford; 0.28 in Gelbvieh; 0.1 in Brahman; 0.25 in Polish and 0.95 in Gry cattle (Zwierzchowski et al., 2001). A heterozygosity of less than 0.5 indicated low variation for these genes in studied populations. It is suggested that the strategies such as migration, introduction of new diversity and crossbreeding for increasing gene diversity and its conservation besides exploration of this potential genetic diversity should be adapted. Although the allele frequency of B is high for some Iranian populations, the AB genotype (favorable genotype) frequency is not too high. Therefore, it is suggested that crossbreeding should be done between these populations and/or with exotic breeds to increase the frequency of the favorable genotype.

**Acknowledgments**

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References


O-MB06

APPLICATION OF RANDOM AMPLIFIED POLIMORPHIC DNA (RAPD) MARKERS FOR DETECTION OF POWDERY MILDEW RESISTANCE GENE IN DIVERSE MELONS

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ABSTRACT

pBUC114 is a random amplified polymorphic DNA (RAPD) primer amplified a DNA band in 1060 bp linked to powdery mildew resistance gene (Pm-W) in PMAR5, a powdery mildew resistant melon cultivar. In this study, the RAPD primer was used for Polymerase Chain Reactions (PCR)-RAPD to detect Pm-W gene in 16 melon cultivars. Results of PCR-RAPD showed that among 16 melon cultivars, 4 cultivars (WMR29, Harukei, Action 434 and Nigeashi) did not obtained a DNA band in 1060 bp linked to Pm-W gene and they are susceptible. Whereas, 12 cultivars (PMAR5, PMR45, PMR5, Action 434, PI 414723, PI 124112, PI 124111, PI 371795, Sunrise, MR1, Nigeashi-2, PI 161375 and Kohimeuri) obtained a DNA band in 1060 bp linked to Pm-W gene and they are resistant. The results of this study revealed that RAPD markers can be used as a tool for rapid screening of resistant melons against powdery mildew.

Key words: melon, Pm-W, powdery mildew, RAPD

INTRODUCTION

Melon (Cucumis melo L.) is a valuable cash crop grown throughout the world. It is a cross-pollinated diploid (2x = 2n = 24) species of African origin and a member of the genus Cucumis, in the family Cucurbitaceae (Robinson and Decker, 1999). Recently, melon is widely cultivated in Indonesia, mainly in Java including Ngawi, Magetan, Madiun, Sukoharjo and Kulonprogo. However, melon cultivation has been faced with plant diseases. One of the most damaging diseases affecting melon crops is powdery mildew (Alvarez et al., 2000). Powdery mildew attacking melon in Indonesia was caused by fungal species Podosphaera xanthii that reduces fruit quality and yield (Aristya and Daryono, 2007). The use of
genetically resistant cultivars will be good option for control virus. Furthermore, new source of resistance to an Indonesian isolate of powdery mildew and a locus (\(Pm-l\)) that confers resistance to the powdery have been successfully determined as a single dominant gene in melon PI 371795 (Daryono and Qurrahman, 2009).

Recently, PCR-based genetic markers have become available. These markers have been identified by either specific primers determined from known DNA sequences or arbitrary primers. Random amplified polymorphic DNAs (RAPDs) have been widely used and are one of the most powerful and fastest ways for tagging resistance genes (Michelmore et al., 1991; Paran et al., 1993; Zheng and Wolff, 2000). A RAPD marker (pUBC4111050) linked to powdery mildew resistant melon has been previously reported in PMAR5 cultivar (Fukino et al., 2004).

The objective of this study was to apply a RAPD marker linked to \(Pm-I\) for detection of \(Pm-I\) in diverse melons.

**Materials and Methods**

**Plant materials**

Sixteen melon cultivars such as: PI 371795; WMR29; Harukei; PMR45; PMR5; Action 434; PI 414723; PI 124112; PI 124111; PMAR5; Sunrise; MR1; Nigeashi-1; Nigeashi-2; PI 161375; Kohimeuri were used in this study. Seeds of each cultivar were planted in plastic pots in growth chamber under continuous illumination (8000 lux) at 26°C-28°C. Healthy leaves were harvested from seedling at 3 to 5 leaves of each individual plant. One gram of fresh leaves of each plant was collected and immediately stored frozen at -20°C. Genomic DNAs of 16 melon cultivars were used as templates for PCR amplification with the SCAR primers.

**DNA isolation**

Healthy leaves were harvested from melon seedling grown in the green house at 3 to 5 leaf stages. Genomic DNAs were extracted from harvested leaves, which have been stored at -20 °C after frozen in liquid nitrogen, and treated according to Daryono and Natsuaki (2002). The DNA samples of the 16 cultivars are each extracted from leaf tissue from 5 individual plants. DNA concentration of 10 ng uL\(^{-1}\) is prepared for all samples for use in PCR and the rest are stored in -80°C.
Random amplified polymorphic DNA

All PCR conditions were optimized and modified from protocols of Genetech and Fastat PCR kit (Roche Co.). Concentration of all DNA samples was diluted to 10 ng uL⁻¹. Amplification is performed on a DNA Thermal Cycler (PTM-100™ Programmable Thermal Controller, MJ Research Inc.) programmed for 1 cycle at 95°C for 5 minutes followed by 45 cycles at 95°C for 1 minute, 36°C for 1 minute and 72°C for 2 minutes. The products were separated by electrophoresis using 2% agarose gel in 1 x TAE buffer. A 100 bp DNA ladder was included as a size marker, and the bands were detected with ethidium bromide staining and observed under UV light. Gel pictures were then scored for the a polymorphic DNA band at 1060 bp linked to Pm-I.

Data analysis

Pictures of gels were then scored for the polymorphic DNA band as resistant (+) for presence of the DNA band and susceptible (-) for absence of the DNA band.

RESULTS AND DISCUSSION

Application of RAPD markers linked to Pm-I gene in diverse melons

A total of 80 PCR reactions was carried out by a single of 10-mer arbitrary primers and only RAPD bands specific for resistance were obtained, while susceptible specific RAPD bands for susceptible cultivars could not be amplified and excluded from the analysis. A RAPD markers, which was amplified by a PCR for the pBUC114 primer migrated around 1060 base pairs (bp) in a gel analysis (Fig.1). The primer was then used for further screening of resistant cultivars against powdery mildew in diverse melons. As result shows in Figure 1, the RAPD marker shown a fragment DNA at the 1060bp which amplified by primers pBUC114 was not only confirmed in the resistant PI 371795, but also showed in others powdery mildew resistant cultivars such as; PMR45, PMR5, PI 414723, PI 124112, PI 124111, PMAR5, Sunrise, MR1, Nigeashi-2, PI 161375 and Kohimeuri (Fig. 1).
A RAPD marker, pBUC114<sub>1060</sub> was used for selecting segregation in melon PI 371795 and its hybrid generation due to obtaining the polymorphic bands between parental resistant PI 371795 and susceptible Action 434. The markers obtained a specific DNA band linked to powdery mildew in resistant PI 371795 and used for further selection of DNA markers in diverse melons.

Identification and selecting markers will be important in breeding program due to lack serological test to find no visible symptoms in resistant cultivars. Furthermore, in this study, the markers associated with resistance was linked tightly to the specific band/ locus and will be extremely valuable for breeding programs using PI 371795 as the source of resistance to powdery mildew compare with conventional markers. Moreover, RAPD analysis was also used to identify RAPD markers linked to plant pathogens such as RAPD markers linked to downy mildew resistance in lettuce (Michelmore et al., 1991), to Rhynchosporium secalis in barley (Barua et al., 1993), to Podosphaera leucotricha in apple (Evans and James, 2003), to black leaf spot (Stegohora ulmea), in Chinese elm (Benet et al., 1995), to Tomato spotted wilt virus (TS WV; Chague et al., 1996), and to Tomato yellow leaf curl virus (TYLCV) in tomato (Chague et al., 1997).

This is the first report of RAPD markers for resistance to powdery mildew in diverse melons. This result confirmed the utility RAPD analysis to quickly identify tightly linked DNA markers that may readily be used in breeding programs. From the result of this study, RAPD as one of molecular techniques seem likely to benefit for application on resistance breeding.
of melon in the future, such as quick detection of resistant cultivars and shortening the breeding process by the more rapid and cost-effective selection of desired character includes virus resistance. Marker-assisted selection may, in future, prove to be particularly useful for selection of resistance genes from wild species, local cultivars or parents with poor agronomic characteristics. On the other hand, screening lines for resistance is expensive and time consuming, so measures to increase the success rate by marker-assisted selection may be a useful approach.

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REFERENCES


O-MB07

DEVELOPMENT OF AN EFFICIENT AGROBACTERIUM-MEDIATED
TRANSFORMATION METHOD FOR SUGARCANE

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ABSTRACT

Sugarcane is a major industrial crop for sugar production and widely cultivated in tropical and subtropical countries. DNA transformation technology serves as an useful and practical tool to introduce particular genes for crops improvement. Agrobacterium-mediated gene transfer is considered as an efficient tool for genetic manipulation. The research objectives are to compare two plasmid vectors (pCL4 and pCAMBIA) and to conduct transformation of gene encoding for sucrose-phosphate synthase (SPS) to investigate spindle leaf (apical meristematic tissue) eksplants for the transformation. Infection of Agrobacterium was conducted by dipping explants with Agrobacterium suspension in MS liquid media in the presence of 100 mg l⁻¹ acetosyringone. After infection and co-cultivation in Agrobacterium, were incubated in MS1 (3 mg l⁻¹ 2,4 D) medium. Callus formed after 3 week in culture media. Histochemical analysis of infected tissue showed the presence of blue spot of GUS transient expression in the spindle leaf, especially surrounding cutting area. The callus developed on MS1 media for 2 weeks were excised and cultured on the regeneration media MS2 (0.2 mg l⁻¹ and 1 mg l⁻¹) for 3 weeks. Putative transformants of sugarcane were found in the two plasmid vectors (pCL4 and pCAMBIA) after 5 weeks infection. PCR analysis was done with different set of primers and genome DNA was isolated from 68 of putative sugarcane transgenic. Only 12 plants that positively containing GUS DNA. Shouthern blot analysis indicated that transgenic sugarcane have one copy gene (dark band).

Keywords: Agrobacterium tumefaciens, Sugarcane, pCL4, PCAMBIA, PCR and SPS

INTRODUCTION

Traditional plant breeding techniques have been widely used to enhance important traits in agronomic crops, but this approach is laborious and time-consuming, especially in species like sugarcane. Transformation of DNA in plant can serve an important function to introduce useful genes into sugarcane, where they would be difficult or impossible by standard procedure.
Recent research indicates that *Agrobacterium*-mediated transformation is possible in monocots such as rice (Raineri et al., 1990; Park et al., 1996), maize (Ishida et al., 1996) and banana (May et al., 1995). This system offers several advantages, such as technical simplicity, minimal genome rearrangement in transformants and the ability to transfer long stretches of DNA. Although the *Agrobacterium*-mediated method has been applied also to sugarcane (Arencibia et al., 1998; Enríquez-Obregon et al., 1998), the lack of a reproducible result has been an obstacle to establish effective transformation protocol for routine genetic manipulation in the plant. The cells being traumatic due to *Agrobacterium* infection and poor survival rate. Oxidative burst, phenolization, and subsequent cell death are frequent phenomena after the infection (De la Riva et al., 1998).

The promoter is a key DNA regulatory element that directs appropriate strength and pattern of gene expression in a constitutive or specific manner, and therefore, plays a crucial role in successful transformation studies. There are some types of promoters that drive strong, constitutive, or organ specificity expression. For example, the viral Cauliflower Mosaic Virus 35S (CaMV 35S) promoter has been widely used in the transformation of many dicot and monocot. However, it has been demonstrated that activity of the promoter was low in sugarcane (Chowdhury et al., 1992; Gallo-Meagher et al., 1993). The rice actin 1 and the Emu elements have shown higher activity than CaMV 35 S in different sugarcane tissues (Gallo-Meagher and Ervine, 1996), and it was recently reported that the rice ubiquitin promoter RUBQ2 has high transgene expression levels in sugarcane (Liu et al., 2003). Thus, it is an important issue to have comparative study on the type of promoters in sugarcane.

**MATERIALS AND METHODS**

**Plant Materials.** *Apical meristematic* (spindles leaf) tissue of the shoots (around 30 cm) were removed from healthy sugarcane, wiped with 70% ethanol and used as explants. The outer leaves were aseptically removed until the inner meristematic leaf tissue was exposed. About 15 spindle leafs were excised transversely from above the apical meristem (3-5 mm in wide) and collected in 50 mL of liquid Murashige-Skoog medium supplied with 3 mg l⁻¹ 2,4 D (MS1). *In vitro* plants were prepared from mature axillary buds from sugarcane stalk. The buds were aseptically isolated from the stalk and placed on shoot formation medium containing MS basal with 0.1 mg l⁻¹ 6-benzyladenin (BA) (MSX) in the light at 26°C for 3 weeks to establish *in vitro* plants. The developed *in vitro* plants was maintained and sub-cultured in same media every 3 weeks.
Plasmid vectors and Agrobacterium strains. Plasmid of pCL4 and pCAMBIA1301 were used as plasmid vectors containing gene *gus* driven by rice ubiquitin and CaMV35S promoter, respectively. A single colony of the *Agrobacterium* containing the plasmid was inoculated in 3 mL liquid YEP medium containing 50 mg l⁻¹ kanamycin and 50 mg l⁻¹ rifampicine and incubated at 28°C on shaker for 2 days. One ml of the culture was added to 50 ml of liquid YEP medium containing the antibiotics and incubated in same condition until the culture reached an OD₆₀₀ 0.8-1.0. The culture was centrifuged at 4000 x g for 10 min and suspended in fresh 2 ml LB medium for infection.

Infection and co-cultivation. *Agrobacterium* infection was performed by addition of *Agrobacterium* suspension to the MS1 medium containing around 30-50 spindle leaves and acetosyringone (100 mg l⁻¹), so that the density of the *Agrobacterium* was 0.3 OD₆₀₀, then incubated on the shaker at 28°C for 10 min. The infected spindle leaves were filtered, washed once with sterile water (optional), and brief dried treatment in laminar flow for 20 min. Co-cultivation was conducted by placing and incubating the infected spindle leaves on solid MS1 medium (callus induction medium) containing acetosyringone in the dark at 28°C for 2 days. For infection of *in vitro* plants, basal segments of *in vitro* plants (around 3 mm) were excised and used as explants for the infection. Collected of the basal segments (100 pieces) were injured slightly by pricking 4-5 times with a sterile needle and immersed in 50 mL MS basal liquid medium. *Agrobacterium* suspension was added to the liquid medium at density of 0.5 OD₆₀₀ and incubated on a shaker at 28°C for 30 min. The infected explants were removed and briefly dried in laminar air flow, then co-cultivated in solid MS basal medium containing acetosyringone (100 mg l⁻¹) at 28°C under light for 3 days.

*Agrobacterium* elimination The co cultivated spindle leaves were washed 3 times with MS1 liquid media containing 500 mg l⁻¹ cefotaxime and briefly dried in laminar air flow for 20 min. To eliminate *Agrobacterium* growth the infected spindle leaves were incubated on MS1 (callus induction medium) containing 500 mg l⁻¹ cefotaxime in dark condition at 28°C for 10 days. To eliminate the *Agrobacterium* growth the infected *in vitro* plants were also washed 3 times with MS basal liquid media containing 500 mg l⁻¹ cefotaxime and briefly dried in laminar air flow for 10 min. The infected *in vitro* plants were cultured in MS basal media containing 50 gram/Lt sucrose, 0.1 mg l⁻¹ BA, 500 mg l⁻¹ cefotaxime and incubated at 28°C under light illumination for 10 days.

Selection and regeneration of transformant. Selection was performed by sub-cultured the infected materials to same media containing appropriate antibiotic marker (either 50 mg l⁻¹ geneticine or 0.25 mg l⁻¹ hygromicin) and 500 mg l⁻¹ cefotaxime. The spindle leaves were sub-cultured in same MS1 medium containing the antibiotic selection markers
and incubated in dark at 28°C for 3 weeks (1 cycle selection on MS1). To regenerate transformants, the developed callus were excised from the spindle and sub-cultured on MS2 medium (shoot induction medium) containing the antibiotic selection markers and 500 mg l⁻¹ cefotaxime at 28°C under light illumination for 3 weeks. The transformants were sub-cultured in same MS2 media containing the appropriate antibiotic marker and 500 mg l⁻¹ cefotaxime every 3 weeks and incubated at 28°C under light illumination. After 5 times successive selection in MS2 media, the transformants were then sub-cultured in rooting media (MS basal-hormone free medium) with no antibiotic selection marker for adaptation before acclimatization.

**Isolation of genome DNA and PCR.** Total genomic DNA was isolated from leaves of putative transformant plants using a DNeasy Plant Mini Kit (Qiagen) according to the manual protocol. PCR analysis was performed with FastStart PCR Master Kit (Roche) using genomic DNA (0.5 µg) as a template and a set primer designed either from gus DNA sequences. The set of primer were: (a) gus-F: 5'-CGTGCTGCTTTGCCATGC-3', gus-R: 5'-ACCATTGGCCAC CACCTGCC-3' (b) The PCR condition was 30 cycles at 94°C for 10 seconds (denaturation), 55°C for 30 second (annealing), 72°C for 1 min (extension), followed by 7 min (final extension) at 72°C. The amplified DNA were analyzed by 1% agarose gel electrophoresis and photographed.

**GUS assay.** The infected materials were assayed for transient expression of gus gene following the histochemical procedure described by Jefferson et al (1987) with some modification. The infected spindle leafs and in vitro plant were assayed a week after infection with Agrobacterium. Samples were washed once with 0.1 M potassium phosphate buffer (pH 7.0) and then incubated with same buffer containing 2 % methanol, 0.3% Triton X-100, 0.5 mM potassium ferricyanide, 0.5 mM potassium ferrocyanide and 0.5 mg ml⁻¹ 5-bromo-4-chloro-3-indolyl-β-D-glucoronide at 37°C for overnight.

**Southern Hybridization.** Total genomic DNA was isolated from leaves of transgenic plants using the CTAB protocol with a little modification (Sambrook et al, 1989). Approximately 10 µg genomic DNA was digested with HindIII at 37°C for overnight. The digested DNA was separated in 1% agarose gel by electrophoresis and blotted onto Hybond N+ nylon membrane. The DNA was capillary blotted onto the membrane (Sambrook et al, 1989), incubated in prehybridization and hybridization at 42°C in the hybridization oven according to the manufacturer’s protocol (KPL, Inc USA). The probe was prepared by PCR amplification of gus coding region with gus-F and gus-R primers (see above section) and as consequence, 0.35 kb fragment gus DNA was resulted. After washing to remove nonspecific
banding of the probe according to the manual mentioned in the Kit, the membrane was exposed to X-ray film (Fuji Film).

RESULTS AND DISCUSSION

To compare the affectivity of promoter for sugarcane transformation, an experiment was conducted with various binary vector and different expression promoter (Table 1).

<table>
<thead>
<tr>
<th>No</th>
<th>Var</th>
<th>Infecting explant</th>
<th>Agro strain</th>
<th>Binary vector</th>
<th>Selection marker</th>
<th>Expression promoter</th>
<th>Exp marker</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>BL</td>
<td>Spindle leaf and in-vitro plant</td>
<td>LBA4404</td>
<td>pCL4</td>
<td>Geneticin</td>
<td>Rice ubiquitin</td>
<td>GUS</td>
</tr>
<tr>
<td>2</td>
<td>BL</td>
<td>Spindle leaf and in-vitro plant</td>
<td>LBA4404</td>
<td>pCAMBIA1301</td>
<td>Hygromycin</td>
<td>CaMV 35S</td>
<td>GUS</td>
</tr>
</tbody>
</table>

Infection of *Agrobacterium* was conducted by spindle leafs with *Agrobacterium* suspension in MS liquid media in the presence of 100 mg l⁻¹ acetosyringone. In case of explants spindle leafs, the infected materials were co-cultivated in the callus induction media (MS1) containing 100 mg l⁻¹ acetosyringone for 2 days at 28°C in dark, then washed 3 times with liquid MS1 medium containing 500 mg/l cefotaxime. Most spindle leafs remained fresh(fig 1A), only a little browning and not developed yet a callus during a week at elimination medium (fig 1B), but the callus started to grow in the next MS1 media containing antibiotic selection marker for 3 weeks incubation (fig 1C). Histochemical analysis of infected spindle leafs showed presence of blue spot of *gus* transient expression in the spindle especially surrounding cutting area (fig 1D).

![Fig. 1. Agrobacterium-mediated transformation using explants meristematic spindle leaf tissue of sugarcane (A). After infection and co-cultivation with Agrobacterium, the spindle leafs were incubated](image)
in *Agrobacterium* elimination medium of MS1 (B), then transferred to the selection medium of MS1 (C). Transient expression of *gus* gene (blue spots) was detected a week after infection by histochemical analysis (see the blue spots in D).

Antibiotic resistant callus developed surround cutting area during 3 weeks incubation on callus induction medium (MS1). The callus were then excised from spindle leaf and incubated on regeneration media (MS2) at 28°C under illumination. Shoot of putative transformants that developed during 3 weeks incubation on the regeneration media were subcultured in same MS2 media (Fig. 2). The callus that not resistant to the antibiotic were browning and died.

![Browning callus](image1)

**Fig.2.** Shoot development on regeneration media of MS2 containing antibiotic selection marker. The developed callus on MS1 media were excised and cultured in the regeneration media for 3 weeks. The callus that not resistances to antibiotic selection marker were turns to brownish and died.

After five successive sub-cultured on MS2 selection media, the shoots were sub-cultured in rooting media for one cycles (Fig.3A), then subjected for acclimatization in pot-pot containing soil at green house condition (Fig. 3B). To have a better growth of the plantlets before acclimatization, the antibiotic selection marker was omitted from the rooting media. This method was implicated just to maintain surviving rate of acclimated plantlet when it was transferred to soil media.

![Transgenic sugarcane](image2)

**Fig. 3.** The growth of antibiotic resistant plantlet in the rooting media (A) and soil media during acclimatization (B).
**PCR Analysis.** PCR reaction was performed using genome DNA isolated from leaf of sugarcane putative transformants that were acclimated in green house. The single band was found at PCR analysis using a set primer from GUS gene (Fig. 4).

![Fig. 4. Electrophoresis separation (1% agarose) of the PCR product using template genome DNA isolated from transgenic sugarcane using a set gus primer, transgenic sugarcane (lines 1-12), wild type (WT) and marker (M)]](image)

**Southern Blot.** to confirm T-DNA integration into the sugarcane genome, Southern blot analysis was performed on total genomic DNA isolated from leaves of the transgenic plants. A 0.35 kb DNA fragment prepared by PCR amplification of ORF gus DNA was used as a probe and labeled with chemiluminescent (non-radioactive). To determine the number of integration sites in the plant genome, isolated DNA was digested with *Hind III* and separated with 1% agarose gel electrophoresis. Figure 5 is showing the southern hybridization results using detector from the kit. The probe was strongly hybridized at line 1,2,3 and low signal at line 5 and 6 and very low signal at line 4.

![Copy number](image)

**Figure 5.** Southern blot analysis of genomic DNA isolated from transgenic plants. Approximately 10 μg of genomic DNA from each transgenic plant were digested with *HindIII*, separated on agarose (1%) electrophoresis, and transferred to Hybond N+ nitrocellulose membrane. Hybridization was performed with a gus probe labeled with AP chemiluminescent detector (KPL, Inc.). Arrow is showing black spots (number of copy gene) of estimated DNA bands.
CONCLUSION

PCR analysis was done with different set of primes and genome DNA was isolated from 68 of putative sugarcane transgenic. Only 12 plants that positively containing GUS DNA. Southern blot analysis indicated that transgenic sugarcane have one copy gene (dark band).

REFERENCES

O-MB08

DIAGNOSTIC SENSITIVITY COMPARISON BETWEEN POLYMERASE CHAIN REACTION-HIGH RESOLUTION MELTING (PCR-HRM) AND SEQUENCING TECHNIQUES TO DETECT KRAS MUTATION

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ABSTRACT

Colorectal cancer is a common type of cancer that has good prognosis if detected early. Mutation in K-Ras gene may contribute to initial development of colorectal cancer and has been proposed as a diagnostic biomarker for early detection. Recently KRAS mutation has been found as a resistance factor to anti-EGFR (Epidermal Growth Factor Receptor) therapy. Therefore detection of KRAS mutation has important clinical utility. Polymerase Chain Reaction-High Resolution Melting (PCR-HRM) is a promising technology to scan subtle genetic and epigenetic changes of cancer biomarkers due to its high sensitivity and technical simplicity. On the other hand, direct DNA sequencing which is known as the golden standard for DNA analysis is more complicated and costly to conduct. Our study aims to compare the sensitivity of PCR-HRM and direct DNA sequencing techniques to detect KRAS mutation. To obtain consistent results, both HRM and DNA sequencing as post-PCR analyses require high quality of a specific PCR product. DNA isolated from samples with previously known KRAS mutation were titrated with wild type KRAS DNA and tested with PCR-HRM. The data suggest that PCR-HRM can detect down to 6.25% mutant DNA in the background of wild type KRAS DNA. These data will be validated against DNA sequencing using clinical isolates.

Keywords: KRAS Mutation, PCR-HRM, DNA Sequencing

INTRODUCTION

Cancer biomarker detection plays important role for cancer early detection and treatment selection. Mutations on RAS gene induce changes of protein conformation, which plays critical role for transducing extracellular signals of cellular proliferation1. In colorectal cancer, more than 90% of mutated RAS gene is KRAS gene 2. Recent many clinical studies involving cancer patients, this KRAS mutation has been discovered as a resistance factor to
anti-EGFR (Epidermal Growth Factor Receptor) therapy. Mutation of KRAS gene is more likely to occur on codon 12, 13, and 61. However, mutation on codon 61 is not as frequent as codon 12 and 134. DNA mutation has been detected by PCR method, which is followed by direct DNA sequencing or real time PCR. Polymerase Chain Reaction-High Resolution Melting (PCR-HRM) is a promising technology to detect subtle genetic and epigenetic changes of cancer biomarkers due to its high sensitivity and relatively easy to do. On the other hand, direct DNA sequencing which has been accepted as gold standard of DNA analysis is a cumbersome technique and costly to conduct. Nevertheless, the combination of these two methods play important role for DNA variations detection. To obtain consistent results, HRM and direct DNA sequencing as post-PCR analysis requires high quality of a specific PCR product.

MATERIALS AND METHODS

Samples Preparation

Cell lines A549 and HCT116 which harbor homozygous genotype of KRAS mutation on codon 12, and heterozygous genotype on codon 13, respectively were used to serve as mutant DNA template controls (Fig 1). MCF-7 cell line was used as KRAS wild type DNA template control. A549 and HCT116 cell lines were mixed with MCF-7 in dilutions of 50%, 25%, 12.5%, and 6.25%.

PCR-HRM Conditions

Primers were designed to span codons 12 and 13 of the KRAS gene. The intercalating dye used was SYTO 9 (Invitrogen). The reaction mixture was made up using HotStarTaq (Qiagen) and consisted of 25 ng of genomic DNA, 1× PCR buffer, 2.5 mM MgCl2 total, 200 nM of each primer, 200 µM of dNTPs, 5 µM of SYTO 9, 0.5 U of HotStarTaq polymerase and PCR grade water in a volume of 20 µL. All PCR reactions were performed in duplicate. PCR cycling and HRM analysis was performed on the Rotor-Gene 6000™ (Corbett Research, Mortlake, New South Wales, Australia). The amplicon was run according to the following conditions; one cycle of 95°C for 15 minutes; 50 cycles of 95°C for 10 seconds, 68°C for 5 seconds, 72°C for 20 seconds; one cycle of 95°C for 1 second, 72°C for 90 seconds and a melt from 72 to 95°C rising at 0.1°C per 2 second.
Fig. 1: Type of KRAS Mutation on Codon 12 and 135

PCR and Nested PCR for Sequencing Primers were designed to span codons 12 and 13 of the KRAS gene but differ from HRM-primers. The reaction mixture was made up using FastStartTaq (Roche) and consisted of 25 ng of genomic DNA, 1× PCR buffer, 1.5 mM MgCl2 total, 1 µM of each primer, 200 µM of dNTPs, 1.25 U of FastStartTaq Polymerase and PCR grade water in a volume of 25 µl. The samples were run according to the following conditions; one cycle of 95°C for 5 minutes; 35 cycles of 95°C for 1 minute, 50°C for 1 minute, 72°C for 2 minute; and one cycle of 72°C for 10 minute. The reaction mixture for nested PCR consisted of 1 µl of 1/10 diluted PCR products from the first PCR, 1× PCR buffer, 1.5 mM MgCl2 total, 500 nM of each primer, 200 µM of dNTPs, 1.25 U of FastStartTaq Polymerase and PCR grade water in a volume of 25 µl. The samples was run
according to the following conditions; one cycle of 95°C for 5 minutes; 38 cycles of 95°C for 1 minute, 55°C for 1 minute, 72°C for 2 minute; one cycle of 72°C for 10 minutes.

DNA sequencing

Nested-PCR products were column purified using the PCR Product Purification Kit (Roche) according to the manufacturer's instructions. The purified PCR product was then used as template in cycle sequencing with the DYEnamic ET Dye Terminator kit (GE Life Science). The reaction mix consisted of 1× DYEnamic ET Dye Terminator premix, 5 pmol primer and 8 µl of cleaned template in a 20 µl total volume. The reactions were run on a C1000 thermocycler (BioRad) according to the following protocol; 40 cycles of 95°C for 20 seconds, 50°C for 15 seconds, 60°C for 1 minutes. The sequencing reactions were ethanol precipitated and ran on a MegaBACE™ (GE Life Science). Sequencing data was analysed using Bioedit Software.

RESULTS AND DISCUSSION

The PCR-HRM data is shown on dif graph while sequencing data is shown on sequence graph. Difference graph from PCR-HRM differs many samples from one template used as background. In this analysis, wild type KRAS sequence which codon 12 and 13 are located from gene bank were then used as background to compare it from mutant template and template from titration between mutant and wild type DNA. The sensitivity between this PCR-HRM and direct DNA sequencing methods were shown on figure 4 and figure 5.

Fig. 2: PCR-HRM dif graph of KRAS mutated Cell Line5

Fig. 3: Wild Type KRAS sequences (NCBI) The wild type sequence for codon 12 is shown as GGT peak and for codon 13 is shown as GGC peak.
As represented in Figure 4, sequencing chromatogram of 100% mutation template (HCT116 100%) showed A and G peaks at second base on codon 13. As the mutation template titrated with wild type template, the A peak began to shrink and started to disappear on fourth titration (HCT 6.25%). Meanwhile the HRM difference graph still shows significant difference between 6.25% mutation template and wild type template. Therefore, HRM remained sensitive to detect mutated KRAS down to 6.25% in comparison to direct DNA sequencing.

Fig. 4: Sensitivity comparison between PCR-HRM and direct DNA Sequencing on KRAS sequence from diluted HCT116

As shown in Figure 5, sequencing chromatograph of 100% mutation template (A549 100%) indicated a change of the original G peak at first base on codon 12 into an A peak. As the mutation template titrated with wild type template, the A peak began to shrink and G peak began to occur. The A peak started to disappear on fourth titration (A549 6.25%). Meanwhile the HRM difference graph still showed robust distinction between 6.25% mutation template and wild type template.

Fig. 5: Sensitivity comparison between PCR-HRM and direct DNA Sequencing on KRAS sequence from diluted A549 In light of this result it is important to isolate DNA where 50% or more tissues are cancerous.

CONCLUSION

The results of comparing these two molecular analyses suggest that PCR-HRM is more sensitive method than using direct DNA sequencing to detect variations on DNA sequence. However, PCR-HRM cannot detect which variation precisely but this PCR-HRM method can be used as early diagnostic for variations on DNA sequences more swiftly. Recent development of PCR using COLD (Co-amplification at Lower Temperature Denaturation) technique may be incorporated to improve sensitivity of direct PCR sequencing7, 8.
ACKNOWLEDGEMENT

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REFERENCES

O-MB09

GENOMIC AND PROTEOMIC CHARACTERIZATION OF INSULIN RECEPTOR (HINSR) OF DM PATIENT

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ABSTRACT

The aim of our research is to characterize genomic and proteomic insulin receptor (hINSR) of Indonesian diabetes mellitus patients. The blood and serum were collected from normal and DM patient at some public clinics and Saiful Anwar Hospital, Malang. DNA and RNA were isolated from blood, and protein was isolated from serum. To find out the genomic and 3D-proteomic hINSR, DNA sequences were analyzed by in silico, and the serum protein characterized by 2D-electroforesis analysis. The result of our study showed that the hINSR gene has two variant as same as GeneID 3643 at GeneBank, at chromosome 19p13.3-p13.2, and has 22 exons with mRNA 4200bp. We found specific protein of DM patient from 2D-protein profile and some type mutation of hINSR and can change the INSR 3D-protein structure. According to our result, we suggested that the INSR protein mutation of DM patient proceed abnormally INSR function against tyrosine kinase and perhaps correlated with genetic syndrome of insulin resistance.

Keywords: Diabetes Mellitus, insulin receptor family, insulin resistance, proteomic.

INTRODUCTION

The pathogenesis of NIDDM has been studied in various ethnic groups. It appears that insulin resistance can precede the clinical onset of NIDDM. The human insulin receptor exists in two isoforms, hINSR -A and hINSR-B, which are generated by alternative splicing of a primary gene transcript and differ by a 12-amino acid insertion sequence in the a-subunit. The two receptor isoforms bind insulin with different affinities and are differentially expressed in human tissues. Mutations of human insulin and insulin receptor family can lead autosomal dominant syndrome on diabetes, fasting hyperinsulinemia, and insulin resistant. Activation of
the insulin receptor on the plasma membrane of cells by binding of insulin is the initial event that triggers the insulin receptor-signaling cascade, leading to the multiple cellular responses induced by insulin (1, 2). The insulin receptor is a tetrameric membrane protein with an α2β2-subunit structure and is encoded by a single gene on chromosome 19 (2, 3). Processing of the primary α-β gene product yields the mature insulin receptor. Next to insulin receptors, most cells also express IGF-I receptors with similar structure and function (4).

Mutations of the human insulin receptor gene have been identified in patients with severe insulin resistance, and studies of these naturally occurring mutants may provide important insights into the relationship between structure and function of the receptor. Although 17 distinct insulin receptor mutations have been identified in 14 human subjects (1-5), there is as yet little known about the ability of natural mutants to alter the normal pattern of insulin-mediated signaling. In contrast, site-directed mutagenesis of residues within the intracellular P-subunit has led to new insights into the consequences of impaired tyrosine kinase activity for receptor signaling and the potential divergence of insulin-stimulated pathways (5).

Biochemical analyses of the various mutations seen in patients with insulin-resistant syndromes provides insight into the residues of the insulin receptor that are critical for correct functioning and processing of the receptor. Further more, by studying multiple patients with the same mutation, insight can be obtained into what extent the genetic background is an important modulator of phenotypic expression of insulin receptor gene mutations. Studies of the signaling properties of natural mutants are also important, not only because of unique insights into structure/function that may emerge, but also because it is possible that the diverse phenotypes associated with severe insulin resistance may in part be due to the ability of some mutations to differentially affect insulin-regulated cellular events.

MATERIAL AND METHODS

The blood samples are patients from Saeful Anwar Hospital Malang and some public clinics. Protein was isolated from serum samples and DNA was isolated from blood samples. To analyze the profile protein of diabetics patient and control, we used tetracell-2D-electrophoresis (BioRad), then the gels were visualized and analyzed by using ChemiDoc-Imaging (BioRad). To analyze the hINSR gene, we used the blood DNAs of patients and control. Blood DNAs were amplified with six pairs of primer that specific for hINSR gene with GeneAmp PCR Systems 9700 (Applied Biosystems). Then, the DNA-amplified were
sequenced by ABI Prism Sequencer (Applied Biosystems). The protein and DNA sequence were analyzed by GeneBank Software application.

RESULT AND DISCUSSION

The result of research showed that the hINSR gene has two variant as same as GeneID 3643 at GeneBank, at chromosome 19p13.3-p13.2, and has 22 exons with mRNA 4200bp. We found specific protein of DM patient from 2D-protein profile and some type mutation of hINSR and can change the INSR 3D-protein structure, but the 2D-protein result should be analyzed by further methods to identify the type of protein. The mutation types of hINS gene exon 22 of some DM patients are point mutation with single base deletion and substitution. We found mutation of single deletion at Met$^{1296}\rightarrow$Cys$^{1296}$ and Glu$^{1300}\rightarrow$Gly$^{1300}$, and also point mutation at Met$^{1296}\rightarrow$Ser$^{1296}$ and Trp$^{1299}\rightarrow$Ala$^{1299}$ and Met$^{1389}\rightarrow$Ile$^{1389}$. These series mutations are made the polypeptides sequence changed as frame shift mutation, actually normal INSR has six amino acids -Met Arg Met Cys Trp Glut- and DM patient has sequence of the five amino acids - Cys Ala Ser Ala Gly, as properly. This domain is suggested as the ATP-binding site of tyrosine kinase of INSR, according to Kodawaki research group (6) were identified two point mutations in the insulin receptor tyrosine kinase domain in subjects with the Type A syndrome of insulin resistance: Trp$^{1200}\rightarrow$Ser$^{1200}$ and Ala$^{134}\rightarrow$Thr$^{134}$ (6). The other research report was one additional naturally occurring amino acid substitution that is linked to an insulin resistance syndrome has been identified within the intracellular P-subunit tyrosine kinase domain (3, 5). This mutation replaced Gly$^{1008}$ within the receptor ATP-binding site and results in complete loss of both tyrosine kinase activity and insulin-mediated biologic signaling; these effects closely resemble site-directed ATP-binding site (Lys$^{1030}$) mutations (3, 6).

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REFERENCE


O-MB10

METAGENOMIC SEQUENCE ANALYSIS REVEALS DIVERSE 16S rRNA GENE IN TERMITE INTESTINE BACTERIA

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ABSTRACT

More than 99% of the bacteria present in many environmental samples cannot be cultivated in the laboratory (or uncultureable) and hence remain obscure for their ecological functions, and unexploited for biotechnological applications. Metagenome permits searches for diversity species or genes by extracting genome DNA directly from varied microbial habitats. The diversity of unculturable bacteria also permits searches for new enzymes and various natural products. The uncultureable bacteria can be found in many bacterial habitats from environments including intestinal termite. There were evidences intestinal bacteria symbioses with termite to produce cellulose (wood digested enzyme) (Moriya and Toshiaki, 1996; Brune and Friedrich, 1998). Here we described metagenome sequence based was carried out and small-subunit rRNA genes (16S rDNAs) directly amplified from the mixed-population DNA of the intestinal termite Hypotermes sp. Wasman by the PCR and were clonally then compared the partial sequences of the genes to explore the uncultureable bacteria phylogenetic diversity.

INTRODUCTION

More than 99% of the bacteria present in many environmental samples cannot be cultivated in the laboratory (or uncultureable) and hence remain obscure for their ecological functions, and unexploited for biotechnological applications. Metagenome permits searches for diversity species or genes by extracting genome DNA directly from varied microbial habitats. The diversity of unculturable bacteria also permits searches for new enzymes and various natural products. The uncultureable bacteria can be found in many bacterial habitats from environments including intestinal termite. There were evidences intestinal bacteria
symbioses with termite to produce cellulose (wood digested enzyme) (Moriya and Toshiaki, 1996; Brune and Friedrich, 1998). Here we described metagenome sequence based was carried out and small-subunit rRNA genes (16S rDNAs) directly amplified from the mixed-population DNA of the intestinal termite Hypotermes sp. Wasman by the PCR and were clonally then compared the partial sequences of the genes to explore the uncultureable bacteria phylogenetic diversity.

MATERIALS AND METHOD

1. Sample Preparation

Intestinal termite was prepared based on method of Moriya & Toshiaki (1996). The termite (Fig. 1) is taken from some trees at Biotech Center Serpong and the intestinal gut was isolated by using a pin set from its abdomen terminal.

![Termite Hypotermes sp. Wasman](image)

Fig. 1: Termite Hypotermes sp. Wasman

2. Genome DNA Isolation and 16S rRNA gene Amplification

Genome DNA was isolated directly from intestinal termite based on Q-Bio Gene method (2004). About 20 intestinal termites were collected and added to the lysing matrix then 16S rRNA gene was amplified to the genome DNA using primer 9F (5’-GAGTTTGATCCTG GGTCAG-3’) and 1541R (5’-AAGGAGGTGATCCAGCC-3’).

3. PCR Cloning and Recombinant DNA Isolation

The PCR reaction conditions were as follows: 98°C for 3 min, 30 cycles at 98°C for 20 s, 56°C for 30 s, and 72°C for 1 min. PCR products corresponding to the expected size of the bacterial 16S rRNA (1.5 kb) were purified on an agarose gel. Amplified gene of 16S rRNA was cloned into pGEM-T Easy vector based on the method (Promega, 1999) with the insert gene and vector concentration ratio is 1:3. Recombinant DNA was isolated by minipreparation DNA plasmid method (Sambrook and Russell, 2001). Bacterial clones were grown on liquid LB contained 100 μg/ml of ampicillin.
4. RFLP DNA Digestion

Plasmids DNA of the recombinant bacterial clone were digested by HhaI dan Rsal (Fermentas) to profile restriction fragment pattern. Reaction of the digestion was carried out according to the manufacturer’s directions.

5. Nucleotide Sequencing and Phylogenetic Analysis

Single-stranded clonal DNAs were prepared from plasmid DNA recombinant bacterial clone and used as templates in sequencing with the universal M13 primer contained in a PRISM Ready Reaction Dye Primer UniCycle Sequencing Kit (Applied Biosystems). Sequencing reactions were performed with an automatic sequence analyzer (Applied Biosystem Genetic Analyzer 3130). Subsequently, the sequences data were analyzed by BLAST program (http://www.ncbi.nlm.nih.gov/blast/blast.cgi). Sequence data were aligned by using the CLUSTAL X 1.83 package and then corrected by manual inspection, and nucleotide positions of ambiguous alignment were omitted from subsequent phylogenetic analyses. Phylogenetic trees were constructed by the neighbor-joining method (Saitou, 1996) with the NJPLOT package. Bootstrap analyses for 1000 replicates were performed to provide confidence estimates for tree topologies.

RESULTS AND DISCUSSION

Amplification and Cloning of 16sRNA gene

DNA was directly extracted from the intestinal gut contents, and 16S rRNAs were amplified from universal primers (9F and 1541R). PCR products were electrophoresed, and bands of the expected size range for prokaryotic 16S rRNA (1.5 kb) (Fig. 2) were recovered

![1.5 kb](image)

Fig. 2: Agarose gel electrophoresis of PCR product from DNA isolated from intestinal termite samples: Selective amplification of the 1.5 kb fragment using 9F/1541R specific primers for 16S rRNA gene. Lane 1: DNA Marker; 2: PCR Product
from the gel and cloned into *E. coli*. Most of the DNA inserts in the clones were the expected full-length amplification products (1.5 kb).

**RFLP Analysis of 16S rRNA Gene Clones**

PCR products of 16S rRNA genes were ligated into the pGEM-T Easy vector and digested with *HhaI* and *RsaI*. Several clones containing an insert are isolated, and one is selected for profiling the gene to assess polymorphism of 16S rRNA gene among intestinal termite guts samples. About 53 of identified cloned has
contained 16S rRNA gene were analyzed by restriction digestion. Nine PCR-RFLP profiles digested by *Hha*I of verified inserted 16S rRNA were different. Meanwhile, 3 PCR-RFLP profiles digested by *Rsa*I enzymes using UPGMA method were also different. It was suggested that by using of the *Hha*I and *Rsa*I enzymes have revealed that both enzymes can be used to profile 16S rRNA. The variety of restriction patterns demonstrates the molecular diversity of DNA clones in the metagenomic library. Figure 3 shows us a dendogram of 9 unique restriction profiles of 2 clones (called A and B clones) obtained from intestinal termite samples using *Hha*I and *Rsa*I digests. Based on the dendogram can be known grouping 16S rRNA profile among samples. Constructed dendogram with UPGMA method formed 2 clusters, cluster A and B while A1 clone is out of both cluster. Cluster A consists of 16S rRNA genes from A6, A7, A21, B2, and B3 clone. Meanwhile, cluster B consists only B4, B5, and B26 clone. This presence of the clustering demonstrates the presence of polymorphisms of 16S rRNA gene among intestinal termite samples. Based on sequencing result of Ohkuma and Kudo (1996), 16S rRNA gene that is in the same cluster has no distinct gene function to the other which it could be reflected genetic variation of 16S rRNA gene. Therefore, this genetic variation demonstrates the molecular diversity of DNA clones in the metagenomic library of intestinal termite samples. The results show that DNA extracted directly from intestinal termite samples is a valuable source of new genetic information and is accessible by using metagenomic libraries.

**Phylogenetic Analysis Based on 16S rRNA Gene Sequence**

A total of 5 clones (A1, A7, B2, B4 and B5 clone) were sequenced and analyzed by BLAST program. As the results, all of the clones were phylogenetically affiliated with of the uncultured bacterium NP (Bacteria domain) and closely related to *Pseudomonas*
acephalitica. More-detailed information concerning the phylogenetic relationships of the clones and known bacteria is shown in Figure 4 and 5. The cluster containing A1, A7, B2, B4 and B5 clone is also deeply branched with Proteobacteria (purple bacteria) but fails to branch with the Fungi. The Proteobacteria is one phylum of Bacteria domain. The phylum is grouped based on 16S rRNA gene sequence (Madigan et al., 2000).

Fig. 4: Phylogenetic position of A1 and A7 clones at bacteria domain using neighbor-joining method (Saitou, 1996) with the NJPLOT package. Bootstrap analyses for 1000 replicates.

Fig. 5: Phylogenetic position of B2, B4 and B5 clones at bacteria domain using neighbor-joining method (Saitou, 1996) with the NJPLOT package. Bootstrap analyses for 1000 replicates.

Sequence alignment results of A1 and A7 clone (Fig.6) have revealed that both clones are different. The A1 clone has gap on the 246-268 base, but A7 clone has gap on the 280-300
base. This result demonstrated that there was insertion or deletion of nucleotide base at both clone. The A7 clone was assigned and sharing a high degree of similarity to previously sequenced of *Achromobacter insolitus* in the gene bank database. The *A. insolitus* and A7 clone sequences wasn't have gap at A1 clone gap position that showed genetic variation between A1 and A7 clone.

In contrast, sequence alignment results of B2 and B5 clone (Fig.7) have revealed that both clones are quite not different and have gap only 14 bases on the 393 base, but it is out group from B4. From these result show that A and B clone is not identical. Additionally, although A1, A7, B2, B4, and B5 clone was came from intestinal termite, they have not been positioned in one cluster (Fig. 4 and 5). Therefore, in conclusion, phylogenetic analysis of clonally isolated 16S rDNAs shows that the intestinal termite consists of diverse microbial species, many of which have not been previously characterized (unculturable). To our knowledge, this is the Indonesian first report of a phylogenetic analysis of the intestinal bacterial diversity of a termite species in which formerly uncultivated bacteria have been found.
Fig. 7: Sequence alignment results of B2, B4 and B5 clone

REFERENCES


O-MB11

GENETIC POLYMORPHISM OF mt-DNA CYTOCHROME B (CYT B) 
IN INDONESIAN DOMESTIC CATTLE

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ABSTRACT

The aim of this research was to identify nucleotide polymorphic sites in a 464 bp region of the cytochrome b (cyt b) mitochondrial gene of Indonesian Domestic Cattle (Bos indicus and Bos javanicus). This region is widely used as a target polymorphism by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) for species identification studies. We used four cattle breed as samples, there were Bali, Madura, Ongole-Grade (PO) and Local Pacitan cattle breeds. The L14735 and H15149 primer pair was used to amplify the cyt b gene. The PCR products were cleaved by TaqI restriction enzyme and then electrophoresed on 2% agarose gels. This research subjected to determine the diversity of domestic cattle base on maternal inheritance. The results showed that Indonesian domestic cattle had three haplotype. The domestic cattle which consist of Bali cattle, Madura cattle and Local cattle of Pacitan included in haplotype A, the PO cattle included in haplotype A and B, and one of Local cattle of Pacitan belong to haplotype C. The haplotype A indicated that the cattle related to Bos javanicus (Banteng), while Haplotype B and C indicated that the cattle related to Bos indicus. Indonesian domestic cattle had genetic diversity based on genetic polymorphism of mt-DNA cytochrome b.

Key words: PCR-RFLP, mtDNA cyt b, Indonesian Domestic Cattle, Haplotype

INTRODUCTION

The developing of domestic cattle is very important for Indonesian livestock industry. Huijtema (1982) reported there domestic cattle has better reproductivity, more adaptable in tropical environment and management, and more resistant from tropical disease. Indonesian have four domestic cattle breed, such as Bali, Madura, PO and Local cattle of Pacitan (native Pacitan cattle).
To study the genetic polymorphism of domestic cattles in Indonesia, we examined the sequence of mt-DNA cytochrome b in several breeds which collected from the differentiation. Mitochondrial DNA had been used as a molecular marker. Mitochondrial DNA evolves much faster than nuclear (nc) DNA and thus contains more sequence diversity compared to nuclear DNA, facilitating the identification of closely related species (Brown et al., 1996). In addition, maternal inheritance of the mt-DNA generally results in lack of heterocigosity. The one of gene that encoded by mitochondrial DNA is cytochrome b gene (Prusak and Grzybowski, 2004). Cytochrome b gene of several vertebrates, including mammals, were mainly investigated for evolutionary, genetic diversity and molecular phylogenetic studies (Wolf et al., 1999).

MATERIALS AND METHODS

Samples collection and DNA extraction. Both blood and ear tissue were collected from Madura Island, Pacitan Regency, Yogyakarta and another region (Bali, Kalimantan and NTB; Bali cattle). The DNA was isolated from blood samples and ear tissue. Blood samples were prepared by using DNA isolation KIT high pure PCR template preparation (ROCHE) appropriate with it’s protocol. Ear tissues were prepared by using standard SDS/proteinase K extraction (Sambrook et al., 1989).

PCR and RFLP analysis. Amplification of the mt-DNA cytochrome b (cyt b) gene was carried out in a final volume of 20 µl in 0.5 ml tubes containing 13.3 µl aquabidest, 2 µl PCR buffer, 1.5 µl MgCl₂, 0.1 µl dNTP mix, 1 µl each primer (L14735: AAA AAC CAC CGT TGT TAT TCA ACT A and H15149: GCC CCT CAG AAT GAT ATT TGT CCT CA) as universal cyt b internal primer pair, designed by Kocher et al. (1989), 0.5 units of Taq DNA polymerase and 1 µl DNA genom. The cycling conditions were as follows: 94°C for 2 min for pre-denaturation, 35 cycles of 36 s at 95°C, 73 s at 51°C, 84 s at 72°C and followed by a final extension step of 3 min at 72°C and 4°C until the next step. PCR products were examined by electrophoresis through a 1% agarose gel in 10X TBE buffer and stained by ethidium bromide. As size reference, Novagen marker was used. PCR product was digested by restriction enzymes as described previously (Verkaar et al., 2002) and electrophoreted on a 2% agarose gel. In this work we used TaqI restriction enzymes.

RESULTS AND DISCUSSIONS

Polymorphism of mt-DNA Cytochrome b based on TaqI-RFLP Analysis

Polymerase chain reaction was carried out in a final volume 20 µl. The PCR product was resulted 464 bp. The PCR product was digested by TaqI restriction enzyme (Fig. 1).
Figure 1. The TaqI-RFLP analysis of indonesian domestic cattle’s mt-DNA cytochrome b. Lane 1 is Bali cattle (similar pattern with Madura and PO cattle from Pacitan Regency), lane 2 is Local Pacitan cattle (Pc11), lane 3 is PO cattle from DIY (PO22) and lane 4 is the PCR product (464 bp) as a negative control.

Figure 1 show TaqI-RFLP pattern of Indonesian domestic cattle. The restriction patterns were grouped at three different haplotype. The first pattern generated 225 bp, 191 bp and 48 bp (lane 1), was haplotype A; second pattern generated 416 bp and 48 bp (lane 2), was haplotype C; and the third pattern generated 372 bp, 48 bp, and 44 bp (lane 3), was haplotype B. The cleaved fragment sizes were determined by individual haplotype sequences analysis with DNAMAN software. The results showed that Bali, Madura and Native Pacitan cattle included to the haplotype A (the complete data not shown), the PO cattle from DIY (Bos indicus) included to the haplotype C, while Local Pacitan cattle, especially Pc11 cattle included to the haplotype B.

**Sequences Analysis of Three Different Haplotype in Indonesian Domestic Cattle**

Three different individual haplotype sequences compared to the some sequences from NCBI. Sequences analysis was performed by DNAMAN software. The results showed that PO cattle (PO22) have similar TaqI-RFLP pattern with the AF492351, AF492350 and NC005971. This results indicated that PO cattle related to Zebu (Bos indicus) and Bos taurus cattle. Prado et al (2005) reported that Bos indicus and Bos taurus have similar TaqI-RFLP pattern and produced three DNA band with 372 bp, 48 bp and 44 bp in sizes (haplotype B).

One of native Pacitan cattle (Pc11) was haplotype C. It produced two DNA band with 416 and 48 bp. it’s sequence and TaqI-RFLP pattern almost similar with the Bos indicus and
Bos Taurus sequences. The differentiation between them due to alteration nucleotide number 93, C→A (Fig. 2, compared lane Pc11 with lane AF492351, AF492350 and NC005971).

![Diagram showing TaqI restriction site and sequences analysis](image)

Figure 2. Polymorphism of Indonesian Domestic cattle sequences in three different haplotype. The box indicated TaqI restriction site. The sequences analysis was performed with the DNAMAN software.

The Bali, Madura and some native Pacitan (complete data not shown) cattle were included to the haplotype A. This result indicated that the cattle had the same maternal lineage (maternal inheritance). The Madura cattle suspected as a result of cross breeding between Zebu and Banteng cattle (Nijman et al., 2003; Wijono and Setiadi, 2004). The Bali cattle was domesticated banteng (Anonimous, 2005). The genetic characteristics similarity due to Bali, Madura and native Pacitan cattle contain Banteng nucleotides in it's mt-DNA cyt
b gene. It supported that Bali cattle had the same sequence and TaqI-RFLP pattern (Fig. 2) with the AY689188 (Bos javanicus/Banteng).

CONCLUSIONS

There were mt-DNA cytochrome b polymorphism in Indonesian domestic cattle based on TaqI-RFLP analysis and the study also have vary haplotype, there were haplotype A, B and C. Sequences analysis indicated that the most Indonesian domestic cattle has Banteng nucleotide in its cyt b gene sequences.

ACKNOWLEDGEMENT

We are thankful to the head of research center and community services Gadjah Mada University for providing Research Grant, Hibah Bersaing XVI 2008. We also thank to the staff Veterinary Services in Pacitan Regency and Madura Island.

REFERENCES


O-MB12

STUDY OF MA-ACS AND MA-ACO GENES EXPRESSION DURING VEGETATIVE AND RIPENING STAGE OF PISANG AMBON (Musa sp. AAA GROUP)

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Pisang Ambon (Musa sp. AAA group) is a banana cultivar exclusive from Indonesia. As a climacteric fruit, Pisang Ambon ripening is regulated by an important plant hormone, ethylene. Ethylene is an internal signal molecule, plays a pivotal role in plant growth and development and significantly affects seed germination, root hair development, flower senescence, abscission, and fruit ripening. Ethylene biosynthesis is regulated by two genes, ACC synthase (ACS) and ACC oxidase (ACO). ACC synthase is encoded by a multigene family. In previous study, nine ACS genes (MA-ACS 1-9) and one ACO gene (MA-ACO) have been detected in pisang Ambon. Until now, no information is available regarding the ethylene biosynthesis and the expression level of MA-ACS and MA-ACO during vegetative and ripening stages of Pisang Ambon. Therefore, in this study we investigated the expressions of ACO gene (MA-ACO) and four ACS genes (MA-ACS1, MA-ACS2, MA-ACS4 and MA-ACS6) during vegetative and fruit ripening. The results of this study will give preliminary data to determine which gene that expressed during fruit ripening and specific fruit promoter candidate. Gene expression analysis by RT-PCR showed that MA-ACS1, MA-ACS4 and MA-ACS6 were expressed specifically in ripening stage (banana peel and pulp) but was not expressed in vegetative stage (roots and leaves), while MA-ACS2 and MA-ACO were expressed in both stages. The quantification of expression level of each gene is still in progress.

Keywords: Pisang Ambon, MA-ACS, MA-ACO, Gene expression

not presented
O-MB13

THE INHERITANCE OF RANDOM AMPLIFIED POLIMORPHIC DNA (RAPD) MARKERS LINKED TO POWDERY MILDEW RESISTANCE GENE IN MELON

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ABSTRACT

The inheritance of resistance to an Indonesian isolate of powdery mildew in melon cultivar PI 371795 was previously determined and it is controlled by a single dominant gene, to which the symbol Pm-W was assigned. However, the origin of Pm-W gene in PI 371795 and their generations were not characterized. In this study we sought to define the inheritance of Pm-W gene among resistant cultivars PI 371795, susceptible cultivar Action 434, F1, F2, and Testcross generations by RAPD marker analysis. As the result, a RAPD marker which produced the specific DNA bands linked to Pm-W was shown only in resistant cultivar of PI 371795, F1 generation, F2 and Testcross resistant. However, the DNA band was not shown in the susceptible cultivars Action 434, F2 and Testcross susceptible, respectively. This result revealed that a specific DNA band linked to Pm-W gene in melon PI 371795 and their generations was derived from the resistant parental cultivar of PI 371795.

Keywords: Melon, powdery mildew, resistance gene, RAPD

INTRODUCTION

Resistance to severe diseases including powdery mildew as well as morphological traits can be manipulated in melon breeding program. Many melon cultivars planted in Indonesia have been severely infected with powdery mildew so far, and the appearances of new Indonesian race Podosphaera xanthii which are different from races from Japan, USA and France has been reported [1]. Therefore, it is important to breed cultivars with resistance to new races. Our previous study showed that the inheritance of resistance to an Indonesian isolate of powdery mildew in melon cultivar PI 371795 is controlled by a single dominant
gene, to which the symbol $Pm-W$ was assigned. Resistant and susceptible plant of $F_1$, $F_2$ and testcross were recognized based on morphological trait, however the origin of $Pm-W$ gene in PI 371795 and its generations has not been characterized.

Currently selection of disease resistance to powdery mildew has been assigned based on disease symptoms or phenotypic screening, which are not practical and time consuming. With advancement of molecular biology, molecular markers have got available in plant breeding and development of markers in breeding for resistance is expected to shorten selection process [3]. The use of molecular markers can improve efficiency of conventional plant breeding by selecting a marker linked to the trait of interest (e.g., diseases resistance) because expression of molecular markers are not fluctuated by environmental condition [7].

RAPD is based on the amplification of genomic DNA using single primer consisting of random sequences, usually with ten bases, to find polymorphic regions within the genome defined by the primer sequence [7]. The products formed and separated by agarose gel electrophoresis reveal sequence variation in the form of variable numbers of bands of variable length which may be characteristic of species and/or cultivars within species. RAPD is also a simple and inexpensive technique, more rapidly and more easily than restriction fragment length polymorphism (RFLP) [8]. RAPDs have been widely used and are one of the most powerful and fastest ways for tagging resistant genes [6]. In the present study, the trait used was resistance to powdery mildew by using arbitrary primers and polymerase chain reaction (PCR) to screen the susceptible and resistant pools of DNA, any differences due to base changes resulting in DNA fragments unique to the resistant pool would be linked to the locus for powdery mildew resistance. The present study was designed to identify of RAPD markers linked to powdery mildew resistant and elucidate the inheritance of RAPD markers linked to $Pm-W$ resistance gene in $F_1$, $F_2$ and Test cross.

**MATERIALS AND METHODS**

**Plant materials**

Twelve cultivars of melon (*Cucumis melo* L.) were used in preliminary identification of RAPD markers linked to Pm-W resistance of melons. Seeds of each cultivar were planted in plastic pots in a glasshouse at 25°C-28°C. Healthy leaves were harvested from melon seedling grown in the green house at 3 to 5 leaf stages. About 5 g of fresh leaves per plant was collected and immediately stored in refrigerator at -20°C.
DNA isolation

DNA samples of 10 plants from each of resistant cultivars PI 371795, susceptible cultivar Action 434, F₁, F₂, and Testcross generations were extracted and purified according to the protocol of DNA extraction kit (illustra PhytoPure series RPN-8511). About 0.1 g frozen leaf tissue of each cultivar was ground in a mortar with liquid nitrogen and the powder transferred to a tube. The sample powder was mixed with 600 µl of Reagent 1 and added to 200 µl of Reagent 2 of DNA extraction kit. Samples were incubated at 65°C in a shaking water bath for 10 minutes and then placed on ice for 20 minutes. Samples were removed from ice and 500 µl of chloroform stored at -20°C was added. Fifty to seventy µl of DNA extraction resin suspension was added and shacked on tilt shaker for 10 minutes at the room temperature. The samples were centrifuged at 3,000 rpm for 10 minutes by high-speed micro refrigerated centrifuge. Without disturbing the nucleon resin suspension layer, samples were transferred into a fresh tube. An equal volume of cold isopropanol was added into tube and was gently inverted until DNA precipitates and centrifuged at 15,000 rpm for 5 minutes. The obtained DNA pellet was washed with 70% cold ethanol and centrifuge at 15,000 rpm for 5 minutes at 4°C. The supernatant was discarded and the DNA pellet was air-dried for 10 minutes. The resulting DNA was then re-suspended in 100 µl Tris-EDTA (TE) buffer, and then stored at -20°C. DNA concentration of 10 ng µL⁻¹ (0.001 g was diluted in 100 µl dH₂O water) was prepared for all samples to use in PCR analysis.

Random amplified polymorphic DNA

All PCR amplifications were carried out in 25 µl reaction volumes in 600 µl tubes. Each reaction mixture containing 2.5 µl of 10x ExTaq buffer, 2 µl of dNTPs, 0.25 µl of Faststart PCR (Roche), 25 picomoles of PMAR 5 primer (5’CAGACAAGCC3’) and 10 ng of genomic DNA. Amplification was performed on a DNA Thermal Cycler programmed for 1 cycle at 95°C for 5 minutes followed by 45 cycles at 95°C for 1 minute, 36°C for 1 minute, and 72°C for 2 minutes. Following amplification, DNA products were mixed with 2 µl 6x loading buffer, and 10 µl of each sample was delivered into wells of 1.5% agarose gel prepared with 1xTris-acetic acid EDTA (1xTAE) buffer for analysis via gel electrophoresis. Gels were stained in ethidium bromide for 3 to 5 minutes, destined in dH₂O for approximately 2 to 3 minutes, and photographed by UV light. Molecular weights were estimated using a 100 bp DNA ladder (Promega).
RESULTS AND DISCUSSION

A total of 120 PCR reactions were carried out by a single of 10-mer arbitrary primers and RAPD bands specific for resistance were obtained. A RAPD marker, which was amplified by a PCR for the PMAR 5 primer migrated around 1058 base pairs (bp) in a gel analysis (Fig.2). This marker was named as PMAR 5\textsubscript{1058}. This primer was then used for further screening of resistant cultivars from in resistant cultivars PI 371795, susceptible cultivar Action 434, and susceptible and resistant F\textsubscript{1}, F\textsubscript{2}, and test cross generations. Samples of parental, F\textsubscript{1}, F\textsubscript{2} and test cross were originally from breeding results in 2007-2008 (see Fig.1.) To characterize the nature of powdery mildew resistance gene in PI371795 and its F\textsubscript{1}, F\textsubscript{2}, and test cross plants were used as template DNAs for RAPD analysis with PMAR 5 primer. Figure 2 showed that DNA amplification using PMAR 5 primer produced a total of 12 polymorphic bands appeared on agarose gel analyses. PMAR 5 primer produced a specific DNA band of 1058 bp linked to powdery mildew resistance gene (Pm-W) in resistant PI371795, resistant F\textsubscript{1}, resistant F\textsubscript{2} individual tested plants, however, not shown in the susceptible Action 434, F\textsubscript{1}, F\textsubscript{2} and test cross, respectively (test cross results not shown). Samples, which showed presence of specific DNA fragment on resistance cultivars shown on RAPD markers PMAR 5\textsubscript{1058} (Fig 2 and Table 1), were also previously shown resistant reaction against powdery mildew by artificial inoculation (Table 2). This result revealed that this specific DNA band at 1058 bp linked to Pm-W gene in melon PI 371795 and their generations was derived from the resistant parental cultivar of PI 371795. This result suggested that a specific DNA band linked to Pm-W resistance in melon was originated from parental PI371795.
Fig. 1. Pedigree of melon cultivar Action and PI371795 and its generation

Fig. 2. Electrophoresis of amplified products obtained with PMAR5 primer, 1:PI371795 2: Action 434, 3: F_{1}AC_{R}, 4: F_{1}P_{R}, 5: F_{2}A_{H}R, 6: F_{2}PB_{T}N_{S}, 7: F_{2}P_{B}B+N_{R}, M: 100 bp ladder (Promega). The arrow indicates specific DNA fragments for Pm-W resistance gene at the 1058 bp.
Table 1. RAPD marker linked to powdery mildew resistance gene ($Pm-W$) in resistant cultivars PI 371795, susceptible cultivar Action 434, and susceptible and resistant F$_1$, F$_2$, and test cross generations

<table>
<thead>
<tr>
<th>Cultivars</th>
<th>Hybrids</th>
<th>Code</th>
<th>Reaction against powdery mildew</th>
<th>RAPD markers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melon PI 371795</td>
<td>-</td>
<td>PI371795</td>
<td>R</td>
<td>+</td>
</tr>
<tr>
<td>Melon Action 434</td>
<td>-</td>
<td>Action 434</td>
<td>S</td>
<td>0</td>
</tr>
<tr>
<td>♂ Action 434 x ♀ PI 371795</td>
<td>F$_1$ ♀ Action 434</td>
<td>F$_1$AC$_R$</td>
<td>R</td>
<td>+</td>
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<tr>
<td></td>
<td></td>
<td>F$_1$AC$_S$</td>
<td>S</td>
<td>0</td>
</tr>
<tr>
<td>♂ PI 371795 x ♀ Action 434</td>
<td>F$_1$ ♀ PI 371795</td>
<td>F$_1$PI$_R$</td>
<td>R</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F$_1$PI$_S$</td>
<td>S</td>
<td>0</td>
</tr>
<tr>
<td>F$_1$ ♀ Action 434 self pollination</td>
<td>F$_2$ ♀ Action 434 green</td>
<td>F$_2$AH$_R$</td>
<td>R</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>F$_2$AH$_S$</td>
<td>S</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>F$_2$ ♀ Action 434 white</td>
<td>F$_2$AP$_S$</td>
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<td>0</td>
</tr>
<tr>
<td>F$_1$ ♀ PI 371795 self pollination</td>
<td>F$_2$ ♀ PI small without net</td>
<td>F$_2$PK$_C$</td>
<td>R</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>F$_2$ ♀ PI medium without net</td>
<td>F$_2$PSD$_R$</td>
<td>R</td>
<td>+</td>
</tr>
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<td></td>
<td></td>
<td>F$_2$PSD$_R$</td>
<td>S</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>F$_2$ ♀ PI big without net</td>
<td>F$_2$PBTN$_R$</td>
<td>R</td>
<td>+</td>
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<td>Testcross Action 434</td>
<td>TC AC$_S$</td>
<td>S</td>
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</tr>
<tr>
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<td>Testcross PI 371795</td>
<td>TC PI$_R$</td>
<td>R</td>
<td>+</td>
</tr>
</tbody>
</table>
From the result of this study, RAPD as one of molecular techniques seem likely to benefit for application on resistance breeding of melon in the future. This technique can overcome screening lines for resistance of melon against powdery mildew by morphological marker, which is expensive and time consuming. In melon, application of RAPD markers was also used to detect resistant cultivars of melon against cucurbit viruses [2]. Similar study to screen lettuce for resistance to downy mildew also showed that RAPD marker assisted selection of downy mildew resistance in lettuce, which is difficult and laborious [4]. The specific DNA fragment found in this study refers to the identification of existing DNA resistance to Pm-W in melon, which can be developed to design SCAR (Sequence Characterized Amplified Region) marker and applied this marker with parental plants and its generations. This will assist greatly in molecular breeding in the future studies.

**ACKNOWLEDGEMENT**

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**REFERENCES**


O-MB14

MUTATIONS ANALYSIS IN D-LOOP REGION OF MITOCHONDRIAL DNA IN HUMAN GASTRIC TISSUES AND ITS INFLUENCE ON THE ROS AND CELL CYCLE

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ABSTRACT

In recent years, the instability, gene mutation or abnormal expression of mitochondrial genome has been detected in many kinds of malignant cancer tissues. The mitochondrial D-loop is a hotspot for gene mutation in cell lines of colonic and rectal cancer. But there is a difference in mutation frequency of the D-loop among different tumors. The of the research is to investigate the mutation in D-loop region of mitochondrial DNA in gastric cancer and its influence on the changes of reactive oxygen species (ROS) and cell cycle. The D-loop region was amplified by PCR and sequenced. Reactive oxygen species and cell cycle were detected by flow cytometry in 20 specimens from gastric cancer and adjacent normal tissues. According to the sequence results, gastric cancer tissue was divided into mutation group and control group. Reactive oxygen species, apoptosis and proliferation in the two groups were compared. Among the 20 gastric cancer specimens, 18 mutations were identified in some patients, the mutation rate being 35%. There were four microsatellite instabilities in the mutations. No mutation was found in the adjacent tissues. Reactive oxygen species, apoptosis, and proliferation in the mutation group were all significantly higher than those in control group. Here we demonstrated that mutation in D-loop region plays a role in the genesis and development of gastric cancer. All publications nucleotide sequence can be seen in the European Molecular Biology Laboratory (EMBL) with the Accession Number: FN298671- FN298671, FN293093-FN293094, FN256288, FN252860.
Keywords: Mutations Analysis, D-loop, mtDNA, Human Gastric, ROS and Cell Cycle

INTRODUCTION

The occurrence and progression of gastric cancer are a multistep process due to multiple factors and gene mutations. Although great success has been achieved in oncogenes and cancer suppressor genes, a lot of questions cannot be explained by alteration of nuclear genes. In recent years, mutation of mitochondrial DNA (mtDNA) has been associated with the occurrence and progression of tumor and is regarded as a possible causitive factor for cancer. Human mitochondrial DNA is a closed double strand circular molecule of 16569 bp, coding 13 proteins, 22 tRNAs and 2 rRNAs. Its D-loop is a non-coding region, containing some important sequences such as the promoter for heavy chain replication. Because of the properties of its structure as well as its mechanism of DNA replication and injury repair, the mutation frequency of mitochondria is 10-100×higher than that of nuclear DNA. In recent years, the instability, gene mutation or abnormal expression of mitochondrial genome has been detected in many kinds of malignant cancer tissues [2-3]. The mitochondrial D-loop is a hotspot for gene mutation in cell lines of colonic and rectal cancer. But there is a difference in mutation frequency of the D-loop among different tumors [4-7].

In this report the region of mtDNA D-loop was amplified by PCR and then sequenced with samples from cancer tissues and corresponding normal mucous membrane of 20 gastric cancer patients. Reactive oxygen species (ROS) and cell cycle were detected with flow cytometry. The samples were divided into mutation group and control group according to the mutations. We compared the level of ROS and cell cycle within two groups to clarify the influence of mutations of D-loop on ROS and cell cycle. Our purpose was to investigate the influence of mutation of D-loop on cell carcinogenesis and progression of gastric cancer on Papuan Polulations.

MATERIALS AND METHODS

Twenty surgical samples of gastric cancer were selected from hospitalized patients in the Department of Gastroenterology Surgery of Dok II Provincial Hospital of Papuan Capital, Jayapura from January to November, 2008. All patients, 13 males and 7 females, were diagnosed by gastroscopy and biopsy before operation. Their ages varied from 39 to 78 years and averaged 57.2 years.
A region without bleeding was carefully selected. A block of 1.0 cm$^3$ fresh cancer tissue was cut and stored in a-70 refrigerator. Other tissues were cleaned with PBS. Cell suspension was prepared by mechanical grinding and filtered through a net and cancer cells were separated by centrifugation. A block of 1.0 cm$^3$ normal gastric tissue was cut and cell suspension was prepared according to the same procedure.

Half of 1 mL cell suspension was put into a test tube, centrifuged for 5 min at 1500 r/min, washed thrice with normal saline water and cell debris were by centrifugation for 3 min at 500-800 r/min. Cells were fixed with 2-4 mL 1% poly-formaldehyde and centrifuged for 10 min at 1 500 r/min. The supernate was discarded and the pallet was resuspended in 2-4 mL 0.1% Triton-X-100 for 3 min and centrifuged. The supernate was discarded and resuspended in 1-2 mL 0.01% RNase, vortexed for 30 min in 37 water bath. One milliliter 0.05% PI solution was added to dye the DNA for 30 min. The cell cycle of cancer cells and normal gastric cells was measured by FACS. The fluorescence signal was processed by multicycle analytical software for cell cycle.

Measurement of ROS level, DCFH-DA (from Sigma Company) was dissolved in 95% ethanol to a concentration of 5 mmol/L and stored at 4 in the dark, diluted to 5 µmol/L with PBS before use. Two hundred microliter cell suspension (1×106/mL) was put into a test tube, washed twice with PBS and centrifuged for 5 min at 1 500 r/min. The supernate was discarded and the pallet was resuspended in 2 mL and 5 µmol/L DCFH-DA (2 mL PBS for the contrast groups), vortexed for 20 min in 37 water bath and centrifuged for 5 min at 1 500 r/min. The supernate was discarded and the cells were resuspended in 600 µL PBS. The intensity of DCF green fluorescence was measured after DCFH-DA reaction with FACS. The wavelength of stimulation sub-laser within the FACS was 488 nm and the power was 10 mW. The results were expressed as mean fluorescence intensity (MFI).

DNA extraction was carried out according to the protocol of the reagent kit. (Promega, USA). PCR replication of mtDNA D-loop region, The sequences of primers are listed in Table 1. The total volume of PCR reaction was 50 µL, including 1 µL of each primer (20 pmol/µL), 5 µL of 10× PCR buffer, 5 µL of dNTPs (2 nmol/L), 0.4 µL of ExTaq DNA polymerase (5 U/µL) and 100 ng of extracted DNA sample. PCR reaction was carried on using PCR instrument (Perkin Elmer 2400, USA). The initial denaturation was at 94 for 5 min, followed by 35 cycles of denaturation at 94 for 45 s, a denaturation at 56 for 45 s, an extension at 72 for 90 s. The final extension was at 72 for 7 min.
Table 1. Primers for amplification and their nucleotide sequences

<table>
<thead>
<tr>
<th>Primers Name</th>
<th>Nucleotide Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1</td>
<td>5'-CACCATTAGCACCACAAAGCT-3'</td>
</tr>
<tr>
<td>HV2R</td>
<td>5'-CTGTTAAAAGTCGATACCGCC-3'</td>
</tr>
</tbody>
</table>

Confirmation of PCR product

Two microliters of PCR product was loaded on 1.5% agarose gel for electrophoresis. If the mtDNA D-loop region was successfully amplified, a picture was taken (Figure 1) and the PCR product was purified with an instant PCR product purification kit and quantified with a spectrophotometer.

Figure 1. PCR amplification of D-loop region in mtDNA; lane 1: PCR product of D-loop region in mtDNA; lane 2: marker.

DNA sequencing. Sequencing reaction was completed with the sequencing kit of end termination by fluorescence labeled ddNTPs (ABI, USA). The total volume of sequencing PCR reaction was 10 μL, including 1 μL of sequencing primer, 6 μL of kit mixture, 3 μL of purified PCR product. Three sequencing primers (Table 2) were used to divide the replicated D-loop region into three overlapped segments. The reaction condition was as follows: the initial denaturation step was at 96 for 1 min, followed by 40 cycles of at 96 for 10 s and at 55 for 2 min. The sequencing reaction product was precipitated by 70% ethanol and loaded on a ABI Prism 310 sequencer (Perkin Elmer, USA).
Table 2 Primers for sequencing and their nucleotide sequences

<table>
<thead>
<tr>
<th>Primers Name</th>
<th>Nucleotide Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1</td>
<td>5’-CACCATTAGCACCCAAAGCT-3’</td>
</tr>
<tr>
<td>M2</td>
<td>5’-GATTTCACGGAGGATGGT-3’</td>
</tr>
<tr>
<td>HV2F</td>
<td>5’-GGTCTATCACCCTATTAACCAC-3’</td>
</tr>
<tr>
<td>HV2R</td>
<td>5’-CTGTTAAAGTGATACCGCC-3’</td>
</tr>
</tbody>
</table>

DNA sequencing of PCR products. PCR products were sent to Macrogen Company, Ltd, Seoul, South Korea for direct sequencing.

Experimental data and statistical analysis

Taken the sequence of mtDNA D-loop from Cambridge sequence as criterion, a comparison was made between the sequences of cancerous tissue and those of normal tissue. If the mtDNA D-loop sequence from cancerous tissue was different from normal tissue, the alteration was regarded as gene mutation. Other data were expressed as mean±SD. Comparison between groups was carried out by t test. P<0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Eighteen gene mutations were found in the cancerous tissue from seven patients, among which four were microsatellite instabilities. Thus the mutation rate of mtDNA D-loop in the specimens of gastric cancer was 35% (Table 3). Cell cycle and apoptosis could be detected by flow cytometry synchronously. In cell cycle, DNA was synthesized in synthesis (S) phase. As a result, the percentage of cells in synthesis phase could reflect cell proliferation. As shown in Table 4, level of ROS, rate of cell apoptosis and proliferation in mutation group were higher than those in control (P<0.05).
Table 3. Mutations in D-loop region of mitochondrial DNA in gastric cancer

<table>
<thead>
<tr>
<th>Location</th>
<th>Cambridge sequence</th>
<th>Cancer nucleotide</th>
<th>Normal tissue nucleotide</th>
</tr>
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<td>16122</td>
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<td>T</td>
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Table 4. ROS level and cell cycle in mutation group and control group (mean±SD)

<table>
<thead>
<tr>
<th></th>
<th>ROS (MFI)</th>
<th>Apoptosis (%)</th>
<th>G0/G1 (%)</th>
<th>S (%)</th>
<th>G2/M (%)</th>
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<tr>
<td>Mutation group n = 7</td>
<td>156.3±9.7a</td>
<td>12.5±1.5a</td>
<td>57.3±5.3</td>
<td>22.3±2.4a</td>
<td>20.6±2.1</td>
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<tr>
<td>Control group n = 13</td>
<td>117.3±10.4</td>
<td>8.6±0.1</td>
<td>65.4±5.6</td>
<td>16.4±1.5</td>
<td>18.1±1.3</td>
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</table>

*P<0.05 vs control group.

There was only 1120 nucleotide in the D-loop of mtDNA, but 18 mutations were detected in the 20 gastric cancer patients, indicating that the D-loop of mtDNA is a fragment with a high mutation rate. Among the 18 gene mutations found in this work, four were microsatellite instabilities. Habano [10] researched the mitochondrial genome instability (mtGI) and nuclear microsatellite instability (MSI ) in 62 gastric cancer tissues and found 10 mitochondrial PolyC instabilities (16%) and 7 MSI, among which 4 existed mtGI. Thus mtGI is correlated with nuclear MSI. Since nuclear MSI induces gene mutation in coding region, mtDNA mutation plays its role in the process of cancer genesis and progression by cooperating with the alteration of some nuclear genes. Although a non-coding region, the mtDNA D-loop contains the initial site of heavy chain replication and the promoters for heavy and light chain transcription. Thus Dloop is responsible for the regulation of mtDNA replication and transcription, its mutation leads to mutations in coding region and change of protein synthesis, and finally affects the function of respiration chain which hampers the energy supply of cells and produces volume of ROS. ROS results in injury to the genome and then induces cancer. High level of ROS is toxic through activating cell apoptosis and causing injuries to the genome. ROS might regulate cell apoptosis in the following ways.
ROS is the message molecule of some transcription factors (such as Apaf-1) and can activate some useful components of cell apoptosis[11]. The increase of ROS is often accompanied with the decrease of intracellular anti-oxidant, resulting in imbalance between oxidant and reductive, which is just the common central step of cell apoptosis[2]. Most people believe that ROS is necessary for cell apoptosis. High level of ROS inspires cell necrosis or drives cells to the way from apoptosis to necrosis. ROS not only participates in the process of cell apoptosis but also is a kinetin for cell division that promotes nuclear DNA mutation, cell mitosis and selective growth of tumor cells. ROS is relatively stable and easy to diffuse within cells and exist universally in various cell types. The formation and elimination of them are under strong cellular regulation. All the above properties make ROS extraordinary proper for second messengers. The level of intracellular ROS increases under extracellular stimulation signals such as cytokine and growth factor. Then they take part in cellular signal transduction. There exist two research hotspots at present as for the relation between ROS and cell proliferation. One is activation of MAP kinase family to promote cell mitosis, the other is activation of transcription factors such as NF-κB to facilitate gene expression. In conclusion, the mutation of D-loop takes part in the carcinogenesis and progression of gastric cancer through the effect of increased ROS.

REFERENCES


O-MB15

STUDY OF SUGAR CANE SUCROSE TRANSPORTER cDNA
BY FUNCTIONAL EXPRESSION IN YEAST

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ABSTRACT

Previous study in transgenic plants demonstrated that sucrose transporter (SUT) was essential for sucrose translocations. Sucrose transporter were isolated from sugarcane (\textit{Saccharum officinarum} L.), the gene designated SoSUT1 and SoSUT2, encodes a protein of 518 and 747 amino acids respectively. It was constructed in to expression plasmid pYES2 for SUT1 and pYX112 for SUT2. cDNA SoSUT2 had also been constructed in plasmid pBIN-At-GFP. The plasmid were transformed in Yeast (\textit{Saccharomyces cerevisiae}) and grown in minimal medium (SD–\textit{urasil}) as selection medium. Polymerase chain reaction was used to confirm the transformed colony. To study the functional expression, yeast grown in YPD medium with 2% Sucrose then the sucrose uptake were measured in number interval time using resorcinol method. The result showed that yeast INVSc1-pYES2-SUT1 and BF264-pYX112-SUT2 had higher ability in sucrose transport compared to the control INVSc1 for SUT1 and control-BF264 for SUT2. Moreover, the result showed that SUT1 have higher ability to transport sucrose than SUT2. Confocal microscope observation showed that SUT2 gene located in mitochondria.

\textbf{Keyword}: Sugarcane, Sucrose transporter, Yeast
INTRODUCTION

Photosynthesis represents the major source of energy required for biological processes. As a primary product of photosynthesis carbohydrates are formed in photosynthetically active cells. In higher plants leaves, and to a certain extent also other parts of the plant, e.g. stem tissue, represent the primary sites for photosynthesis. In contrast other parts of the plant e.g. roots, seeds or tubers, do not contribute significantly to the energy gained by photosynthesis but rather are dependent on carbon dioxide fixed in photosynthetically active parts of the plant.

Sucrose is the major product of photosynthesis in many higher plants and is transported from the source tissue (mature leaves) through the phloem to various sink tissues to support plant growth and development. Most plants studied contain multiple sucrose transporters (SUTs), also known as sucrose carriers (SUCs), which likely have different functions in phloem loading and/or unloading or in the import of sucrose into sink tissues. Based on phylogenetic analysis (Barker et al. 2000) states that sucrose and sucrose transporter transporter like proteins are divided into three families, namely 1 SUT, SUT 2, and SUT 4. While SUT 3 is found only in tobacco, and according its homolog sequences included in the family SUT 1 (Kuhn et al., 1999). In plant, sucrose uptake kinetics involve high- and low-affinity components. A family of low- and high-affinity sucrose transporters (SUT) was identified. SUT1 serves as a high affinity transporter essential for phloem loading and long-distance transport. SUT4 is a low-affinity transporter with an expression pattern overlapping that of SUT1. (Riesmeier et al., 1994; Kühn et al., 1996). Using *Saccharomyces cerevisiae* strain,a cDNA from sugarcane encoding a sucrose carrier was identified functional expression. Yeast strains that allow the phenotypic recognition of a sucrose carrier activity were constructed in to yeast. Previous study in transgenic plants demonstrated that sucrose transporter (SUT) was essential for sucrose translocations. Sucrose transporter were isolated from sugarcane (*Saccharum officinarum* L.). Recently, a number of genes which encode enzymes of sugar metabolism or sugar transport in sugarcane have been identified. Together with a functional analysis of these enzymes, information on the localization of their expression and activity will enable us to build a model of the pathways and control points for sugar movement into the storage sink. According to the references above it is needed a research about study of cloning of cDNAs encoding sucrose transporter proteins from sugarcane.

MATERIALS AND METHODS
The following bacterial and yeast strains were used: DH5α, INVSc1 and BF264. Construction-SUT1 cDNA in pYES2 plasmid (Invitrogen) starting with the formation of Kozak consensus nucleotide sequence for translation initiation. cDNA-SoSUT1 amplified by PCR using forward primer containing the nucleotide sequence of Kozak consensus,

5'-gttacatggtcgctgagctgacggc-3' and reverse primer 5'-tcagtgccgccccgctgac-3'. DNA PCR products inserted into pGEMT plasmid and transformed into E. coli DH5α. After restriction using EcoR1 and Kpn1 enzyme at N-and C-terminal than cDNA SoSUT1a restriction products were inserted into pYES2 plasmid and transformed back into E. coli DH5α.

Whereas cDNA of SUT2 was amplified by PCR using tagged primer Sall for forward primer 5'-gtc gac atg tcg gga gct gct ctt-3' and Xho1 for reverse primer 5'-gag ctc cta ggc ctt tgc aac cgc-3'. DNA PCR products were cloned into pGEM T-Easy vector (Promega). After digestion with Sall and Xho1, cDNA SUT2 fragments were cloned into pYX112. Another plasmid pBin-AtBI-GFP after digestion using Sall and Ncol, was constructed by inserting of cDNA SUT2 coding region site of Sall and Ncol. Transformation constructs pYES2-SoSUT1 into the yeast cell strains INVSc1, and pYX112-SoSUT2 and pBin-SoSUT2-GFP into yeast cell strain BF264 conducted using ion Li (lithium) in accordance with the methods mentioned in the instructions from Invitrogen Inc. Yeast transformants were plated on SD-glucose-Ura− medium and incubated at 30°C for two days.

To confirm the transformants, clone yeast inoculated and cultured in proper medium. 3 ml overnight cultures of the respective yeast strains were harvested, washed in yeast lysis buffer resuspended in 100 ul of the same buffer and after addition of an equal volume of glass beads (0.5 mm diameter) the suspension was vortexed five times for 15 s, centrifuged and the supernatant was analyzed using PCR and 'forward primer 5'-agccctggcaggctacctcctc-3' and reverse primer 5'-ggagatcttgggcagcaggaa-3' for SoSUT1 whereas for SoSUT2 using forward primer 5'-gatggtttgctcagcaagggc-3' and reverse primer 5'-gcgacctgttggaaggatgtg-3'

In order to study the functional expression, yeast grown in YPD medium with 2% sucrose than the sucrose uptake were measured in number interval time using resorcinol method. Yeast cells of BF264 harboring pBin-SUT2-GFP were cultured for two days in SD-glucose-Ura−. After two days, GFP fluorescence was observed using a confocal fluorescence microscope (Zeiss, Germany) with a 488-nm excitation wavelength.

RESULTS AND DISCUSSION
cDNA expression in yeast are required to insert Kozak consensus nucleotide on start codon for translation initiation. Kozak consensus created using PCR so that sequence ACCATGG obtained at start codon of SoSUT1a cDNA, and then cloned into pGEMT plasmid. Therefore, digestion using EcoR1 and Kpn1 enzyme produced SoSUT1a cDNA which contain start and stop codon with size of 1.5 kb and contains Kozak consensus.

![Fig. 1: Separation using electrophoresis gel agarose (1%) on pYES2-SoSUT1 after digestion using PstI and SphI](image)

In order to study function of SoSUT1a genes isolated from sugarcane, gene pYES2-SoSUT1a constructs were transformed and expressed into yeast cells INVSc1 strains. Transformation performed using Li + ions as the method mentioned in the Invitrogen manual. Yeast transformant are selected on minimal selection media that do not contain amino acids uracil. Transformant yeast gene constructs containing pYES2-SoSUT1a grow on media selection and control yeast that do not contain constructs are not able to grow on selection medium.

![Fig. 2: Yeast growth control (left) and transformant yeast (right) on selection medium without uracil amino acid at temperature of 30°C for two days. Gene constructs pYES2-SoSUT1a transformed into yeast cells and grown strains INVSc1 on selection medium without uracil (right), while the control yeast without transforming gene constructs were grown in selection medium (left).](image)
Tagged cDNA SUT2 using PCR and primer contain sequence nucleotide of restriction site of Sall and Xhol was done and PCR product showed as in figure 3. Figure 3 showed that PCR product has size about 2.3 kb and it indicates that cDNA SUT2 which have been tagged could be used in transformation into yeast.

Fig. 3: PCR product cDNA SUT2 was tagged using Sall and Xhol before used in tranformation into yeast (A); Restriction of plasmid pBin-AtBI-GFP using Sall/Ncol and pYX112 using Sall /Xhol (B)

As showed in figure 4, in lane 2-3 showed result of digestion of pBIN-AtBI-GFP using restriction enzyme Sall and Ncol, whereas in lane 4-7 showed digestion result of pYX112 using Sall and Xhol. This result of digestion then were isolated from gel agarose and used for transformation.

Fig. 4: Transformant yeast on selection medium without uracil amino acid at temperature of 30°C for two days. Gene constructs pYX112-SoSUT2 transformed into yeast cells grown on selection medium without uracil

Yeast cell contain pYX112-SUT2 were used in this experiment can grow in selection medium without uracil. Therefore, yeast cell which did not contain plasmid suppressed growth of yeast cell. It can be determined that colonies growth on selection medium are transformant yeast cells. To confirm that yast cell are transformant, then single colonie of yeast transformat cultured in liquid medium. Culture was harvested and plasmid were isolated
using vortex after addition of glass bead. Supernatant was analyzed using PCR and primer for amplification of fragment of cDNA SUT1 and SUT2.

![Image](image1.png)

A

![Image](image2.png)

B

Fig. 5: Confirmation of yeast transformant of SUT1 and SUT2; amplification of fragments of cDNA - SUT1 in size 450bp (A), amplification of fragments of cDNA – SUT2 in size 1048bp (B)

In order to observe DNA inserted, transformant yeast grown in YPD liquid medium containing 2% sucrose, cultured at temperature of 30°C. Incubation time adjusted as treatment time used, so harvesting depends on the desired incubation time.

Observation of sucrosee transport indicates transport / absorption of sucrose by both yeast transformant yeast strain INVS or BF 264. Sucrose absorption measurements using method of Resocinol yeast transformant strains INVS-SoSUT1 with pYES2-interval 6-hour incubation.

![Image](image3.png)

A

![Image](image4.png)

B

Fig. 6: Sucrose uptake by wild type and transformant yeast (pINVS-SoSUT1) (A); transformant yeast (pYX112-SoSUT2) (B). Duration interval of incubation of yeast is 6 hours.
Figure 6.A showed that absorbent increased in 24 hours after culture of yeast transformant SoSUT1, while absorbent of wild type of INVS showed decreasing in 24 hours after culture even though absorbent increase in 18 hours after culture. This result followed by decreasing of absorbent of medium which used for culture of yeast. It can be conclude that gene SUT1 has physiological activity in sucrose transport. Whereas observation on gene SUT2 (Fig. 6. B) showed that there was no increasing of absorbent of SUT2. It was equal absorbent value between SUT2 and wild type yeast BF 264. It was tend indicate that there no physiological activity in gene SUT2. This result indicate that SUT2 was cDNA putative sucrose transporter type 2A that there no physiological capability in sucrose transport. (Casu et. al., 2002).

Further observations performed to explore intracellular localization of SUT2 in yeast cells. pBin plasmid-AtBI-GFP is used to observe the location of the SoSUT2 cDNA-transformed into yeast. Plasmid was cut with restriction enzyme Ncol Sall and, restriction using these enzyme allows ligation of cDNA SUT2 which has tagged using nucleotide sequencing according to the site and region Sali Ncol enzyme. After transformant yeast cultured in liquid medium, fluorescence pBin-SUT2-GFP was observed using confocal microscope. Fluorescence observations showed that the SUT2 transformed into yeast cells located in the mitochondria. The presence of SUT2 in mitochondria indicate that transformation SUT2 was run well and it could be used for suggest that it will increasing absorption sucrose in medium.

![Images of yeast cells with GFP localization](Image)

**A**  **B**  **C**  **D**

**Fig. 7:** The cellular localization of SUT2-GFP in yeast cell. The fluorescence of SUT2-GFP was examined using confocal microscop (A) light (B); Green big spot was fluorescence distribution of GFP (C) light (D)

In summary, we have identified and determined localization a sucrose transporter, which SUT1 is active sucrose transport process in yeast. Identification of sucrose transporter showed that SUT2 was putative. We hope that this research will have important
implications for understanding of sugar transportation in sugarcane plant. It was important point to suggest that measurement of sucrose uptake by yeast carry out use HPLC.

REFERENCES


O-MB16

CLONING AND SEQUENCE ANALYSIS OF STEAROYL-ACYL CARRIER PROTEIN DESATURASE (SAD) GENE FROM OIL PALM (Elaeis guineensis Jacq.)

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Stearoyl-acyl carrier protein desaturase (SAD) is an important enzyme of fatty acids biosynthesis in higher plants. SAD plays a key role in determining the ratio of saturated to unsaturated fatty acids in plants. Engineering of fatty acids biosynthesis in Elaeis guineensis Jacq. will be important to manipulate the composition of the oil for various reasons, such as for biofuel or for cooking oil. The SAD gene has been known as a member of a small gene family and highy expressed in fruit and seed. DNA genomic of Elaeis guineensis Jacq. was isolated by CTAB method. The isolation of SAD gene was done by PCR with genomic DNA as a template. PCR from genomic DNA produced fragment with 1800 bp, 1200 bp and 1000 bp in size (E-SAD). Only 1000 bp fragment was successfully cloned to pGEMT-Easy (Promega) and delivered to E. coli strain DH5α prior to sequencing with SP6 dan T7 primer. Sequence alignment using BLASTN indicates that E-SAD has a high similarity with SAD gene from some other plants. Sequence analysis using Geneious software showed that the E-SAD was a partial gene consisting of two exons dan 1 intron. Translation analysis of the E-SAD gene fragment using Genious shows 166 amino acid residue. 3D structure and motif analysis showed that E-SAD gene fragment has two pairs of iron binding helices. Alignment using BLASTp showed high similarity with other stearoyl acyl carrier protein desaturases.

Keyword: SAD gene, PCR, Elaeis guineensis Jacq., fatty acids biosynthesis
O-MB17

CLONING AND TRANSIENT EXPRESSION OF PROMOTER FROM ELONGATION FACTOR 1 ALPHA GENE (MeEF2) FROM CASSAVA (Manihot esculenta Crantz.)

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Elongation factor-1α (EF-1α) is an essential factor for protein synthesis in eukaryotic cells. It catalyzes the binding of aminoacyl tRNA to the A site of the ribosome. Previous evidences showed that this protein is encoded by a gene family which consists of more than one copy of EF-1α genes. EF-1α gene had been isolated from cassava (Manihot esculenta Crantz.) and promoter from this gene had been cloned into pGEMT-Easy Vector (MeEF1α2). In order to study the activity, MeEF1α2 promoter had been subcloned into pCAMBIA 1303 vector containing GUS reporter gene. The recombinant plasmid had been transformed transiently into cucumber seeds (Cucumis sativus L.) using Agrobacterium tumefaciens GV 3101. The activity of MeEF1α2 promoter had been detected by GUS reporter gene in cucumber seeds using histochemical staining. The result showed positive expression of GUS reporter gene in cucumber seeds. MeEF1α2 promoter has a putative wound responsive element. In this study we still investigate the role of putative wound box by site-directed mutagenesis.

Keywords: MeEF1α2, GUS reporter gene, putative wound box, site-directed mutagenesis, Cassava (Manihot esculentaCrantz.)
O-MB18

GENETIC RELATIONSHIP BETWEEN ONGOLE HYBRID CATTLE AND BOS JAVANICUS IN INDONESIA BASE ON PARTIAL D-LOOP MTDNA

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ABSTRACT

Bos indicus such as Ongole and other zebu cattle had been developed in Indonesia during Dutch Colonial period. The pure Ongole was brought to Sumba island and became the Sumba-Ongole. The Sumba-Ongole at Java had been crossed with the Javanese cattle (Bos javanicus) and formed the Ongole-grade (Peranakan Ongole). Ongole crossbred (PO) is the most number of local cattle in Indonesia. The introduction of exotic breeds (Simmental and Limosin) in Indonesia started since 1970s. We have analyzed the genetic material of the Simmental, Limosin, Ongole crossbred and the hybrids of those cattle (Simpo and Limpo) using D-loop mtDNA sequences. The sequence type of D-loop mtDNA for the hybrid of PO and exotic breeds was closer to PO than to exotic breeds. To distinguish between several type mtDNA sequence of crossbreds, the obtain PCR products were cloned to pGEM-T easy vector and cut with different restriction enzyme resulting in species specific restriction fragment length polymorphism (RFLP). We determined that Simmental and Limousin have similarity in restriction pattern of D-loop mtDNA sequence, but Limpo and Simpo have similarity in restriction pattern of D-loop mtDNA with PO. This result suggests that D-loop mtDNA sequences in Ongole hybrid are maternally inherited from Bos Javanicus, and we found three haplotypes of Ongole hybrid cattle.

Key words: D-loop, mtDNA, Bos indicus, Bos javanicus, Ongole hybrid Cattle.

INTRODUCTION

Ongole breed has been entered to Indonesia in 1906 at Java Island, Sumatera, Kalimantan, and North Sulawesi, in which crossed with local breed to improve the quality as working cattle and meat produced (Joshi and Phillips, 1953). Ongole breed are used in crossbreeding programs as they can adapt to hot and humid climate and a good type of working (Koger, 1980; Turner, 1980). As grading up the crossbreeding between Ongole with
local breed in Java Island resulted cattle’s that has similarity with Ongole breed, which called as Ongole crossbred (PO=Peranakan Ongole). This was recorded in the 19th century using Ongole bulls and small sized local Java-breeds (now considered extinct) in East Java.

The local breed has a good adaptability to the harsh condition. However, there is a tendency that farmers prefer to keep crossbred cattle instead of local breed recently. The crossbreeding programs tend to be overlap between breeds; two or three breeds are crossed with the local without pay attention on their blood composition. Controlling crossbreeding is needed to prevent the worst condition (Hartatik, 2005). Up to now, there are no complains reported from the farmers regarding the quality of calves produced by crossbred, because it is still in the first generation (F1). However, it must be considered that crossing with the exotic breed may have adverse effect on the second and later generation (Udo, 1994). Therefore, investigation the genetic introduction of each crossbreed produced is needed, in order to quantify genetic diversification of cattle in Indonesia.

Many crossbreeding programs using exotic bulls (Bos Taurus) or frozen semen (artificial breeding) in these regions were failed to yield desirable results (Martojo, 2005). Success occurred only where Ongole (Bos Indicus) or PO crossbreeding (Bos Indicus-Bos Javanicus) was utilized. This program cause the genetic mixture of Bos Indicus-Bos Javanicus and Bos Taurus. There is potential to threaten the genetic identity of indigenous breed types. The aim of this research is to identify the genetic relationship between Ongole hybrid cattle (Bos Indicus-Bos Javanicus- Bos Taurus) and Bos Javanicus in Indonesia.

**MATERIAL AND METHODS**

**DNA Samples.** DNA was isolated from the whole blood and ear tissue using standard SDS/proteinase K extraction, with 730 µl lysis buffer (50 mM Tris-HCl pH 8.0, 100 mM EDTA, 100 mM NaCl, 1% SDS) and 20 µl Proteinase K (20 mg/ml) (Sambrook et al., 1989).

**Polymerase Chain Reaction (PCR).** A fragment of the mtDNA D-loop was amplified from 50 ng templates DNA with the primers D-loop-L (5’-AAAAATCCCAATAACTCAACACAG-3’; positions 15848-15870) and D-loop-R (5’-TACAATAGATGCTCCGGTCAG-3’; position 126-105). PCR was performed in 20 µl reaction mixture containing 1 µl of 10 pmol for each primer, 1.5 µl of 25 mM MgCl₂, 2 ml of 10x mgCl₂ free buffer, 0.1 ml dNTP and 0.1 ml Taq polymerase (promega) with the following program: predenaturation for 2 min at 92°C, 30 cycles of 15 s at 92°C, 45 s at 52°C, 45 s at 72°C and followed by a final extension step of 7 min at 72°C and 4°C until the next step. PCR product were seperated by 1% agarose gel electrophoresis in 1x TBE buffer and the
excised fragments were purified with the QIAquick isolation system (Qiagen, USA) and inserted in the pGEM-Teasy vector (Promega, USA). Clones were purified with the Qiagen Midiprep kit and 300 ng of pGEM Teasy positive clones were sequenced using T7 and SP6 primers with the following program: 96°C 1 min, 25 cycles of 96°C 30 s, 50°C 15 s and 60°C 4 min, BigDye Terminator v3.1 Cycle sequencing Kit and an ABI Prism™ 310 sequencer Systems (Applied Biosystems).

**Sequence analysis.** Bos Javanicus sequences were retrieved from the NCBI with BlastDNA search. The appropriate database from Genbank was compare to the partial D-loop mtDNA in this study. The alignment of the sequence was analyzed by the program Bioedit (Hall, 2002) and homology tree was constructed by using DNAMAN program.

**RESULTS AND DISCUSSION**

The DNA samples were consist of 5 samples of PO, 3 samples of Limpo, 5 samples of Simpo, 4 samples of Limousin semen and 4 samples of Simmental semen. Figure 1 shows the process to obtain the positive mtDNA D-loop clone in pGEM-T easy vector. The PCR product of mtDNA D-loop (Figure 1A) shows 600 bp. After purification with QIAquick isolation system (Qiagen, USA), the PCR products were cloned into pGEM-T easy vector (Figure 1B). The analysis of mtDNA D-loop with Ssp1 restriction enzyme was described at the Figure 1C. Analysis sequence of mtDNA D-loop using Ssp1 restriction enzyme shows that the pattern of restriction site for Simpo, Limpo and PO (lane 2, 3, and 4) was similar, another pattern also found at restriction site for Limosin (lane 1) and Simmental (lane 5) compare with the control of pGEM-Teasy vector (lane 6). Thus, identification of Ongole hybrid cattle (*Bos Indicus-Bos Javanicus*) compare with *Bos Taurus* can be recognized by Ssp1 restriction enzyme.

![Figure 1. Polymerase Chain Reaction (PCR) product of partial D-loop mtDNA (A), cloning the PCR product into pGEM-T easy vector (B) and digestion with Ssp1 restriction enzyme(C).](image-url)
Base on partial mtDNA D-loop sequence, there are three haplotypes of Ongole hybrid cattle (see Table). The mtDNA D-loop sequences of Ongole hybrid cattle were compared to the *Bos indicus*, *Bos taurus* and *Bos javanicus* mtDNA D-loop sequences from NCBI. There are three groups base on homology of mtDNA D-loop sequences. The first sub tree was *Bos taurus* group. The second sub tree was *Bos indicus* group, where is almost all of ongole hybrid cattle included in, and the third group was *Bos javanicus* group.

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<td>A</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>OHS(1)</td>
<td>A</td>
<td>A</td>
<td>G</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

Hyphen indicates the identical nucleotide with reference sequence.

<sup>a</sup> Number written vertically show nucleotide base position of mtDNA D-loop.

<sup>b</sup> Reference sequence Zebu (AF1262484).

<sup>c</sup> Number in parenthesis indicate number of animal observed.

OH = Ongole Hybrid , OHS = OHxSimmental, OHL = OHxLimousine

The results showed that all ongole hybrid cattle samples does not include in *Bos taurus* group. It indicated that mtDNA D-loop was inherited by maternal line. Therefore, almost all of Ongole hybrid cattle have genetically homology to the *Bos indicus*. Since ongole hybrid cattle were derived from *Bos Javanicus, Bos indicus and Bos Taurus*, some of Ongole hybrid cattle were included in *Bos javanicus* group. These results suggest that a part of Ongole hybrid cattle has genetically identity with *Bos javanicus*.
Figure 2. Homology Tree of Ongole-hybrid cattle.
Mitochondrial DNA is a marker of maternal lineage, which in cattle corresponds to the history of the herd (Nijman et al, 2003). So cows of the hybrid of Simpo and Limpo were used from Ongole hybrid. Exotic breeds were introduced to Ongole hybrid mostly by artificial insemination (Hartatik et al 2006). The results were reliable, because almost of the mtDNA D-loop sequences of the hybrids revealed similarity mtDNA D-loop sequences of the Ongole hybrid. Our data indicate that the Ongole hybrid cattle closer to Bos Indicus than to Bos javanicus. This explorative observation may become essential molecular basic data for the future breeding plan in cattle.

REFERENCES

O-MB19

A DNA PRIMER FOR RECOGNIZING PORCINE IN MEAT PRODUCTS

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ABSTRACT

DNA of raw or precooked meat containing pork has been investigated by Polymerase Chain Reaction (PCR) to prepare a procedure for determining this undesirable substance in commercial meat products.

The DNA was isolated and purified before the treatment through several steps involving cell tissue lysis, protein and RNA removal, and concentrating the DNA. Afterward, the isolated DNA was identifying qualitative- and quantitatively by electrophoresis.

In this research, we developed a DNA primer based on sequence alignment of several components of pork's fragments. This synthetic primer was used as promoter in the amplification of the DNA, comparatively with the application of commercial primer. The result showed that the A₂₆₀/A₂₈₀ ratio of the DNA was between 1.82 and 1.97, and its concentration was between 512 g/ml and 2570 g/ml.

The PCR indicated that the synthetic primer qualified in detecting pork DNA inside 439 bp fragments. Significantly, it was able to recognize the existence of 1% w/w fresh or cooked pork blend with beef or chicken meat.

Keywords: DNA primer, meat products, PCR, pork, sequence alignment

INTRODUCTION

Meat adulteration in food product has become a most attracted matter for public in Indonesia. Customers keep worrying about the frequent reports on the contamination of beef with pork, or meatballs made by flesh of rat. Identification of the species origin in meat products is relevant to consumers for several reasons: (a) personal dislike, (b) possible economic loss from fraudulent substitutions or adulterations, (c) medical requirements of individuals who might have specific allergies, and (d) religious reasons (Lenstra et al., 2001).

The conventional methodology used for the determination of species origin in meat products has been primarily based on the immunochemical and electrophoretic analysis of proteins (Kim and Shelef, 1986). However, proteins lose their biological activity after an
animal dies, and their presence and characteristics depend on the cell types. Furthermore, most of them are heat labile. Thus, for species identification, DNA analysis would be preferable to protein analysis.

More modern techniques now allow the identification of species specific DNA sequences, which has two major advantages over protein analysis: samples heated to as high as 120 °C can still be analyzed and discrimination between related species, such as sheep and goat or chicken and turkey, is possible (Hames and Higgins, 1995).

An accurate measurement of small amounts of pork DNA is possible with PCR technique. PCR (polymerase chain reaction) is a method for amplifying a fragment of DNA enzimatically under specific thermal cycling conditions (Bloch, 1991). Successful PCR amplification yields sharp bands on agarose gel electrophoresis. PCR has become an easier process for multiplying the quantity of DNA without continuos adding of enzyme within the duration of reaction.

A specific oligonucleotide primer is needed to serve as starting points for DNA synthesis in PCR. The primer is a key component to enable selective and repeated amplification. During PCR reaction, the primer recognizes a fragment of DNA sample, annealing specifically to the fragment, and then DNA polymerase extends the primer and synthesizes a new complementary DNA strand. Once we could find a primer that recognizes distinctly a DNA of pork, it can be used in detection of porcine contaminant in meat products.

In this study, we are designing a primer for pork DNA by searching any sequence similarity among several biological components of pig. The ability of the synthetic primer in recognizing porcine in meat products through PCR technique is validated with the application of a commercial primer.

**MATERIALS AND METHODS**

**Reagents**

Sodium dodecyl sulphate, Tris-hcl, EDTA, nacl, isopropanol, agarose, dntp, DNA marker (DNA λ/hindiii), ethidium bromide, TE buffer, proteinase K, Taq DNA Polymerase, commercial primer (5’- TCA GTT TAC ACT CAC CTG ATA GCA TCT -3’).

**Meat Samples**

To find the primer, five biological components of pig were examined. They are flesh, skin, bone, liver, and colon of pig.

To validate the primer, 24 compositions of meat products were analyzed. Six samples were meatballs and sausages; each made from beef, chicken, and pork. These meat products
were analyzed without cooking. Samples containing 0, 0.1, 0.5, 1.0, or 100 % pork in beef and 0, 0.1, 0.5, 1.0 % pork in chicken were taken, each with a total weight of 100 g. These mixture meat samples were heated in autoclave at 121 °C and 1.5 atm for 20 minute. Raw samples were also analyzed.

**DNA Extraction**

The DNA was extracted from meat samples according to a previously described procedure (Sambrook *et al.*, 1989). This includes the adding of lysozyme to the samples for cell tissue lysis, precipitating the DNA with ice-cold ethanol, and purifying the DNA.

**Quality and purity of DNA**

Horizontal submarine agarose gel electrophoresis was performed to check the quality of genomic DNA using 0.8% w/v agarose gel. The purity of DNA was checked using a UV spectrophotometer. The DNA samples (OD260:280 ratio) greater than 1.82 were considered good and were used for PCR amplification.

**Synthetic Primer of Pork DNA**

DNA sequencing was performed to each of the isolated DNAs, which were extracted from the five biological components of pig. The procedure involves the cutting of the DNA into several fragments using restriction enzyme BamHI, sequencing the fragments, and reconstructing the genome sequence.

The five genome sequences were aligned to find regions that are similar in sequence by previously described method (Smith and Waterman, 1981). A highly conserved pattern that found among the aligned sequences is the inquiry fragment. This specific fragment of 20-40 nucleotides length was synthesized chemically as a “primer”.

**PCR Cycle**

The PCR cycle is composed of three reactions, all of which are performed at different temperatures. In the first reaction, the isolated meat DNA (template DNA) is denatured to single strands at high temperature for a short time (94.0°C for 1 minute). In the second reaction, the temperature is lowered to allow the synthetic primer to anneal to the template DNA, again for a short time (62.0°C for 0.5 minute). In the third reaction, the temperature is raised to the optimal temperature for the *Tag* polymerase to synthesize DNA (72.0°C for 1 minute). It is necessary to repeat this cycle forty five times in order to synthesize enough DNA to analyze by agarose gel electrophoresis.
RESULTS AND DISCUSSION

Designing the Synthetic Primer

Most of the DNA is expressed as proteins or RNAs. Some regions of the DNA sequence are devoted to control mechanisms, and other parts of the sequence to be 'junk' (because we do not yet understand its function). It is believed that there are highly conserved sequence regions that shared among various part of species-specific DNA. The regions are unique, are thought to have functional value, and could be used as reference to the species.

Our bioinformatics program carried out the multiple sequence alignment across the five DNA sequences to identify a conserved sequence region. It works by combining the results of pairwise sequence comparisons to form a pattern that match the subsets of the sequences. The output from the program with window of 64 nucleotides in length is exhibited in Figure 1.

In designing the PCR primer, we considered several constraints. Firstly, the optimal length of the primer. This length is long enough for adequate specificity, and short enough for the primer to bind easily to the template at the annealing temperature. Secondly, the distribution of G’s and C’s in the primer. The presence of G or C bases within the last five bases from the end of primer helps promote specific binding at that end. Thirdly, the primer was checked for self-complementary to avoid the formation of hairpins and primer dimers. Fourthly, we avoided the primer with long runs of a single base, as they can misprime (Deffenbach et al., 1995).

We selected consecutively 40 nucleotides from the pattern (or consensus) sequence as ‘pork primer’. The sequence has 55 % G+C content and its maximum number of runs is 2
bp. The sequence was synthesized using ABI DNA Sequencer (from Applied Biosystems), and produced a synthetic primer with melting temperatures (Tm) in the range of 52-58 °C.

**PCR using Commercial Primer**

The amplified DNA of beef, chicken, and pork (as raw or cooked meats) with the application of the commercial primer exhibited several bands in gel electrophoresis, as established in Figure 2 (left). The amplification of the 647 bp DNA of pork samples are shown in lanes 4a and 7a. The same result is also shown in lanes 5a and 8a for the amplification of the 647 bp DNA of chicken samples. Whereas lanes 3a and 6a containing beef samples did not show any existence of DNA fragment.

![Gel electrophoresis showing the binding of the commercial primer to meat samples.](image)

**Fig. 2:** Gel electrophoresis showing the binding of the commercial primer to meat samples. Lane 2a: molecular marker, Lane 3a: Raw Beef, Lane 4a: Raw Pork, Lane 5a: Raw Chicken, Lane 6a: Cooked Beef, Lane 7a: Cooked Pork, Lane 8a: Cooked Chicken, Lane 2b: molecular marker, Lane 3b: Beef Sausage, Lane 4b: Pork Sausage, Lane 5b: Chicken Sausage, Lane 6b: Beef Meatball, Lane 7b: Pork Meatball, Lane 8b: Chicken Meatball.

The amplified DNA of beef, chicken, and pork (as sausages or meatballs) with the application of the commercial primer exhibited a similar pattern in gel electrophoresis as the amplified DNA of raw samples, as established in Figure 2 (right). Lanes 3b and 6b containing beef sausage and beef meatball samples did not show the presence of DNA fragments. The amplification of the 647 bp DNA of pork sausage and pork meatball samples are shown in lanes 4b and 7b. The same length of DNA fragments of chicken sausages and chicken meatballs are shown in lane 5b and 8b.

This fact illustrates the unsuccessful detection of pork elements in samples. We are expecting that the function of the commercial primer as starting fragment in PCR reaction is
work only for samples containing pork. The primer did not recognize the DNA of beef samples, as we want, but it did recognize the DNA of chicken samples. Therefore, the commercial primer can not be use in determination of pork contaminant in meat samples because it did not specifically discriminate pork and chicken.

The inability of the commercial primer to discriminate DNA of pork and chicken is probably caused by genotype similarity between pig and chicken although they have notably different phenotype. This fact has been confirmed by sequence alignment of three DNA of their peptides. DNA sequences of nanopeptide Y, peptide YY, and pancreatic peptide of pig and chicken are very similar (Bromée et al., 2006).

**PCR using Synthetic Primer**

The amplified DNA of beef, chicken, and pork (as raw or cooked meats) with the application of the synthetic primer exhibited a different figure in gel electrophoresis than the one we have from the application of the commercial primer. The gel electrophoresis did not show any existence of DNA fragment for samples containing beef or chicken. Sharp bands appear from the amplification of the 439 bp DNA of pork samples only. This fact was confirmed by the yields of amplified DNA after PCR process, as presented in Table 1.

The amplified DNA concentration are 1580 ng/ml and 1260 ng/ml, consecutively for raw pork and cooked pork samples.

<table>
<thead>
<tr>
<th>Sample</th>
<th>DNA concentration</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DNA concentration</td>
<td>Isolated (ng/ml)</td>
<td>Amplified (ng/ml)</td>
</tr>
<tr>
<td>Raw meat:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beef</td>
<td></td>
<td>176</td>
<td>-</td>
</tr>
<tr>
<td>Pork</td>
<td></td>
<td>249</td>
<td>1580</td>
</tr>
<tr>
<td>Chicken</td>
<td></td>
<td>174</td>
<td>-</td>
</tr>
<tr>
<td>Cooked meat:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beef</td>
<td></td>
<td>157</td>
<td>-</td>
</tr>
<tr>
<td>Pork</td>
<td></td>
<td>161</td>
<td>1260</td>
</tr>
<tr>
<td>Chicken</td>
<td></td>
<td>165</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 1: DNA concentration of raw and cooked meats before and after PCR with the application of the synthetic primer.
The heating process did not considerably influence to DNA analysis. Although the amount of the isolated DNA of cooked pork is decrease comparing with those of raw pork, the amplification factor of their PCR products is almost the same. It explains that the sterilization temperature did not damage DNA sample. With the application of the synthetic primer, we obtained the similar results for the amplified DNA of sausage and meatball samples. Gel electrophoresis shows the DNA fragments of pork samples only. There is not any existence of DNA fragment for beef or chicken samples. The amplified DNA concentration of pork sausage is 2570 ng/ml, and the amplified DNA concentration of pork meatball is 1090 ng/ml, as presented in Table 2.

Table 2: DNA concentration of sausages and meatballs before and after PCR with the application of the synthetic primer.

<table>
<thead>
<tr>
<th>Sample</th>
<th>DNA concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Isolated (ng/ml)</td>
</tr>
<tr>
<td>Beef sausage</td>
<td>172</td>
</tr>
<tr>
<td>Pork sausage</td>
<td>332</td>
</tr>
<tr>
<td>Chicken sausage</td>
<td>190</td>
</tr>
<tr>
<td>Beef meaball</td>
<td>78.3</td>
</tr>
<tr>
<td>Pork meatball</td>
<td>105</td>
</tr>
<tr>
<td>Chicken meatball</td>
<td>37.7</td>
</tr>
</tbody>
</table>

PCR products of 12 meat mixtures verified the successful in detection pork element in the samples. There are 512 ng/ml to 886 ng/ml DNA of raw meat mixtures and 502 ng/ml to 749 ng/ml DNA of cooked meat mixtures which has been amplified during PCR process, as presented in Table 3.
Table 3: DNA concentration of the mixtures of raw and cooked meats before and after PCR with the application of the synthetic primer.

<table>
<thead>
<tr>
<th>Sample</th>
<th>DNA concentration</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Isolated (ng/ml)</td>
<td>Amplified (ng/ml)</td>
</tr>
<tr>
<td>Raw meat:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beef-Pork (99 : 1)</td>
<td>170</td>
<td>825</td>
</tr>
<tr>
<td>Beef-Pork (99.5 : 0.5)</td>
<td>179</td>
<td>886</td>
</tr>
<tr>
<td>Beef-Pork (99.9 : 0.1)</td>
<td>148</td>
<td>745</td>
</tr>
<tr>
<td>Chicken-Pork (99 : 1)</td>
<td>87.6</td>
<td>870</td>
</tr>
<tr>
<td>Chicken-Pork (99.5 : 0.5)</td>
<td>70.2</td>
<td>697</td>
</tr>
<tr>
<td>Chicken-Pork (99.9 : 0.1)</td>
<td>68.3</td>
<td>512</td>
</tr>
<tr>
<td>Cooked meat:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beef-Pork (99 : 1)</td>
<td>142</td>
<td>738</td>
</tr>
<tr>
<td>Beef-Pork (99.5 : 0.5)</td>
<td>156</td>
<td>719</td>
</tr>
<tr>
<td>Beef-Pork (99.9 : 0.1)</td>
<td>157</td>
<td>578</td>
</tr>
<tr>
<td>Chicken-Pork (99 : 1)</td>
<td>75.2</td>
<td>749</td>
</tr>
<tr>
<td>Chicken-Pork (99.5 : 0.5)</td>
<td>57.7</td>
<td>600</td>
</tr>
<tr>
<td>Chicken-Pork (99.9 : 0.1)</td>
<td>35.6</td>
<td>502</td>
</tr>
</tbody>
</table>

It should be noticed that the mixture samples containing as little as 0.1 % pork could be measured by DNA analysis. The isolated DNA containing 0.1 % pork in the mixture is the lowest but the amplification factor of their PCR products is not low. Hence, PCR is the suitable technique to analyze extremely small amounts of meat sample.

From the determination of 24 meat samples, it can be assured that the synthetic primer performs specifically in the amplification of DNA by PCR. The synthetic primer recognizes the DNA sequence of pork only and did not bind to the DNA sequence of beef or chicken. The synthetic primer can be used to detect pork contaminant in meat products because it able to discriminate pork and other meats.

CONCLUSIONS

By mean of a multiple sequence alignment to five DNAs of biological components of pig, we found a 40 bp length of DNA primer which able to recognize porcine in meat
products. The specificity of the synthetic primer has been validated by PCR using commercial primer as a reference.

The PCR indicated that the synthetic primer qualified in detecting pork DNA inside 439 bp fragments. Significantly, it was able to recognize the existence of 0.1 % w/w raw or cooked pork blend with beef or chicken meat.

REFERENCES

O-MB20

KARYOTYPE VARIATION OF SWAMP EELS, *Monopterus albus* ZUIEW 1793
(PISCES : SYNBRANCHIDAE)

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ABSTRACT

Swamp eel is native to Indonesia and commonly inhabits in paddy fields. The freshwater fish has an economic potency in aquaculture due to few of fish spines, good taste, and high reproductive capability. Even though the fish is extensively exploited to meet market demands, any comprehensive study on chromosome has not been investigated yet. This study therefore was to report the basic study of karyotype on the fish species collected from two different populations: Ngantenan, Canan, Klaten, Central Java and Kayutrejo, Widodaren, Ngawi, East Java according to its chromosome morphology during prometaphase of mitosis. Chromosome preparation of the fish species investigated in this study was a splash method with blood culture cells.

The result revealed that the swamp eels from Ngantenan, Canan, Wedi, Klaten, Central Java and Kayutrejo, Widodaren, Ngawi, East Java had similar diploid chromosome number (2n) = 24. However, the swamp eels collected from the two different population had different karyotype formula. Chromosomes of the swamp eels from Ngantenan, Canan, Wedi, Klaten, Central Java was classified as 2 (1 pair) submetacentric chromosomes and 22 (11 pairs) of telocentric chromosomes whereas the chromosome of swamp eels in population Kayutrejo, Widodaren, Ngawi, East Java was consisted of 24 (12 pairs) telocentric chromosomes. Therefore, the karyotype formula of swamp eels from Ngantenan, Canan Klaten, Jawa Tengah was 2n = 24 = 2 sm + 22 t while the karyotype formula of swamp eels from Kayutrejo, Widodaren, Ngawi, East Java was 2n = 24 = 24 t.

Keywords: *Monopterus albus*, chromosom, karyotypee

INTRODUCTION

Fish is the most important source of protein and is especially significant in developing countries. Over exploitations of wild fish stocks and environmental degradation has meant that the world’s demand for fish protein can no longer be met from traditional fishing. Thus aquaculture production and sustainable management of fish stocks that have not collapsed are of critical importance for food security and enhanced income. Based on that condition, genetic information is now considered essential for sustainable exploitation, management,
and conservation of commercially important of fish species. Such information is not only valuable for determining genetic diversity but also for investigating genetic improvement programs.

Fish of the family Synbranchidae group is an economically important freshwater fishes in worldwide. Within this family, the genus *Monopterus* are especially popular due to their reputation as delicious food item and their ability to survive and grow in poorly oxygenated waters (Archipchuk, 1999; Collins *et al*., 2002). Approximately 10 species of *Monopterus* are currently recognized, with the majority of species occur in Africa and Asia. Of the 9 species presently recognized in Asia, one species is native to Indonesia which is *Monopterus albus* Zuiew. The fish species is broadly distributed in Java, Bali, Sumatra, Sulawesi, and some of the smaller islands. The fish species is found mainly in warm freshwaters locations such as muddy ponds, swamps, canals, and rice fields. In addition, the fish is known to reach about 40 cm in length and recognized by cylindrical snake-like body with tapered tail and small eyes. Its body color is brown above and white or light-brown below. In adults, paired fins are lacking, and the dorsal, caudal and anal fins are reduced. The gill openings are merged into a single slit underneath the head while the mouth is large and protracile and both upper and lower jaws have tiny teeth for eating fishes, worms, crustaceans, and other small aquatic animals at night (Kottelat *et al*., 1993; Nelson, 1994; Archipchuk, 1999).

The fish species is considered to be non-migratory species which is likely to have any limited powers of dispersal. A consequence of this life cycle is therefore reproductive isolation between populations leading to genetic divergence and over sufficiently long periods of time can lead to speciation (Ye *et al*., 2007; Cai *et al*., 2008). Indeed this life cycle is thought to be important factor to account for high diversity of the swamp eels in Indonesia. Thus even within the Indonesian archipelago, *Monopterus albus* may consist of a large number of endemic cryptic species. Alternatively the species may be have high levels of genetic similarity among widely separated populations. It is also possible that both factors, cryptic speciation and translocation causing complicated geographic patterns of genetic variation.

Within the context of aquaculture and conservation, this research investigated chromosome characters of the fish species collected from two populations (Ngantenan, Canan,Wedli, Klaten, Central Java and Kayutrejo, Widodaren, Ngawi, East Java) as a preliminary research on genetic variation of the fish species in those populations. This information is a prerequisite for strategies for future conservation and sustainable use of the fish species.
MATERIALS AND METHODS

1. Sample collection

Specimens of swamp eels were collected from Ngantenan, Canan, Klaten, Central Java (07°45'35"S, 110°34'56"E; 30 samples) and Kayutrejo, Widodaren, Ngawi, East Java (07°26'21"S, 110°13'31"E; 30 samples). The specimens were then maintained at Laboratory of Genetics, Faculty of Biology, Gadjah Mada University in well aerated aquariums.

2. Chromosome preparation

Chromosome preparation of the fish species used in this study was a splash method with blood culture cells described by Amemiya et al. (1984) and Session (1996). Around 0.5 ml of the blood sample of each individual was placed in a flask and cultured in 7 ml growth medium. The growth medium was made from 10 ml FBS (Fetal Bovine Serum), 2 ml Streptomycin, 0.5 ml Fungison, and 87.5 ml Dulbecco’s Modified Eagle Medium (DMEM) solution containing 10.4 gr DMEM, 2 gr NaHCO₃, and 2 gr 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) dissolved in 1000 ml aquades and adjusted to pH 7.2-7.4 with 0.1 N HCl. Each blood sample in a flask was then added with 0.1 ml Phytohaemaglutinin (PHA) and incubated in incubator with 5% CO₂ level for 72 hours at 37°C. Next, the samples were monitored daily for cell attachment and growth.

After 2-3 days, the cell growth reached two-thirds confluence in a flask and can be karyotyped. Two hours prior to harvesting blood cultured cells for karyotyping, 2 ml of 20% colchicine was added to each flask to arrest mitotic activity. The harvested cells from each flask were then transferred to a conical centrifuge tube and centrifuged at 750 G for 10 minutes. Next, the supernatant was removed and the cell pellet was resuspended in 4 ml of hypotonic solution (0.56% KCl). Hypotonic incubation was for 15 minutes at 30° C, after which the suspension was sentrifuged at 750 G for 10 minutes. The supernatant was then removed and the cells were fixed for 5 minutes in carnoy solution (methanol : acetic acid =3:1). Next, the fixed cells were centrifuged at 750 G for 10 minutes and the fixative was replaced at least twice prior to preparing slides. Slides were made by dropping suspended cells from a height 2-3 cm onto wet slides. Air dried preparations were then stained in 20% Giemsa solution for 30 minutes and after that they were rinsed briefly in distillated water and dried. The slides were then photographed using Olympus C-35-AD-4 and Kodak ASA 100 film.
3. **Chromosome analysis**

The measurement of chromosome size was done using AutoCAD Map 2000i for Windows program. Centromere position of chromosome was classified by centromeric index calculated by short arm/total length following Levan et al. (1964) classification: metacentric chromosome with centromeric index of 37.50–50.00; submetacentric chromosome with centromeric index of 25.00–37.49; subtelocentric chromosome with centromeric index of 12.5–24.99; and telocentric chromosome with centromeric index of 0–12.49. Data of chromosome size and centromere position of chromosomes were then arranged to construct karyotype using Adobe Photoshop CS2 for Windows program, and idiogram using CorelDRAW Graphic Suite X3.

**RESULTS AND DISCUSSION**

The result revealed that *Monopterus albus* collected from Ngantenan, Canan, Wedi, Klaten, Central Java and Kayutrejo, Widodaren, Ngawi, East Java had similar diploid chromosome number (2n) = 24 (Table 1 and Fig.1). The chromosome number of the two populations is in agreement with Archipchuk (1999), who recorded the diploid chromosome number of the fish species collected from Hubei (China), Manipur (India), and Nara (Japan). However, he gave no further information about the chromosome size of the fish species. Even though *M.albus* sampled from Ngantenan, Canan, Wedi, Klaten, Central Java and Kayutrejo, Widodaren, Ngawi, East Java exhibited similar diploid chromosome number, they appeared to have differences in chromosome size and the centromeric index displaying variation in karyotype formula (Table 2, Fig.1 and Fig.2).

As shown in Table 1 and Fig. 2, it can be seen that chromosome size of *M.albus* from Kayutrejo, Widodaren, Ngawi, East Java was larger than that of *M.albus* from Ngantenan, Canan, Wedi, Klaten, Central Java. In addition, length of long arm chromosomes and chromosome total length of the fish species from each population were similar. This was due to the position of centromere in most chromosomes of the fish species identified in this study were in the terminal region. Moreover, the proportion of the largest chromosome total length with the smallest chromosome total length (R) of *M.albus* from Kayutrejo, Widodaren, Ngawi, East Java and Ngantenan, Canan, Wedi, Klaten, Central Java was 1.764 ± 0.012 and 2.596 ± 0.416 respectively. This data revealed that chromosome size of *M.albus* from Ngantenan, Canan, Wedi, Klaten, Central Java had more variation compared to chromosome size of *M.albus* from Kayutrejo, Widodaren, Ngawi, East Java eventhough *M.albus* from Ngantenan, Canan, Wedi, Klaten, Central Java exhibited much smaller chromosome than *M.albus* from Kayutrejo, Widodaren, Ngawi, East Java did.
Table 1. Chromosome characters of swamp eels sampled from Kayutrejo, Widodaren, Ngawi, East-Java and from Ngantenan, Canan, Klaten, Central Java

<table>
<thead>
<tr>
<th>Chromosome Characters</th>
<th>Population Kayutrejo, Widodaren, Ngawi (East-Java)</th>
<th>Population Ngantenan, Canan, Klaten (Central Java)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromosome number</td>
<td>2n = 24</td>
<td>2n = 24</td>
</tr>
<tr>
<td>Length of Short Arm (µm)</td>
<td>0.00</td>
<td>0.00 – 0.261</td>
</tr>
<tr>
<td>Length of Long Arm (µm)</td>
<td>0.660 – 1.207</td>
<td>0.319 – 1.022</td>
</tr>
<tr>
<td>Total Length (µm)</td>
<td>0.660 – 1.207</td>
<td>0.319 – 1.022</td>
</tr>
<tr>
<td>Karyotype Formula</td>
<td>2n = 24 = 24t</td>
<td>2n = 24 = 2sm + 22t</td>
</tr>
<tr>
<td>Centromeric Index</td>
<td>0.00</td>
<td>0.00 – 30.242</td>
</tr>
<tr>
<td>R Value</td>
<td>1.764 ± 0.012</td>
<td>2.596 ± 0.416</td>
</tr>
</tbody>
</table>

Fig. 1. Karyotype of swamp eels (A. Population from Kayutrejo, Widodaren, Ngawi, East-Java and B. Population from Ngantenan, Canan, Klaten, Central Java). Number below the chromosomes reveal chromosome pairs and letters below the number reveal chromosome shapes (sm=submetacentris, t=telocentris).
The result also revealed that all chromosomes of *M. albus* from Kayutrejo, Widodaren, Ngawi, East-Java appeared to have the centromere in the terminal region displaying no short arm chromosomes and were thus classified as a telocentric according to Levan *et al.* (1964) displaying karyotype formula 2n=24=24t (Table 1, Fig. 1A, and Fig. 2). This karyotype data was similar to the karyotypes of the fish species caught from Hubei (China), Manipur (India), and Nara (Japan) (Archipchuk 1999). Conversely, the Giemsa-stained karyotype of diploid metaphase complement of swamp eels from Ngantenan, Canan, Klaten, Central Java contained 12 pairs of chromosomes with comprising 1 pair of submetacentric and 11 pairs of telocentric chromosomes displaying karyotype formula 2n = 24 = 2sm + 22t. The divergence of the karyotype formula of swamp eels from the two populations investigated in this study seemed to reveal genetic variation a due to their establishment of their habitat and their life history as a non-migratory species.

On the basis of chromosome characters of swamp eels from the two populations investigated in this study, it is recommended that further chromosome studies combined with molecular genetic data on other populations of the fish species in this country should be done to identify more precisely genetic identity and genetic diversity of the fish species of this country. This is due to understanding properly the biodiversity of this group of fish and implementing conservation strategies for genetic divergent forms with restricted populations will allow the aquaculture potential of the fish species to be properly developed and an appropriate genetic improvement programs implemented.

![Idiogram showing comparison chromosome size between swamp eels from Kayutrejo, Widodaren, Ngawi, East-Java (population 1) and from Ngantenan, Canan, Klaten, Central Java (population 2)](chart)

**Fig.2.** Idiogram showing comparison chromosome size between swamp eels from Kayutrejo, Widodaren, Ngawi, East-Java (population 1) and from Ngantenan, Canan, Klaten, Central Java (population 2)
REFERENCES


O-MB21

CHROMOSOME CHARACTERIZATION OF *Citrus nobilis* Lour, *Citrus microcarpa* Bunge, and *Citrus amblycarpa* (Hassk.) Ochse

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Laboratory of Genetics, Faculty of Biology, Gadjah Mada University
Jl. Tehnika Selatan, Sekip Utara, Yogyakarta 55281, Indonesia

ABSTRACT

Three species of the genus *Citrus* were karyomorphologically studied during prometaphase mitosis using a squash method. This research was conducted in order to report the chromosome characters of *Citrus nobilis*, *Citrus microcarpa*, and *Citrus amblycarpa*. The result exhibited that the three *Citrus* species had similar diploid chromosome number (2n) = 18. However, the three *Citrus* species revealed different karyotype formula and chromosome size. The prometaphase karyotypes of the three *Citrus* species were formulated as follows: *C. nobilis*: 2n = 18 = 10m + 8sm; *C. microcarpa*: 2n = 18 = 18m; and *C. amblycarpa*: 2n = 18 = 16m + 2sm. In addition, *C. nobilis* revealed much smaller chromosomes than the other two *Citrus* species did. The chromosomes of the complement in *C. nobilis* exhibited total length of 0.667 μm to 2.718 μm while the chromosomes of the complement in *C. microcarpa* and *C. amblycarpa* displayed total length of 0.938 μm to 3.592 μm and between 1.237 μm and 3.427 μm respectively. The findings of this research are expected to enrich valuable information concerning genetic diversity of the genus *Citrus* used for improving *Citrus* quality through breeding program in Indonesia.

**Keywords**: *Citrus* species, karyotype, chromosome size

INTRODUCTION

Among the Family Rutaceae, the genus *Citrus* is the most important economically with a high diversity of species, cultivars and clones (Filho et al., 1998). Most species of the genus *Citrus* are well known for providing adequate vitamin C as per recommended dietary allowance (RDA) and their flavonoids are effective in improving blood circulation and use for antiallergic, anticarcinogenic, and antiviral properties (Grieve and Scora, 1980; Filatova and Kolesnova, 1999). However, genetic diversity of *Citrus* species in Indonesia is gradually eroding making evaluation of genetic diversity and plant exploration difficult. In addition, improvement of the genus *Citrus* is extremely slow and costly because of the long-term nature of breeding and unusual combination of reproductive characteristics (Muhammad Usman et al., 2002; Chen et al., 2004; Terol et al., 2007). Therefore, a chromosome study of
the genus *Citrus* is absolutely needed due to their implications not only for determining its genetic diversity but also for improving its quality through breeding program in Indonesia.

The three species of *Citrus* (*C. nobilis*, *C. microcarpa*, and *C. amblycarpa*) are widely scattered in cultivation, and are commonly planted on a large scale in Indonesia due to their pleasant flavour and high vitamin C content. *C. nobilis* and *C. microcarpa* is usually eaten as fresh fruit or used for juicy drinks while *C. amblycarpa* is commonly utilized for spices and traditional herbs (Grieva and Scora, 1980; Matsumoto and Okudai, 1986; Filatova and Kolesnova, 1999). Even though demands of the three *Citrus* species always increase every year, any comprehensive study on chromosome has not been examined yet. This study therefore was to report the basic study of chromosome characters on the three *Citrus* species according to their chromosome morphology during prometaphase of mitosis.

The objective of this research was to determine chromosome number, chromosome size, and karyotypes of *C. nobilis*, *C. microcarpa*, and *C. amblycarpa*. The findings of this research are expected to enrich valuable information concerning the chromosome characters of the three *Citrus* species which can evaluate genetic diversity of the genus *Citrus* used for improving *Citrus* quality through breeding program.

**MATERIALS AND METHODS**

1. **Sample collection**

   The three *Citrus* species were collected from several regions shown in Table 1.

   **Table 1. Area sampling of the three *Citrus* species investigated in this research**

<table>
<thead>
<tr>
<th>No.</th>
<th><em>Citrus</em> Species</th>
<th>Area sampling</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Citrus nobilis</em></td>
<td>Kutambelu, Leu Baleng, Tanah Karo, North Sumatra</td>
</tr>
<tr>
<td>2</td>
<td><em>Citrus microcarpa</em></td>
<td>Sukoreno, Sentolo, Kulon Progo, Yogyakarta</td>
</tr>
<tr>
<td>3</td>
<td><em>Citrus amblycarpa</em></td>
<td>Panggungharjo, Sewon, Bantul, Yogyakarta</td>
</tr>
</tbody>
</table>

2. **Chromosome Preparation**

   A chromosome preparation procedure of the three *Citrus* species investigated in this study followed those described by Session (1996). Seeds of *C. nobilis*, *C. microcarpa*, and *C. amblycarpa* were sown and germinated in petri-dishes at Laboratory of Genetics, Faculty of Biology, Gadjah Mada University, Yogyakarta. Chromosome preparation were conducted at 07.00 a.m. to 11.30 a.m. (WIB) at 15 minute intervals. Fresh root tips from germinated seeds of the three *Citrus* species were pre-treated in cold water at 4°C for 1 hour before they were fixed in 45% acetic acid at 4°C for 15
minutes. Fixed root tips were then macerated in 1 N hydrochloric acid for about 5 minutes at 55°C. The root tips were stained in 1% aceto-orcein before they were squashed. The slides were then photographed using Olympus C-35-AD-4 and Kodak ASA 100 film.

3. Chromosome Analysis

The measurement of chromosome size was done using AutoCAD Map 2000i for Windows program. Centromere position of chromosome was classified by centromeric index calculated by short arm/total length following Levan et al. (1964) classification: metacentric chromosome with centromeric index of 37.50–50.00; submetacentric chromosome with centromeric index of 25.00–37.49; subtelocentric chromosome with centromeric index of 12.5–24.99; and telocentric chromosome with centromeric index of 0–12.49. Data of chromosome size and centromere position of chromosomes were then arranged to construct karyogram using Adobe Photoshop CS2 for Windows program, and idiogram using CorelDRAW Graphic Suite X3.

RESULTS AND DISCUSSION

The result exhibited that C. nobilis, C. microcarpa, and C. amblycarpa had similar diploid chromosome number (2n) = 18. This count was similar to the previous studies reported by Krug (1943), Darlington and Wylie (1955), and Guerra et al. (1997), who recorded the diploid chromosome number but gave no further information about the chromosome size and karyotypes of the three Citrus species. Even though they revealed similar diploid chromosome number, they appeared to have differences in chromosome size and the centromeric index displaying variation in karyotype formula (Table 2, Fig.1 and Fig.2).

As shown in Table 2 and Fig. 2, it can be seen that chromosome size of C. nobilis was smaller than that of C. microcarpa and C. amblycarpa. Length of short arm of C. nobilis, C. microcarpa, and C. amblycarpa was 0.244-1.275 µm, 0.442-1.711 µm, and 0.572-1.46 µm respectively whereas length of long arm of the three Citrus species was 0.375-1.443 µm, 0.496-1.881 µm, and 0.665-1.730 µm respectively. In addition, chromosome total length of C. nobilis, C. microcarpa, and C. amblycarpa was 0.667-2.718 µm, 0.938-3.592 µm, and 1.237-3.427 µm respectively. This result exhibited that chromosome size of C. microcarpa and C. amblycarpa was almost similar to C. hystrix while chromosome size of C. nobilis was larger than that of C. aurantifolia examined in the previous study (Arisuryanti et al., 2007).
Table 2. Chromosome characters of *Citrus nobilis*, *Citrus microcarpa*, and *Citrus amblycarpa*

<table>
<thead>
<tr>
<th>Chromosome characters</th>
<th><em>Citrus nobilis</em></th>
<th><em>Citrus microcarpa</em></th>
<th><em>Citrus amblycarpa</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromosome number</td>
<td>2n=18</td>
<td>2n=18</td>
<td>2n=18</td>
</tr>
<tr>
<td>Length of Short Arm (µm)</td>
<td>0.244 – 1.275</td>
<td>0.442 – 1.711</td>
<td>0.572 – 1.46</td>
</tr>
<tr>
<td>Length of Long Arm (µm)</td>
<td>0.375 – 1.443</td>
<td>0.496 – 1.881</td>
<td>0.665 – 1.730</td>
</tr>
<tr>
<td>Total Length (µm)</td>
<td>0.667 – 2.718</td>
<td>0.938 – 3.592</td>
<td>1.237 – 3.427</td>
</tr>
<tr>
<td>Centromeric Index</td>
<td>30.036 – 49.141</td>
<td>38.433 – 49.835</td>
<td>31.804 – 49.995</td>
</tr>
<tr>
<td>Karyotype Formula</td>
<td>2n=2x=18=10m+8sm</td>
<td>2n=2x=18=18m</td>
<td>2n=2x=18=16m+2sm</td>
</tr>
</tbody>
</table>

The result also revealed that all chromosomes of *C. microcarpa* appeared to have the centromere in the median region and were thus classified as a metacentric according to Levan *et al.* (1964) displaying karyotype formula 2n=2x=18=18m (Table 2 and Fig.1). This karyotype formula was similar to *C. aurantifolia* and *C. hystrix* even though the chromosome size and the centromeric index of *C. microcarpa* were different from *C. aurantifolia* and *C. hystrix* (Arisuryanti *et al.*, 2007). The finding of metacentric chromosomes in *C. microcarpa* revealed that this *Citrus* species investigated in this study had symmetry karyotypes. The other two species, *C. nobilis* and *C. amblycarpa* had different karyotype formula compared to *C. microcarpa*. Karyotype formula of *C. nobilis* was 2n=2x=18=10m+8sm with the position of submetacentric chromosomes were in chromosome pair number 2, 4, 6, and 8 whereas karyotype formula of *C. amblycarpa* was 2n=2x=18=16m+2sm with the position of submetacentric chromosome was in chromosome pair number 2. The finding of submetacentric chromosomes in *C. nobilis* and *C. amblycarpa* revealed that the two *Citrus* species investigated in this study had asymmetry karyotypes.
Fig. 1. Karyotype of the three Citrus species (A. Citrus nobilis, B. Citrus microcarpa, C. Citrus amblycarpa). Number below the chromosomes reveal chromosome pairs and letters below the number reveal chromosome shapes (m=metacentris, sm=submetacentris). Scale bar correspond to 0.5 μm.

Fig. 2. Idiogram showing comparison chromosome size between C. nobilis, C. microcarpa, and C. amblycarpa.

On the basis of chromosome characters examined in this study, karyomorphology and chromosome size of the three Citrus species can be used to distinguish between one and another Citrus species. Divergences of chromosome size and karyomorphology of the three Citrus species are also valuable to add information about genetic diversity of the genus Citrus. Moreover, the data can be utilized to improve the three
Citrus species quality through breeding program in Indonesia. This is due to Citrus industry is now critically depend on genetically superior cultivars to improve fruit quality, yield, disease resistance, and ability to tolerate environmental stresses (Singh, 1994; Guerra et al., 1997; Guerra et al., 2000; Brasileiro-Vidal et al., 2007). The breeding program which can be applied for the Citrus species may through an allotetraploid somatic hybrid or autotriploid. This program can gain beneficial agronomic traits from both parents for rootstocks or scion cultivar improvement of the Citrus. Citrus somatic hybrid, if fertile, could serve as pollen parents in interploid sexual hybridization to generate new and improved seedless triploids (Oiyama et al., 1991; Muhammad Usman et al., 2002; Chen et al., 2004).

Further chromosome studies on other Citrus species from Indonesia will be needed to identify more precisely genetic characters of Citrus species of this country. This is due to plant breeders have always been appreciating enhancement of the existing gene pool, and chromosome studies would be helpful in the enrichment of the existing Citrus germplasm resources of the country to develop Citrus industry.

REFERENCES

O-MB22

INSERTION OF ACTIVATION TAG INTO BATUTEGI AND KASALATH RICE CULTIVAR

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ABSTRACT

Indonesia has many rice varieties with many interesting traits that spread out among its land. The genetic resources have been utilized and provided sources of important traits for traditional rice breeding for centuries. Isolation of genes involved in important traits, therefore, is very desirable. Once isolated, the genetic resources of rice can be manipulated for developing new rice varieties by genetic engineering.

As the attempt to study gene functions related to drought tolerance, we are developing mutant insertion population of Indonesian drought tolerant rice cultivars Batutegi using Kasalath as transformation control. Activation tagging populations were developed using the \(Ac/Ds\) transposon that harbour on pMOG22 with \(hpt\) gene as selection marker. The rice transformation with the activation-tag construct was conducted by modified Hiei method (Hiei and Komari, 2006), a highly efficient transformation method via \(Agrobacterium\) for Indica rice. So far we have transformed 146 calli of immature embryos for Kasalath and 797 calli of immature embryos for Batutegi. Hygromycine selection of the transformed callii obtained 46 and 86 resistant embryos from Kasalath and Batutegi, respectively. Two plantlets were already obtained from Kasalath. The putative transformants were analyzed by PCR with a pair of \(hpt\) gene specific primers, that amplifying 492 bp fragment of \(hpt\) gene. This result indicate that the insertion of the \(Ac/Ds\) transposon is succeed.

Keywords: transformation, activation-tag, \(Ac/Ds\) transposon, Batutegi,

INTRODUCTION

Rice is the most important crop in Indonesia, one of the centers of origin of rice. It has many rice varieties with many interesting traits such as tolerant to biotic and abiotic stresses. One of potential Indonesian cultivar is Batutegi. This cultivar belongs to upland rice, which means being sown in dry land and grown in rain feeding or limited irrigation condition but not requiring coverage of water layer in fields for their whole life cycles. (Gupta & O’Toole, 1986). The upland rice can save water and decrease the water pollution.
Spreading around the equator, Indonesia as agrarian country has tropical climate which bring the drought and wet season. There are only about 2 million hectare of 11 million hectare marginal areas had been planted in 2006 (Wurjandari and Syam, 2007). In principle there are sufficient dry lands available, but the prerequisite is the development of suitable drought-tolerant cultivars. Therefor, isolation of genes involved in important traits such as drought tolerant, is very desirable. Unfortunately, gene discovery attempt to study gene functions of Indonesian rice cultivar have not been established.

The inter- and intra-species genetic diversity, such as the diversity among Indica, Japonica and Javanica subspecies or the diversity within subspecies themselves provide important genetic resources. The genetic resources have been utilized and provided sources of important traits for traditional rice breeding for centuries. Isolation of genes involved in important traits, therefore, is very desirable. Once isolated, the genetic resources of rice can be manipulated for developing new rice varieties by genetic engineering.

Insertional mutagenesis was first used with well-characterized transposable elements in Drosophila melanogaster (Bingham, P. M. et al., 1981). In plants, the same strategy was used when transposable elements of maize (Ac/Ds) and snapdragon were characterized (Walbot, V., 1992). Different kinds of inserts besides transposons have been used. For example, T-DNA from Agrobacterium tumefaciens, or retrotransposons like the Tnt1 element from tobacco is functional in Arabidopsis (Feldmann, K. A., 1991; Grandbastien, M.-A., 1992; Lucas, H. et al., 1995).

Activation Tagging is featured by the use of inserts containing of 4 tandem repeat enhancer sequences, from the consecutives promotor of cauliflower mozaik virus CamV 35S that can increase expression level of neighboring genes. The over expressed gene will cause phenotypic changes that may indicate the function of the gene. This approach has been applied successfully in isolating genes from Arabidopsis (Kakimoto, 1996; Weigel et al., 2000; Borevits et al. 2000; Ito and Meyerowitz, 2000,), petunia (Zubko et al, 2002) and Catharanthes roseus (van der Fits and Memelink, 2000).

In rice, activation tagging has also been applied in gene discovery attempt. Jeong et al. (2002) developed activation tagging population in rice with T-DNA insertion on Japonica rice while Nugroho et al. (2006) used Ac/Ds transposon on Japonica rice cv Nipponbare. In the use Ac/Ds transposon, DNA insert fragments (TDNA) are harboring Ac transposon and Ds transposon elements. Ds transposon carries activation tag fragment which is an overexpression induced factor. Once activation tag fragment inserts to a site of genom, it will activate expression of the neighbouring genes. Overexpression of these genes are expected to initiate a new creation of rice phenotype.
This research is a preliminary research of developing rice mutant population carrying activation-tag for gene discovery in order to develop a mutant population of an Indonesian drought tolerant rice cultivar carrying insertion of activation-tag in unique chromosomal position using the Ac/Ds transposable element. The local Javanica rice cv Batutegi, which is an upland drought tolerant cultivar is used in this study. Kasalath, an Indica rice cultivar, was used as control of the transformation.

MATERIALS AND METHODS

Explants
We used sterilized immature embryos of Javanica rice cultivar: Batutegi and of Indica rice culivar, Kasalath-as the control.

Plasmid and Agrobacterium
Recombinant pMOG22 plasmids containing Ac/Ds element were obtained from Dr. Andi Pereira (Plant Research International), Wageningen University, Netherland. Plasmids were confirmed by restriction using BamHI and HindIII enzymes and were cloned in E. coli strains DH5α. The plasmids then were transfered to Agrobacterium tumefaciens strains EHA105 and were confirmed by restriction. Figure 1 describes the construction of pMOG22

![Plasmid construction of pMOG22](image)

Figure 1. Plasmid construction of pMOG22. Selection markers in transformation are hygromycin (HYG) gene and green fluorescent protein (GFP) gene. Activation tag (4Enh) is carried by Ds transposon element (ADE) which can be transposed by the presence of Ac element (AcTPase)

Transformation
Infection Suspension
A suspension of 3-day-old Agrobacterium EHA104::pMO22 on solid LB medium containing 20 mg/L rifamycin and 50 mg/L kanamycin at 28 ºC we used to make infection suspension. The cells were mixed with AA-infection medium contained 0.1 M
acetylsyringone. The density of the mixture (infection suspension) was $3 \times 10^5 \text{ cfu/ml} \ (OD_{600} \sim 0.3$)

Co-cultivation

Both *Batutegi* and *Kasalath* were transformed based on modification of Hiei & Komari (2006) method.

*Batutegi*. The sterile seeds were minced with tweezers in order to get the immature embryos of the seeds out. The immature embryos were then placed -with scutellum side up- on NB medium containing 1 mg/L acetylsyringone and were soaked into a drop (5 µL) of infection suspension. The infected embryos were incubated in the dark at 25 °C for a week. *Kasalath*. After incubation at 25 °C for 3 days in the dark, Kasalath embryos were soaked into a drop of infection suspension and were incubated again in the similar condition for 5 days.

Selection and regeneration

After co-cultivation, elongated shoots were removed from both immature embryos and cultured on NBM medium containing 250 mg/L cefotaxime and 100 mg/L carbenicylin with the scutellum side up for a week at 30 °C under whitelight. Then for selection, the immature embryos were transferred to NBM medium containing 30 mg/l hygromycin and were cultured for 3 weeks at 30 °C. The hygromycin-resistant calli then being transferred in the same medium for 10 days. Calli derived from scutellum that clearly resistant to hygromycin were transferred and cultured on NBPR medium containing 40 mg/l hygromycin, 250 mg/l cefotaxime for 10 days at 30 °C. Calli with the green spots were transferred to and cultured on R05 medium 10 days at 30 °C. After the plantlets formed roots, they were transferred to and cultured on MS medium at 25 °C for 10-14 days, or until plantlets were ready to be transferred to the soil.

*PCR*

Isolated DNA genom of pMOG22, control plants leaves and putative transformant calli were analyzed by amplification of *hpt* gene with sequence of (5'-GATGCCTCCGCT-CGAAGTAGCG-3') as the forward primer and (5'-GCATCTCCGCGCGTGAC-3') as the reverse primer. The PCR was amplificated for 30 cycles.
RESULTS AND DISCUSSION

Transformation

Modification on Hiei method (2006) focusing on infection and co-cultivation day has aim to get the optimal result of transformants' number. Infection of agrobacterium gave effect on the weakness of immature embryos and lead to necrosis and cell death. Delaying infection until 4 days after embryos had been isolated or shortening the co-cultivation day from 7 days into 4 days, the embryos tissue could survive and regenerate. This modification was conducted for Kasalath only.

Table 1. Cycling conditions used for *hpt* gene amplification

<table>
<thead>
<tr>
<th>Step</th>
<th>Temperature(°C)</th>
<th>Minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>denaturation</td>
<td>95</td>
<td>5</td>
</tr>
<tr>
<td>denaturation</td>
<td>95</td>
<td>1</td>
</tr>
<tr>
<td>annealing</td>
<td>55</td>
<td>1</td>
</tr>
<tr>
<td>extension</td>
<td>72</td>
<td>1</td>
</tr>
<tr>
<td>extension</td>
<td>72</td>
<td>10</td>
</tr>
<tr>
<td>Storing</td>
<td>4</td>
<td>∞</td>
</tr>
</tbody>
</table>
Figure 2. Description of immature embryo transformation steps in development of mutant library carrying transposons Ac/Ds
a. Rice immature embryos on NBAs medium with scutellum side up
b. Infection of rice immature embryos by soaking into a drop of Agrobacterium EHA105::pMO22 suspension
c. Pre-selection step on NBM (resting culture)
d. Embryogenic calli on hygromycin selection medium
e. Embryogenic calli with greenspots in preregeneration step
f. Shoot development in regeneration step
g. Plantlet in MS medium

Table 2. Transformation data of Kasalath and Batutegi Cultivar

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>event</th>
<th>∑ of immature embryo</th>
<th>∑ of selected calli</th>
<th>Efficiency of Transformation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kasalath</td>
<td>1</td>
<td>50</td>
<td>1</td>
<td>5%</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>69</td>
<td>14</td>
<td>20.3%</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>32</td>
<td>14</td>
<td>43.75%</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>12</td>
<td>6</td>
<td>50%</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>33</td>
<td>11</td>
<td>33.33%</td>
</tr>
<tr>
<td></td>
<td>∑=196</td>
<td>∑=46</td>
<td></td>
<td>average=30.47%</td>
</tr>
<tr>
<td>Batutegi</td>
<td>1</td>
<td>109</td>
<td>10</td>
<td>9%</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>127</td>
<td>2</td>
<td>1.5%</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>331</td>
<td>46</td>
<td>13.9%</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>78</td>
<td>5</td>
<td>6.4%</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>130</td>
<td>16</td>
<td>12.3%</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>22</td>
<td>7</td>
<td>31.8%</td>
</tr>
<tr>
<td></td>
<td>∑=797</td>
<td>∑=86</td>
<td></td>
<td>average=12.49%</td>
</tr>
</tbody>
</table>

Transformation efficiency can be calculated after the hygromycin selection by media selection had been done. The latest data of transformation efficiency (Table 1) shows that we had 46 proliferating embryogenic calli for Kasalath and 86 for Batutegi. Kasalath transformation efficiency (30.47%) is larger than Batutegi (12.49%). So far, we had obtained 2 plantlets from Kasalath and no plantlet from Batutegi. Kasalath is one of Indica landrace model plant which has good response on tissue culture and transformation, but not as Batutegi which is difficult to be transformed and having low response on tissue culture. Probably, Batutegi needs longer time to regenerate than Kasalath does. Some modification in hygromycin and hormon contents in media might be needed to shorten the time of regeneration.
**hpt gene amplification**

We use hpt (hygromycin phosphotransferase) gene as selection marker. The calli which survive to grow on selection media can be predicted as hygromycin tolerant calli and as putative transformants. To prove that the transformation or the insertion is succeeded, we amplify the hpt gene of the survived calli. We use the pMOG22 as the positive control and wild type leaves negative control. The electroforegram of the amplification (Fig. 3) shows that the 492 bp hpt band could be amplify in survived transformant calli of Kasalath and Batutegi. It means that the insertion of activation tag is succeeded.

**CONCLUSION**

We conclude that pMOG22 is successfully inserted into Kasalath and Batutegi because the 493 bp of hpt gene can be amplified from the Kasalath and Batutegi transformation. Thus, we assume that study in the development of rice mutant population of an Indonesian drought tolerant rice cultivar (Batutegi) can be conducted by inserting activation tag with modification of Hiei method.

**REFERENCES**


O-MB23

STUDY ON THE INTERACTION BETWEEN OsKANADI1 AND A FLORIGEN Hd3a IN RICE

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ABSTRACT

Transition from vegetative phase to flowering involves many genetic pathways that interact with the external signals, such as day length and temperature, and internal signals such as hormones and developmental controls. In order to understand the mechanism of various signals leading to flowering, our study has been focused on studies of genes in rice flowering.

Recently, the nature of Hd3a protein in rice and its ortholog FT in Arabidopsis as a florigen has been proposed. However, molecular mechanism of its function remains to be investigated. Therefore, it is important to search their interaction partners to better understand their signaling in flowering. As a long-distance signal that moves along leaf cells and the vascular system of leaves and stem and exerts its action in apical buds located far from the leaf, it is important to determine the possible mediators of such common responses activated by Hd3a. Therefore, we have performed yeast two-hybrid screening to search Hd3a partners by using a cDNA library from rice leaf blade harvested 35-40 days after sowing (at ZT 0.2 and 4) under SD conditions when the transition from vegetative phase to reproductive phase occurred and Hd3a was highly expressed.

We identified OsKANADI1 as an Hd3a interacting protein. Results of study on the interaction between Hd3a and OsKANADI1 using yeast two hybrid assay and in vitro pull down confirmed their interaction.

Keywords : OsKANADI1, Hd3a, protein interaction, florigen, rice.

INTRODUCTION

Flowering is a critical stage in higher plant growth and reproduction, and is controlled by both environmental and endogenous conditions. One of the most important factors that control flowering is the plant’s response to daylight or photoperiod (Imaizumi and Kay, 2006). Arabidopsis thaliana and Oryza sativa are used as models to study the regulation of flowering time in long-day plant (LDP) and short-day plant (SDP), respectively. Three genes which constitute the major genetic pathway in the photoperiodic regulation of
flowering in rice have been isolated: OsGI (O. sativa GIGANTEA), an ortholog of Arabidopsis GI, Hd1 (Heading date 1), an ortholog of Arabidopsis CO (CONSTANS), and Hd3a (Heading date 3a), an ortholog of Arabidopsis FT (FLOWERING LOCUS T). Although these three genes are conserved between rice and Arabidopsis, differences in their regulation result in either SDP or LDP. The major difference between rice, an SDP, and Arabidopsis, an LDP, is in the regulation of Hd3a/FT by Hd1/CO. Under LD conditions, this regulation is positive in Arabidopsis but negative in rice (Hayama et al., 2003).

Hd3a was first identified as a quantitative trait locus (QTL), which promotes flowering of rice under SD conditions (Yamamoto et al. 1998, Monna et al. 2002). Overexpression of Hd3a protein with the constitutive promoter (Kojima et al. 2002) or vascular specific promoters (Tamaki et al. 2007) results in an early-flowering phenotype, and suppression of Hd3a with RNA interference (RNAi) delays flowering (Komiya et al. 2008). Hd3a is a member of a large gene family consisting at least 13 genes in rice genome (Chardon et al. 2005) and at least two paralogs, RFT1 (Rice FLOWERING LOCUS T1) and FTL (FT-Like)(Izawa et al. 2002, Komiya et al. 2008).

FT encodes a ~23-kDa protein whose sequence is similar to Raf kinase inhibitor protein (RKIP) and phosphatidylethanolamine binding protein (PEBP). It is a powerful promoter of flowering, activating the floral meristem identity gene AP1 (APETALA1), and is the target of several pathways that control flowering time (Simpson, 2003). The crystal structures of the Hd3a orthologs FT and TERMINAL FLOWERING LOCUS 1 (TFL1), an antagonist in Arabidopsis, showed no specific domain but revealed a potential binding pocket that is believed to specify interaction with their partners (Ahn et al. 2006).

Most recently, the nature of Hd3a and its ortholog as a florigen, a mobile flowering signal, has been proposed (Tamaki et al. 2007, Corbesier et al. 2007, Jaeger and Wigge et al. 2007, Mathieu et al. 2007, Lin et al. 2007, Notaguchi et al., 2008, Tsuji et al. 2008). The next question that should be addressed is the mechanism of Hd3a function. One important step in the characterization of Hd3a function is to identify other proteins with which it interacts. We focus here on searching for Hd3a interacting proteins using yeast two-hybrid screening, which has been widely used to identify protein-protein interactions. In this study we have performed a yeast two-hybrid screen to search for Hd3a partners and identified a transcription factor, designated as OsKANADI as an Hd3a interactors. The interaction between Hd3a and OsKANADI1 has been confirmed using several methods.
MATERIALS AND METHODS

Yeast two-hybrid screen

Independent clones (~1.6 x 10^6) were screened for interaction with Hd3a. Double transformants were spotted on SC+His, SC-His and SC-His plus 2.5 mM 3-AT. Clones that showed the activation of His3 (histidine synthase), therefore able to grow in SC media without His and containing 3-AT were selected, and DNA was isolated according to Zymo Research’s protocol. To retrieve the positive interactors, the DNA isolated from yeast was electroporated into Escherichia coli, and DNA minipreps was performed (Invitrogen, Carlsbad, CA). The resulting plasmid were retransformed into yeast strain L40 and the DNA was isolated from yeast. The DNA was amplified using forward and reverse primer, 5’AGTTTGACGAGATGGTTACC3’ and 5’CGACGTTGAAAACGACGGCCAGT3’, respectively. The PCR products were purified according QIAGEN’s protocol for sequencing reactions. The resulting sequences of the cDNA clones were BLAST searched against the Rice Annotation Project Data Base/RAP-DB (http://rapdb.dna.affrc.go.jp/) and NCBI database (http://blast.ncbi.nlm.nih.gov/).

A swapping experiment, using full-length constructs of OsKANADI1 and Hd3a either as bait or as prey, was performed. Interactions were tested on SC medium lacking histidine (-H) or lacking histidine and containing 2.5 mM 3-AT.

In vitro pull down assay

Hd3a and OsKANADI1 were cloned into pMAL-c4X and pDEST17 vectors (Invitrogen), respectively. GST, MBP-Hd3a and GST-OsKANADI1 were expressed in E. coli BL21 (DE3) and purified with glutathione-agarose and Amylose resin high flow (New England Biolabs) columns according to the manufacturers’ instructions. In vitro binding assays were performed as follows. The concentration of each fusion protein was determined by Coomassie staining. The equal amount of GST-OsKANADI1 protein coupled to glutathione sepharose 4B beads and MBP-Hd3a were incubated in TEDM buffer. The beads were then washed four times with binding buffer. Bound proteins were eluted in 1x SDS sample buffer by boiling for 5 min, separated by 10% SDS-PAGE, transferred to PVDF membrane and subjected to western blotting with -MBP antibody (New England Biolabs).
RESULTS AND DISCUSSIONS

One important step in the characterization of Hd3a function is to identify other proteins with which it interacts. We focus here on searching for Hd3a interacting proteins using yeast two-hybrid screening, which has been widely used to identify protein-protein interactions.

OsKAN1 is a novel transcription factor that interacts with Hd3a in yeast two-hybrid screening

In a yeast two-hybrid screen, we identified a myb transcription factor-like protein as an Hd3a interacting protein in a BLAST search, and designated it as OsKAN1 because of its amino acid similarity with other KANADIs in Arabidopsis within the GARP domain (Table 1).

Table 1 Isolation of OsKAN1, an Hd3a interacting protein, in a yeast-two hybrid screen

<table>
<thead>
<tr>
<th>Number</th>
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<td>IV-B1</td>
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<td>OsKANADI1/ GARP domain</td>
<td>147</td>
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<td>VI-G2</td>
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<td>VI-A3</td>
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*Length in amino acids

Analysis of Hd3a interacting proteins in yeast two-hybrid screening identified a novel putative transcription factor belonging to the KANADI domain protein family, namely OsKANADI1 (OsKAN1). In Arabidopsis, KANADI genes function in lateral polarity in organs including roots, leaves and flowers. KANADI is required for abaxial identity in both leaves and carpels. It encodes a nuclear-localized protein in the GARP family of putative transcription factors (Riechmann et al. 2000). GARP homologs constitute a large family of DNA-binding proteins in plants that may be needed for a variety of key cellular functions including regulation of transcription, phosphor transfer signaling and differentiation. A GARP motif was also found in the identified KANADI product (Kerstetter et al. 2001).

Hd3a and OsKAN1 interact in vitro and in the yeast system

Only partial fragment the C-terminal region (220-364) was identified from the initial yeast-two hybrid screen. An experiment using full-length Hd3a and full-length OsKAN1, either as bait or as prey, further confirmed that Hd3a and OsKAN1 interact in the yeast system (Fig. 1).

To further study the interaction between Hd3a and OsKAN1 in vitro, we performed a GST pull-down assay. A GST-OsKAN1 fusion protein was pulled down with MBP-tagged
Hd3a, as shown in Fig. 2, indicating that Hd3a interacts with OsKAN1 *in vitro*. Results of this experiment were thus consistent with the results of the yeast two-hybrid experiment.

**Figure 1.** Identification of OsKANADI1 as an Hd3a interacting protein in yeast. The swapping experiment using full-length OsKANADI1, either as bait or prey suggested a strongly interaction with Hd3a. The growth of yeast colonies on the plates lacking histidine (-H) or lacking histidine plus 2.5 mM 3-aminotriazole (3-AT) indicates a positive interaction between Hd3a and GF14c. Two independent clones used as replication.

**Figure 2.** *In vitro* interaction of Hd3a with OsKANADI1. (A) Hd3a-MBP interacts with GST-OsKANADI *in vitro*. Hd3a-tagged MBP was pulled down by OsKANADI1-tagged GST. Hd3a was detected with an a-MBP antibody.
Few reports have been published about the function of KANADI family members in rice (Luo et al. 2007, Yan et al. 2008) and maize (Candela et al. 2008). RL9 (Rolled leaf 9) was identified by map-based cloning in two rice allelic rolled-leaf mutants which displayed very similar phenotypes, with completely adaxialized leaves and malformed spikelets. RL9 encodes a GARP protein, and is an ortholog of Arabidopsis KANADIs. Therefore, further studies of KANADI family members in rice and their interaction with Hd3a will contribute to assessment of the possible function of OsKANADIs in Hd3a signaling.

ACKNOWLEDGEMENT

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O-MB24

EXPRESSION OF PARTHENOCARPIC GENE, *DefH9-iaaM* ON TRANSGENIC TOMATO LINES

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Tomato (*Lycopersicon esculentum*) is an important vegetable in Indonesia. The demand of this vegetable always increase year to year but the supply is not enough by the farmer. The productivity of this crop is still low compared to the global tomato productivity. One of the problem is that tomato only suitable for highland. Tomato plant is very sensitive to high temperature so it's not suitable for lowland. The development of tomato cultivars that suitable for lowland is very needed. The development of tomato cultivars suitable for lowland have been conducted by using the parthenocarpic technology. Some transgenic parthenocarpic tomato lines have been developed through genetic transformation using *Agrobacterium* binary vector that contained the parthenocarpic gene, *DefH9-iaaM*. Those transgenic tomato lines were then further evaluated in biosafety containment greenhouse in ICABIOGRAED, Bogor. The evaluation were included molecular and phenotypic analysis to see the stability and expression of the parthenocarpic gene on tomato lines. Result indicated that all tomato lines were grown very well in the greenhouse. They still contained the parthenocarpic gene according to Polymerase chain reaction analysis and they showed parthenocarpic phenotypic, such as better fruitsetting and seedless. Three tomato lines showed better performance, i.e OvR1#14-4, OvM2#10-1 and OvM2#6-2. Further molecular analysis using Real Time PCR, those lines expressed the parthenocarpic gene (*iaaM*). Lines OvR1#14-4 showed highest gene expression, followed by OvM2#10-1 and OvM2#6-2. These lines also showed better expression of parthenocarpic gene. This year, these three transgenic tomato lines will be evaluated in the confined field trial for stability and productivity analysis.

**Keywords**: transgenic tomato, gene expression, parthenocarpic gene, defh9-iaaM.

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O-EC01

LOW BIOMASS OF MACROBENTHIC FAUNA AT A TROPICAL MUDFLAT: AN EFFECT OF LATITUDE?

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The macrobenthic animal biomass of the intertidal area of the Sembilang peninsula of South Sumatra, Indonesia, has been studied in 2004. Monthly (March – August) 21 core samples were taken at each of six sampling stations. Macrobenthic fauna was identified at the lowest taxonomical level possible and counted. Biomass was measured as ash-free dry mass (afdm). The average biomass over all stations and months was 3.62 g afdm m⁻², the highest biomass (47.45 g afdm m⁻²) found at a station in one month was due to abundant occurrence of the bivalve Anadara granosa. Low biomass of macrobenthic fauna at Sembilang peninsula cannot easily be explained but is in line with low biomasses found elsewhere in the tropics. For that reason we analyzed a data set of 268 soft-bottom intertidal biomasses collected world-wide to look for a relationship with latitude. It was shown that average biomass of intertidal macrobenthic fauna in the tropics was significantly (p<0.05) lower than that at non-tropical sites. A significant second-order relationship between biomass of macrobenthic fauna and latitude was established.

Keywords: biomass, macrobenthos, intertidal fauna, tropics, Indonesia, Sembilang

not presented
O-EC02

DENSITY AND BIOMASS OF THE MACROBENTHIC FAUNA OF THE INTERTIDAL AREA IN SEMBILANG NATIONAL PARK, SOUTH SUMATRA, INDONESIA

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Intertidal area of the Sembilang peninsula of South Sumatra, Indonesia had been studied in 2004. Three replicated transect lines were located at each of six sampling stations. One transect line consisted of randomly 7 cores sampling monthly. Macrobenthic fauna was identified at the lowest taxonomical level, and counted. Biomass was measured as ash free dry weight. The data compiled by applying statistical analyzing. Stations and months variables showed significant difference. The most abundance of macrobenthic animals found was bivalve Tellina timorensis. Biomass of the total macrobenthic fauna was not significantly different over the 5 months sampling period. However, Anadara showed the highest biomass value.

Key words: biomass, macrobenthos, intertidal fauna, Sembilang, Indonesia

not presented
O-EC03

SOIL ENZYMATIC ACTIVITIES OF NATURAL FOREST IN PERMANENT PLOT OF LOW LAND NATIONAL PARK “GUNUNG GEDE PANGRANGO”

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ABSTRACT

In general, natural forest is a region that has relatively high plant diversity. The diversity of plants affect the diversity of organic and inorganic materials produced in soil. Consequently each plant will have influence with other plants and soil microorganisms diversity depending on the organic material produced and nutrition needs of plants. Diversity of organic materials will impact on the diversity of activities of microbes in the soil because microbes produce specific enzymes depending on the available organic material in soil. This study aims to determine the relationship urease enzyme activity, selulase, and fosfomonoesterase, with local forest conditions. Forest habitat that is studied in this research is the permanent plot of forest in Gunung Gede Pangrango on the altitude of 718-802 m above sea level, 1 Ha plots was divided into 100 subplots, with the order of 10 vertical plots A to J direction of height (slope) and the horizontal subplot (1-10) with the large of each plot 10 m². From the horizontal plot, 5 plots were observed (one plot sampling interval), so the total samples were 50 sampling sites. Fifty samples were analyzed for the rate of soil respiration, activity of fosfomonoesterase, urease and selulase. The Results showed that the existence of the tree in the forest of Gunung Gede National Park Pangrango not influenced by the topographic slope of land. Respiration activity which is a reflection of soil microbiological activities, are determined by the slope of the land environment, especially effected by the intensity of light. The highest respiration activity was 4048 mg CO₂.h⁻¹.g⁻¹ in the top of the plot and the lowest activity was in the lowest plot experiment. The highest urease enzyme activity was in the plot of the lower part with the ranges about 111,764 units.g⁻¹ to 126,6619 units.g⁻¹. Data indicate the actual organic soil have more important role on the urease activity compared to the influence of high light intensity on the ground. Similarly was observed in the PMEase and selulase activity.

Key words: Soil enzymatic activity, natural forest, Pangrango National Park
INTRODUCTION

Soil microbes, such as fungi and bacteria have the different population density and types in each environment. Microbial biomass as a component of life in soil organic matter, there is a direct interaction through the exchange of plant nutrients and its cycle. At the direction of flow of nutrients occurs some time parasitism by microbial patterns, whereas other conditions prevailing in a symbiotic relationship [1]. In this population, developing a symbiotic microbes are more dominant than the microbial parasites.

Dynamics of soil microbial biomass has a direct relationship with the vegetation on it environment and form a system of related eco-physiological metabolic status of microbial biomass [1,2,5]. Therefore, the dynamics of soil microbes is important for plant growth. Application of microbes in the soil to produce enzymes, that can overhaul the organic material outside the cell. Enzymes produced by microbes have an important role in the provision of plant nutrients for enzymes involved in the nutrient cycle in plant soil systems. Nutrient cycling can be viewed through microbial activity which is reflected in the activity of enzymes found in the soil.

Microbes produce enzymes that have an important role in providing nutrients for plants is directly involved in the nutrient cycle in plant soil systems. Enzyme urease change organic nitrogen into inorganic nitrogen to hydrolyze urea into ammonia and carbon dioxide [4]. Phosphatase enzymes play a role and accelerate the production of organic orthophosphate P [5]. Phosphomonooesterase is one of the phosphatase group of enzymes that play a role in phosphate ester bonds hydrolyze in soil that is available in various forms of phosphorus that is needed by plants.

This study aimed to determine the relationship soil respiration rate, urease, cellulase, and fosfomonoesterase enzyme activity, with plants and its habitat. Studies on plant diversity effects on enzyme activity was carried out in permanent plots of natural reserve of Mount Gede, Pangrango National Park which has approximately 822 m above sea level. Administratively, the location of field study was in the village of Cicurug, Sukabumi District. This research was a joint project with several field of research in Indonesian Institute of Sciences (LIPI) include botany, zoology and microbiology with the total of experimental area of 1 ha.

MATERIALS AND METHODS

Soil Sampling

Soil samples were taken from permanent plots of lowland forest of Mount Gede Pangrango. The total large plot was 5000 m² and was divided into 50 experimental sub-
plots. From each experimental sub-plot, soil samples were taken as much as 1 kg from 5 sampling points with about 15 cm depth and then mixed well. The collected soil sample were kept in cool box before transferred to deep freezer (-20°C). Prior respiration rate and soil enzyme activities measurement, the soil was conditioned air dried.

**Soil enzymes activity**

Soil respiration, Phosphomonoesterase, Urease and Celulase activity were measured as described by standard method [3].

**RESULTS AND DISCUSSION**

It has been suggested that enzymes produced by soil microbes soil is strongly influenced by environmental conditions (temperature, pH, humidity and other environmental conditions) and the type as well as population density of plants growing on the forest habitat (Andrea 2000).

**Respiration**

Soil respiration is due to the degradation process of organic materials in the soil. This process indicates the total soil microbial activity, which plays an important role in the biological process. From the results of measurements of respiration, the highest microbial activity is in plot A of 4048 mgCO$_2$/g/h and activity gradually decreased to the lowest plot (plot J, 0.314422 mgCO$_2$/g/h). Here was respiration rate from the plot A to plot J respectively 4048 mg mgCO$_2$/g/h, 3366 mgCO$_2$/g/h, 2662 mgCO$_2$/g/h, 1562 mgCO$_2$/g/h, 0.288212 mgCO$_2$/g/h, 0.302247 mgCO$_2$/g/h, 0.29547 mgCO$_2$/g/h, 0.284333 mgCO$_2$/g/h, 0.301136 mgCO$_2$/g/h, and 0.314422 mgCO$_2$/g/h. Surprisingly the data indicate that the total Fungi and amount of organic material from plot to plot did not reflect to the soil respiration rate. It seemed that the respiration rate correlated with the intensity of sun light (open area). These respiration data were not correlated with other enzymes activities.

**Urease**

It was reported in our previous study that urease activity was not related to the condition respiration [2]. The highest urease activity was in the lower plot (plot of G, 163,877 unit/g). It was clear that urease activity from the plot A to plot E increased, different pattern with the measurement of respiration. However, statistical tests based on urease activity of the plot A to plot E was not significantly different with statistical tests on the results of analysis of organic material N in the soil samples (data not shown). Urease activity of the
plot A to plot E, respectively, 74.66762 unit/g, 64.63674 unit/g, 46.94919 unit/g, 45.98405 unit/g, and 58.01282 unit/g. Significant differences occurred between the five top plot with five bottom plot. The five bottom plots have a higher urease activity than the plot of the top five. In addition, urease activity seemed to be influenced by the number of soil microbes population. Urease activity of the plot until the plot M J, respectively, 111,764 unit/g, 163,877 unit/g, 145.8574 unit/g, 116.8091 unit/g, and 126.6619 unit/g. This proves that the C-organic and number of microbes population in the soil determine the outcome of this enzyme activity.

**Phosphomonoesterase (PMEase)**

Similar with urease activity, the activity of phosphomonoesterase was also not related with respiration rate but C organic and fungal population. However PMEase activity was not significant differences in the top five with five plots in the bottom. Plot A to plot E had very little activity and was not significantly different (0.183477 unit/g, 0.184231 unit/g, 0.176696 unit/g, 0.259581 unit/g, and 0.288212 unit/g). Thought the plot of F to J was not significantly different, but PMEase activity was much higher than the plot A to plot E (3.022472 unit/g, 2.954742 unit/g, 2.843332 unit/g, 3.011359 unit/g, and 3.144217 unit/g).

![Fig. 1: Celullase activity of soil of “Gunung Gede Prangango” permanent plot](image)

**Cellulase**

Cellulase is the enzyme, which degrades cellulose into glucose. Cellulase enzymes are water-soluble enzymes, but able to hydrolyze insoluble molecules such cellulose. Like the urease activity and PMEase activity, cellulase activity seemed to be strongly influenced by the amount of microbial populations in soil. The highest cellulase activity found on the plot E of 0.002816 unit / g. The results of this cellulase activity was relatively small compared to other enzyme activity, since cellulase is an enzyme that is difficult to be extracted. In this experiment cellulose was extracted from the soil but not from the litter.
(fallen leaves the ground). Cellulase activity as a whole was not significantly different. Only plot C, which has a higher cellulase activity than any other plot with the amount of 0.000658 unit/g. As for the other plots (except plot C) has a cellulase activity was not significantly different which were 0.00213 unit/g (A), 0.001868 unit/g (B), 0.002079 unit/g (D), 0.002816 unit/g (E), 0.002605 unit/g (F), 0.002447 unit/g (G), 0.002447 unit/g (H) 0.002711 unit/g (I), and 0.002071 unit/g (J).

Tabel. 1: Number of culturable soil fungi of “Gunung Gede Prangango” permanent plot

<table>
<thead>
<tr>
<th>No</th>
<th>Plot</th>
<th>CFU/gr</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A</td>
<td>69200</td>
</tr>
<tr>
<td>2</td>
<td>B</td>
<td>51000</td>
</tr>
<tr>
<td>3</td>
<td>C</td>
<td>44000</td>
</tr>
<tr>
<td>4</td>
<td>D</td>
<td>67000</td>
</tr>
<tr>
<td>5</td>
<td>E</td>
<td>50200</td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>60500</td>
</tr>
<tr>
<td>7</td>
<td>G</td>
<td>74750</td>
</tr>
<tr>
<td>8</td>
<td>H</td>
<td>98000</td>
</tr>
<tr>
<td>9</td>
<td>I</td>
<td>88000</td>
</tr>
<tr>
<td>10</td>
<td>J</td>
<td>92000</td>
</tr>
</tbody>
</table>

REFERENCES


O-EC04

THE DISTRIBUTION PATTERN AND ABUNDANCE OF ASTEROID AND ECHINOID AT RINGGUNG WATERS SOUTH LAMPUNG

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* Department of Biology, Faculty of Mathematics and Natural Sciences, Sriwijaya University. 0818561648

ABSTRACT

The research of the distribution pattern and abundance of Asteroid and Echinoid was carried out in 28 June-11 July 2008 at Ringgung waters South Lampung. The aim of the research is to collect information about distribution pattern and abundance of Asteroid and Echinoid at Ringgung waters. The research was made by applying square transect method at five stations. The result was analyzed descriptively. The research showed that there were 2 species of Asteroid which consist of Archaster typicus and Culcita novaeguineae and 2 species of echinoid which consist of Diadema setosum and Aganum sp. with distribution patterns is clumped and the abundance of Asteroid and echinoid is about 0,2-5.7 ind/m2. The factors that influenced distribution pattern were differences habitat and living habits of each species.

Key words: distribution pattern, abundance, astroidea and echinoidea, ringgung waters

not presented
O-EC05

THE UTILIZATION OF THE SPACE FIELD BY SUNDANESE
AT RPH CIRangsad, BKPH JASINGA, KPH BOGOR, WEST JAVA

Atus Syahbudin 1), 3), Katsuya Osozawa 2), Ikuo Ninomiya 2), Dwi Tyaningsih Adriyanti 3), Sapto Indrioiko 3) and Mohammad Na’iem 3)

ABSTRACT

A test plantation is one of the conventional improvement techniques. It provides early important information to the selection program. Nevertheless, it needs a long time to gain conclusion and must guarantee in secure location. Limited field in KPH Bogor causes the selection process meet some obstacles. Recently, a part of the candidate location of experimental forest has been cultivated by Sundanese. The species types of Sundanese plantation can possibly influence to the safety of the experimental forest further. In order to know the kind of Sundanese plants and their utilization, this research was done on March and April 2006 at RPH Cirangsad, BKPH Jasinga, KPH Bogor, West Java, Indonesia. The study area which constitutes a candidate location located at the center of three villages belongs to Sundanese. The data were gained by making track and exploring. All targeted species were recorded, characterized, documented, sampled, and were then identified in the laboratory. In order to find out local community activities use cultivated plants, the observation and short interviews were also done. There were twenty four species in four groups according to main function cultivated by Sundanese. They are food plants (18 species), wood plants (3 species), fiber plant (1 species) and medicinal plants (2 species). Perennial tree dominated by fruit species, cash crop, building material and fuel wood which scatter in many locations. While annual species consist of medicinal plant and vegetables by sugarcane plantation as dominated species. Therefore, the determination of experimental forest location should select areas which have annual species or minimum in perennial tree in order to minimize the risk of the failure of experimental forest establishment further and land preparation cost. The utilization of location that vegetation appears due to succession is another alternative in order to avoid local community conflict.

Keywords: space field, Sundanese, utilization, experimental forest, plants species.

INTRODUCTION

A test plantation is one of the conventional improvement technique. This program be able to provide early information that is very important to the selection program further. Nevertheless, the conventional improvement usually needs a long time to gain a conclusion and must guarantee in secure location (Anonim, 2000; Charrier et al., 2001; Anonim, 2002). In 2005-2007 and continue until 2010, Faculty of Forestry, Universitas Gadjah Mada (UGM)
Yogyakarta and Bogor Agricultural University (IPB) are working together with Ministry of Forestry, Republic of Indonesia and Korean Forestry Services to establish experimental forest which covers progeny test, demonstration plot, seed orchard, species trial, and coppice garden in three locations. RPH Cirangsad, BKPH Jasinga, KPH Bogor, Perum Perhutani, West Java is one of them (Anonim, 2008).

The nominated location of experimental forest ideally is a plain and wide field without endanger activity both by Perum Perhutani and local community for a certain time. Limited field in KPH Bogor actually cause the selection process meet obstacles. Most of locations had been previously established company plantations, but partly unsuccessful and due to limited budget, they were neglected and then used by Sundanese (Asep and Bambang, 2006). The existence of Sundanese cultivated species can possibly influence to the safety of experimental forest further. This research aimed to know the kind of Sundanese plants within candidate location and their utilization. It will be important as a basic information to the next study and the continuation of experimental forest.

MATERIALS AND METHODS

This study constitutes field research which used materials such as notebooks, compass, pen, pencil, digital camera, list of interview, cigarettes, snack, and collection equipment, like sasak, sasak belt, newspaper, alcohol 70%, sprayer, branch scissor, and label. Whereas, in order to make herbarium and identification were used oven, wavy alunium, ivory and casing paper, tread, tape, glue, black pen, and identification book (Pudjoarinto, 1984; Martin, 1998; Hardin et.al., 2001).

The study location was in compartments 29 and 30, RPH Cirangsad, BKPH Jasinga, KPH Bogor, Unit III Perum Perhutani of West Java (Figure 1a). RPH Cirangsad actually has 13 compartments with 3,231.90 hectares; lays between 92º80’134”–92º82’079” East Longitude and 67º19’78”–67º33’15” South Latitude; and located at the center of three villages belong to Sundanese, namely Cibugis, Cirangsad, and Panggeleseran (Supriadi, 2005). Compartments 29 and 30 have hilly topography and 151.7 hectares in wide (Figure 1b), then will be selected into 84 hectares for candidate location of experimental forest which cover progeny test, demonstration plot, seed orchard, and species trial (Anonim, 2008).

According to early study and company data (anonym, 2006), these two compartments consist of three types of vegetation condition. First, company plantation that successful growing or remaining. Such as mangium (Acacia mangium), pulai (Alstonia scholaris), and big leaf mahogany (Swietenia macrophylla). Second, vegetation that appears due to succession. It commonly occurs after unsuccessful company plantation and local Sundanese
do not cultivate the location yet. It was recorded that there is difference in succession phase, but this study do not research about the vegetation composition in each phase. Third, vegetation which be cultivated by Sundanese to satisfy their need. Most of their distribution were observed in the edge of compartment, main way, sub-main way or closed to canal or irrigation. Whereas these locations suitable for experimental forest where requires high accessibility for maintenance and show window (Anonim, 2000; Charrier et.al., 2001; Anonim, 2002). This occurs because of limited budget for replanting unsuccessful company plantation previously. Finally, these appropriate areas became neglected and then planted by Sundanese (Asep and Bambang, 2006).

This study gained the data only in the third type of vegetation condition by making track and exploring method (explore each side of location for gaining the data) (Rugayah et.al., 2004). Some certain tracks with 20 m in broad were observed with accompanied by two staff of RPH Cirangsad (Figure 2). All targeted species were recorded, characterized, documented, sampled to assist in their identification, and were then identified in the Laboratory of Dendrology, Faculty of Forestry, UGM (Bridson and Forman, 1999; Hardin et.al., 2001; Khrisnamurty, 2003; Singh, 2004; Tjitrosoepomo, 2005).

![Map](image1.png)

Fig. 1: The map of candidate location of experimental forest, RPH Cirangsad, BKPH Jasinga, KPH Bogor, (within green line) scale 1:10,000 (map source: Anonim, 2006) (a) and its landscape and topography (b)

In order to find out local community activities use cultivated plants, the observation and short interviews were done (Abbas et.al., 1995; Mathias, 1995; Martin, 1998). Beside to Sundanese who work in the field, interviews were also done with the key informants who
responsible for PHBM in RPH Cirangsa, namely Mr. Usup and Mr. Asep. PHBM is a program designed to solve the land problem around the plantation area due to involving of the local community participating on the forest establishment.

Fig. 2: Black line constitutes some certain tracks of the study area, 20 m in broad where the data were gained

RESULTS AND DISCUSSION

Twenty four species have been identified as Sundanese plantation within the candidate location of experimental forest in RPH Cirangsa, BKPH Jasinga, KPH Bogor, Perum Perhutani of West Java (Table 1). They are grouped based on their main function.

Food plants

Food plant not only provides energy in the form of carbohydrates, proteins, and fats, but also minerals and vitamins (Arnett and Braungart, 1970). Figure 3-18 show some food plants were documented in the study area. Rice (Oryza sativa) is cultivated in the valley where water canals or irrigation available. While fruit trees, like mangosteen, mango, avocado, guava, and water apple are generally planted near huts in the field. Karyono in Christiany et.al. (1986) approved these conditions. Normally field with rice are dominated by fruit plants and which non-rice planted by tubers crops, like cassava, ganyong, gembili, sweet potato, etc. Banana and jengkol constitute the most common cultivated trees. Banana trees grow in the edge of canal or irrigation and also cultivated in a part of the slope (Figure 14). Sundanese consume them themselves or sell in the local market, for instance in Cigudeg. While jengkol is daily dish of raw vegetables; and commonly mixed with chili in
order to raise appetite of eating (Suriawinia, 1987). This species included multipurpose tree species (Jensen, 1995).

Vegetable cane (*Saccharum edule*) is the most dominated Sundanese vegetable which grows at the slope of study area. They do not require intensive watering and very suitable to grow in dry season. Differ with Soreang and Ciwidey in Southwest of Bandung where lablab beans (*Dolichos lablab*) and cucumber become the major crops and Majalaya in Southeast of Bandung, they are tobacco and onion (Soemarwoto, 1984), it can say that vegetable cane become the major crop in the Cirangsad. Vegetable canes used for self consumption and traded to other cities in West Java. In other hand, the jackfruit, durian, and coffee can also be sold to local brokers or merchants for adding income. While guava, water apple, mango, kuwini mango, mangosteen, katuk and tomato were harvested for self consumption.

**Wood plants**

Differences in the cell structure and in the amount of gum and resin in the wood determine the quality of the wood and the use to which it is put (Arnett and Braungart, 1970). There are three wood plants were found, namely sengon (*Paraserianthes falcataria*), manii (*Maesopsis eminii*) and Chinese guger tree (*Schima wallichii*) (Figure 19-21). Sundanese will wait the wood plant in certain diameter base on utilization (Asep and Bambang, 2006). Afterward, they can use it themselves or sold it in Cigudeg Subdistrict, about 9 km or 0.5 hour by local transportation (Figure 22). Some sawmills then process it become some products, like board, fuel wood, etc. (Figure 23).

Table 1. Plant species which cultivated by Sundanese within the candidate location of experimental forest, RPH Cirangsad, BKPH Jasinga, KPH Bogor, Perum Perhutani of West Java

<table>
<thead>
<tr>
<th>No.</th>
<th>Scientific names</th>
<th>Family</th>
<th>Common names</th>
<th>Main function *)</th>
<th>Main function **)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Oryza sativa</em></td>
<td>Poaceae</td>
<td>Rice</td>
<td>Food plants</td>
<td>Food crops</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(18 species)</td>
<td>(2 species)</td>
</tr>
<tr>
<td>2</td>
<td><em>Manihot esculenta</em></td>
<td>Euphorbiaceae</td>
<td>Cassava</td>
<td></td>
<td>Vegetables</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(6 species)</td>
</tr>
<tr>
<td>3</td>
<td><em>Arachis hypogaea</em></td>
<td>Fabaceae</td>
<td>Peanut</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td><em>Saccharum edule</em></td>
<td>Poaceae</td>
<td>Vegetable cane</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td><em>Pithecellobium jiringa</em></td>
<td>Fabaceae</td>
<td>Jengkol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td><em>Dolichos lablab</em></td>
<td>Fabaceae</td>
<td>Lablab bean</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td><em>Sauropus androgynus,</em></td>
<td>Phyllanthaceae</td>
<td>Katuk</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td><em>Solanum lycopersicum</em></td>
<td>Solanaceae</td>
<td>Tomato</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td><em>Durio zibethinus</em></td>
<td>Bombacaceae</td>
<td>Durian</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td><em>Musa paradisiaca</em></td>
<td>Musaceae</td>
<td>Banana</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

204
<table>
<thead>
<tr>
<th></th>
<th>Species</th>
<th>Family</th>
<th>Common Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>Mangifera indica</td>
<td>Anacardiaceae</td>
<td>Mango</td>
</tr>
<tr>
<td>12</td>
<td>Mangifera odorata</td>
<td>Anacardiaceae</td>
<td>Kuwinin mango</td>
</tr>
<tr>
<td>13</td>
<td>Syzygium aqueum</td>
<td>Myrtaceae</td>
<td>Water apple</td>
</tr>
<tr>
<td>14</td>
<td>Psidium guajava</td>
<td>Myrtaceae</td>
<td>Guava</td>
</tr>
<tr>
<td>15</td>
<td>Artocarpus heterophyllus</td>
<td>Moraceae</td>
<td>Jackfruit</td>
</tr>
<tr>
<td>16</td>
<td>Garcinia mangostana</td>
<td>Gutteraceae</td>
<td>Mangosteen</td>
</tr>
<tr>
<td>17</td>
<td>Persea americana</td>
<td>Lauraceae</td>
<td>Avocado</td>
</tr>
<tr>
<td>18</td>
<td>Coffea robusta</td>
<td>Rubiaceae</td>
<td>Robusta coffee</td>
</tr>
<tr>
<td>19</td>
<td>Maesopsis eminii</td>
<td>Rhamnaceae</td>
<td>Umbrella tree</td>
</tr>
<tr>
<td>20</td>
<td>Paraserianthes falcataria</td>
<td>Fabaceae</td>
<td>Sengon</td>
</tr>
<tr>
<td>21</td>
<td>Schima wallichii</td>
<td>Theaceae</td>
<td>Chinese guger tree</td>
</tr>
<tr>
<td>22</td>
<td>Ceiba pentandra</td>
<td>Malvaceae</td>
<td>Kapok</td>
</tr>
<tr>
<td>23</td>
<td>Zingiber officinale</td>
<td>Zingiberaceae</td>
<td>Root ginger</td>
</tr>
<tr>
<td>24</td>
<td>Curcuma domestica</td>
<td>Zingiberaceae</td>
<td>Curcuma</td>
</tr>
</tbody>
</table>

Note: *) Arnett and Braungart (1970); **) Hadikusumah in Christiany *et al.* (1986)

**Fiber plants**

Fiber plant is essential to many manufacturing processes. After suitable chemical treatment these species also sources of rayon, paper, cellulose, lacquers, cellophane, and many other products used in modern industry (Arnett and Braungart, 1970). There is only one species found in the candidate location (Figure 24), that is kapok (*Ceiba pentandra*). This species recorded only one grows in the edge of main way.

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**Fig. 3:** Rice (*Oryza* sp.) and huts in the field

**Fig. 4:** Rice is naturally drying in the village

**Fig. 5:** Cassava (*Manihot esculenta*)
Fig. 6: Peanut (Arachis hypogaea)
Fig. 7: Vegetable cane (Saccharum edule)
Fig. 8: Local Sundanese harvesting vegetable cane

Fig. 9: Jengkol (Pithecellobium jiringa)
Fig. 10: Lablab bean (Dolichos lablab)
Fig. 11: Katuk (Sauropus androgynus)

Fig. 12: Tomato (Solanum lycopersicum)
Fig. 13: Durian (Durio zibethinus)
Fig. 14: Banana (Musa paradisiaca)

Fig. 15: Mango and kuwini (Mangifera indica, M. odorata)
Fig. 16: Water apple (Syzygium aqueum)
Fig. 17: Jack fruit (Artocarpus heterophyllus)
Medicinal plants

Medicinal plants have contributed greatly to the welfare of Sundanese. Roosita et al. (2008) reported that Sundanese villagers have depended heavily on herbal medicine. Medicinal plants were used in two-thirds of illness cases, either through the villagers’ self-treatment (60.9%) or by the healers (6.5%). In the study area, root ginger (Figure 25) and curcuma (Figure 26) are found and documented.

If compared to other Sundanese kebun-talun and homegardens (see Table 2), species number of Sundanese plantation in RPH Cirangsad approximately only 10-21%. Perennial tree dominated by fruit species, cash crop, building material and fuel wood which scatter in many locations. While annual species consist of medicinal plant and vegetables by sugarcane plantation as dominated species. Therefore, the determination of experimental
forest location should select areas which have annual species or minimum in perennial tree in order to minimize the risk of the failure of experimental forest establishment further and land preparation cost. The utilization of location that vegetation appears due to succession is another alternative in order to avoid local community conflict.

Table 2. The comparison of species number which planted by Sundanese between RPH Cirangsad and others.

<table>
<thead>
<tr>
<th>Main function of Plants</th>
<th>Number of species</th>
<th></th>
<th>Note</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RPH Cirangsad</td>
<td>Sadu Village *)</td>
<td>Bantarkalong **)</td>
</tr>
<tr>
<td>Food crops</td>
<td>2</td>
<td>5</td>
<td>14</td>
</tr>
<tr>
<td>Vegetables</td>
<td>6</td>
<td>15</td>
<td>29</td>
</tr>
<tr>
<td>Fruits</td>
<td>9</td>
<td>20</td>
<td>28</td>
</tr>
<tr>
<td>Cash crops</td>
<td>1</td>
<td>7</td>
<td>-</td>
</tr>
<tr>
<td>Building materials and fuelwood</td>
<td>4</td>
<td>47</td>
<td>10</td>
</tr>
<tr>
<td>Medicinal</td>
<td>2</td>
<td>5</td>
<td>21</td>
</tr>
<tr>
<td>Ornamentals</td>
<td>--</td>
<td>8</td>
<td>67</td>
</tr>
<tr>
<td>Spices</td>
<td>--</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Others</td>
<td>--</td>
<td>--</td>
<td>53</td>
</tr>
<tr>
<td>Total</td>
<td>24</td>
<td>112</td>
<td>228</td>
</tr>
</tbody>
</table>

*) Plant species in the home-garden and kebun-talun in Legokkole Hamlet, Sadu Village, West Java, Indonesia (Hadikusumah in Christanty et al. 1986) and **) Plant species in 37 Sundanese home-gardens in Bantarkalong, West Java, Indonesia (Abdoellah in Christanty et al., 1986)

REFERENCES


O-EC06

SOIL RESPIRATION RATE UNDER Shorea peltata ASSOCIATION IN TENGGAROH FOREST RESERVE, MERSING, JOHOR

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Soil respiration is a crucial process that regulates carbon cycling and climate change in the earth ecosystem especially in tropical forests. However, despite the large C efflux from soil in tropical forests there are few reports on soil respiration in tropical forests compared with other climate regions especially for Southeast Asia. This study was conducted to determine and to compare soil respiration rate on topographically distinct locations at Shorea peltata association endemic species in Mersing, Johor. Total of 1.25ha study area, consists of five main plots varies by topographically distinct locations that have been established in Tenggaroh Forest Reserve, Mersing, Johor. Five points per plot data of soil respiration characteristics recorded with five replications; all data and the environmental factor variables on air temperature, relative humidity, light intensity and wind speed were recorded and analyzed using Analysis of Variance. Topographically, soil respiration rate at the flat area is the maximum compared to other variable plots; North-East facing slopes in Net Carbon Exchange Rate and also for delta Carbon dioxide exchange rate; the approach is to compare each factor variables related with topographically distinct locations. Overall, the main influence factors on soil respiration were temperature and water based on the topographical plot. All of the information in the study can be used for future reference of tropical forest specifically for endemic species research on Shorea peltata association spatial needs of soil classification.

Keywords: Soil respiration, Shorea peltata, vegetation association, Endemic species, Malaysia

not presented
O-EC07

VEGETATION ASSOCIATION OF SHOREA PELTATA AS ENDEMIC TREE SPECIES AT LOWLAND DIPTEROCARP FOREST IN MALAYSIA

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Shorea peltata is one of an endemic species that has been found at lowland forest in Malaysia, where it is easy distinguish by its intermediate peltate leaves and growth lean to aside. Facing the challenges of this species, research on the vegetation association of Shorea peltata is needed and very important because its role as endemic species where the habitat is very limited. The importance of vegetation association is to describe the population of each species studied and how they relate to each other in the same community. The study plots was laid on site to record the distribution, density, sociability and coverage of plant species. Plant community was determined on the basis of Importance Value Index (IVI) and each was named after the foremost species. The tree species diversity and the evenness in the same plot are calculated using the Shannon-Weiner’s Index. A total number of 67 Families, 154 Genera and 414 Species are recorded. The major plant species recognized associated with Shorea peltata are Rourea rugosa, Rourea fulgens, Elatriospermum tapos, Hopea beccariana, Melanochyla sp. and Strychnos sp. The natural vegetation of the forest was mainly classified as Secondary Forest but high in density of trees with presenting 31 Families, 72 Genera and 193 Species with diameter up to than 5 cm and above. The value of Shannon –Weiner’s Index is 1.945, in term of richness. Due to the availability of high value endemic plant, this site was gazetted as High Conservation Valued Forests.

Keyword: Shorea peltata, endemic species, vegetation association, lowland Dipterocarp forest
O-EC08

HABITAT MANIPULATION IN THE RICE FIELD USING AQUATIC WEEDS FOR INCREASING THE AGROECOSYSTEM STABILITY

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ABSTRACT

The positive effect of some wild and weed plants in the rice field were considered for increasing the stability of its agroecosystem by their attractiveness for any natural enemies of rice pests. For those purpose the aquatic weeds i.e. Ipomoea aquatica F, Marsilea crenata P, Pistia stratiotes L, and their combination have been tested to attract some natural enemies of the rice pest both in laboratory and semi field experiment. In the experiment, the coccinellids Adalia bipunctata L, and Coccinella transversalis F, the syrphids Eristalis tenax L, and the sphecid Sphex maxillosus L were selected for their preference to those plants. For the laboratory test, the four arms olfactometer was used for the preference test of the natural enemies toward the plants. In the semi field experiment, the visual control method was conducted for the selected plants from the laboratory test. The results showed that Marsilea crenata P was the most attractive for the coccinellid and syrphid. The Pistia stratiotes L had the second rank but this plant was the most attractive for the sphecid. The combination of Marsilea crenata P with the other plants were not more attractive for the natural enemies tested than if the plant solely planted in field

Key words: weeds, natural enemy, rice, agroecosystem stability

INTRODUCTION

The positive effect of some wild and weed plants in the rice field that were considered for increasing the stability of its agroecosystem by their attractiveness for any species of earth worms and any natural enemies, were studied (Yanuwiadi 2000 a,b, Yanuwiadi et al. 2000, Yanuwiadi 2003, Sukaromah and Yanuwiadi 2006, Aini et al. 2006, Dewi et al. 2006, Yanuwiadi et al. 2009). Those plants provide a number of important resources for parasitoids and predatory arthropods such as permanent vegetation cover suitable for over-wintering, refuges from disturbance, as well as resources such as alternative prey, pollen and nectar (reviews in Landis et al., 2000; Cronin and Reeve, 2005; Bianchi et al., 2006).
Those natural enemies contributing significantly to pest control must have a rapid numerical response to herbivore density. For this to occur, they must either be present in the cultivated habitat, be easily recruited from nearby, or colonize from a larger distance. That is why, in the agricultural field, especially in non-crop habitat types such as hedgerows, field margins, fallows and meadows, are temporally more stable and heterogeneous environments than in the (annual, arable) crops types. Root (1973) stated that increasing plant diversity within agroecosystem will lead to higher predator abundance and diversity, and thus improved biological control. There is much evidence in the literature that habitat manipulation can increase densities of natural enemies (Barbosa, 1998; Landis et al., 2000; Pickett and Bugg, 1998), although the link between higher predator densities and improved pest suppression has been more difficult to establish (Andow, 1991; Bommarco and Banks, 2003).

Successful biological control efforts involve intentional alterations of important variables associated with consumer-resource interactions and induce trophic cascades in which certain natural enemies effectively reduce the abundance of particular pest organisms and thereby provide enhanced protection for crops or other organisms of benefit to man. Sometimes these efforts involve direct augmentation of populations of parasites, predators, or pathogens (augmentation biological control), but they can also involve manipulations of various biotic or abiotic habitat variables that favor increased populations of natural enemies (conservation biological control), either of which can have the desired effect of suppressing pest populations (Lewis et al., 1998; Stuart et al., 2006).

By the facts above, for the same purpose, following aquatic weeds i.e. *Ipomoea aquatica* F, *Marsilea crenata* P, *Pistia stratiotes* L, and their combination were tested to attract some natural enemies of the rice pest both in laboratory and semi field experiment. Those plants abundantly grew in the rice field so that if their function related with the natural enemies population can be observed, their statues as a weeds can be reduced and their role in agroecosystem stability can be taken in to account increase.

**MATERIALS AND METHODS**

In this work, the aquatic weeds i.e. *Ipomoea aquatica* F, *Marsilea crenata* P, *Pistia stratiotes* L, and their combination have been tested to attract some natural enemies of the rice pest both in laboratory and semi field experiment. In the laboratory test, the coccinelids *Adalia bipunctata* L, and *Coccinella transversalis* F, the syrphids *Eristalis tenax* L, and the sphicid *Sphex maxillosus* L were tested for their preference to those weed plants by using the four arms olfactometer. This test involved 50 individu for each species of those natural enemies for the replication and involved the same amount of weeds biomass used. Since
the learning behavior belong to the natural enemies, each individual of the them were tested only once. The percentage of the insects entering to each plant were recorded and the differences of the mean of duration time of their orientation behavior to chose the plants odor were statistically tested.

In the semi field experiment, the modification of visual control method was conducted for the selected plants from the laboratory test (Freie and Manhart, 1992). Since the Marsilea crenata P was determined as the most favorite weed for the natural enemies tested, so that this weed was used to be planted in the rice field compared with the combination with another weeds. Each combination of the aquatic weeds were planted in the 30 cm diameter of clay pots and placed in the rice field with the distance each other ca.10 meter. The combination of the weeds were: Ipomoea aquatica F and Marsilea crenata P; Marsilea crenata P it self ; Marsilea crenata P and Pistia stratiotes L; the empty pot as a control of the designed experiment. The population size of the natural enemies that visited the plant from 7.00 a clock in the morning until 4.00 a clock in the evening were identified and counted for 15 minutes duration of observation in every 1 hours. The observation were conducted for 14 days and the mean differences of the visiting natural enemies were statistically analyzed.

RESULTS AND DISCUSSION

The results of the laboratory test showed that Marsilea crenata P was the most attractive for the coccinelid and syrphid. The Pistia stratiotes L had the second rank but this plant was the most attractive for the sphecid (Tabel 1). In this case, Ipomoea aquatica F was not so favorite for the coccinelid and for syrphid but it was still attractive for the sphecid although. It seem that there were preference of the natural enemies to come to the certain plants. One species of plant may be not attractive for one species of natural enemies, but it become interesting for another natural enemies. This is the basic idea how important of the diversity of the plant or crops in the agriculture.

All natural enemies mentioned in this article were only four insects that exist abundantly in the rice field. Some of them are predator for the whole of their life (coccinelid) or a part of their life are predator and the rest of their life are pollinator (syrphid and sphecid). In this observation, they were all interested in weeds because of its the odor. It is also possible that they may be attracted to the plants because of its colour or the habitus (form) of them. It is still open to search those phenomena.
Tabel 1. Preference and mean of orientation duration of the natural enemies toward the aquatic weeds

<table>
<thead>
<tr>
<th>Natural enemies species</th>
<th>Percentage and mean of orientation duration of insects toward each weeds</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ipomoea aquatica F</td>
</tr>
<tr>
<td>Adalia bipunctata L, (Coccinellide, Coleoptera)</td>
<td>10% 2 Minutes (a)</td>
</tr>
<tr>
<td>Coccinella transversalis F (Coccinellide, Coleoptera)</td>
<td>5% 2.5 Minutes (a)</td>
</tr>
<tr>
<td>Eristalis tenax L, (Syrphidae, Diptera)</td>
<td>7% 45 Seconds (a)</td>
</tr>
<tr>
<td>Sphex maxillosus L (Sphecidae, Hymenoptera)</td>
<td>15% 30 Seconds (a)</td>
</tr>
</tbody>
</table>

Note: The mean of orientation duration followed by different alphabet in the similar rows showed the significantly differences

In the semi field experiment, the result showed that the combination of Marsilea crenata P with the other plants were not more attractive for the natural enemies tested than if the plant solely planted in field. Tabel 2 demonstrated the sharply differences of the mean of individual natural enemies visited the weeds. It seem that laboratory test gave the similar results with the semi field results. In this case, the combination of the Pistia stratiotes L and Marsilea crenata P were so attractive as in the laboratory test to the sphecids that were attracted to the Pistia stratiotes L. It can be said that this weed were attractive enough to sphecids both if they were together with Marsilea crenata P or they grew lonely.
Tabel 2. : The mean of individual natural enemies visited the weeds and its combination for 15 minutes observation duration

<table>
<thead>
<tr>
<th>Natural enemies species</th>
<th>The mean of individual natural enemies visited the weeds in 15 Minutes</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>Ipomoea aquatica</em> F and <em>Marsilea crenata</em> P</td>
<td></td>
</tr>
<tr>
<td><em>Adalia bipunctata</em> L, (Coccinellid, Coleoptera)</td>
<td>0.45a 0.82b 0.25c 0.0d</td>
<td></td>
</tr>
<tr>
<td><em>Coccinella transversalis</em> F (Coccinellid, Coleoptera)</td>
<td>0.43a 0.73b 0.44a 0.0d</td>
<td></td>
</tr>
<tr>
<td><em>Eristalis tenax</em> L, (Syrphidae, Diptera)</td>
<td>0.61a 0.91b 0.63a 0.0d</td>
<td></td>
</tr>
<tr>
<td><em>Sphex maxillosus</em> L (Sphecidae, Hymenoptera)</td>
<td>0.25a 0.73b 0.82c 0.0d</td>
<td></td>
</tr>
</tbody>
</table>

Note: The mean of individual natural enemies followed by different alphabet in the similar rows showed the significantly differences

In conclusion, the results of present study showed a combination of laboratory test and semi field experiment on the olfactory attractiveness of the aquatic weeds on the population densities of the visiting predators and pollinators can be taken into account to be practiced in the rice field. The *Pistia stratiotes* L and *Marsilea crenata* P at least can be let to accompany rice in field because of their function to stimulate the coccinelids *Adalia bipunctata* L, and *Coccinella transversalis* F, the syrphids *Eristalis tenax* L, and the sphecid *Sphex maxillosus* L entering toward rice
REFERENCES


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O-EC09
PHYTOREMEDIATION POTENTIAL OF Salvinia molesta AND Eichornia crassipes IN THE WATER THAT CONTAMINATED BY SIDOARJO MUDFLOW

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Abstract

The continue discharging of “Sidoarjo mud flow” into the river and marine would be dangerous to aquatic biota. The phytoremediation is predicted would eliminate or minimize hazardous matter from the water body that contaminated by mud flow. The aim of this research was to assess the phytoremediation potential between Salvinia molesta and Eichornia crassipes in the water that contaminated by Sidoarjo mudflow, especially the heavy metal content such as Chromium and Cadmium, and water quality such as : turbidity; salinity and sulfur content. Investigation used ex-situ method that required removal of contaminated water for treatment on site. Twenty four plastic containers that filled with 200 liter mixture of freshwater and liquid Sidoarjo mudflow (abbreviation= flsm) with ratio 1:1, were used as culture media for S. molesta and E. crassipes for one and two weeks. Water samples were taken at the start and every week throughout the experiment and analyzed physic, chemical water quality parameters like salinity, temperature, pH, turbidity, total nitrates, sulphure were measured following protocols as detailed in APHA (1998), and Cd and Cr content were measured using AAS. According the ANOVA result, it was found that S. molesta and E. crassipes one and two weeks were significantly influenced the turbidity, salinity; sulfur and heavy metal content in the media flsm. The best phytoremediator for flsm is S. molesta, that as it showed improvement water quality, such as: Cd and Cr contents in flsm decreased about 50% to 70 %, meanwhile the turbidity and salinity decrease about 30%, and sulfur content in mostly treatments were totally eliminated until 0 mg/ liter. These treated effluents may be potential to reused in the aquaculture activity.

Keynote: Sidoarjo mudflow; Phytoremediation; Salvinia molesta and Eichornia crassipes

INTRODUCTION

Mud flow in Porong Sidoarjo that begin since May 29 2006 has been generating many problems and disadvantage such as social, economic and ecological impact. Mud flow or mud volcanoes in Sidoarjo are geological phenomena due to subsurface over-pressurized mud layers. The cause of the eruption has not yet been established. However, it may be

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linked to the gas exploration activities by Lapindo Brantas at the Banjar Panji I well. The mud volcano emits mud at an average rate of more than 40,000 m$^3$/day and almost 12,000. Medical treatments have been carried out, mainly for people affected by the release of hydrogen sulfide. Mud flow consists of 30% mud and 70% water. The heavy metal content i.e.: mercury (Hg) 9,6- 14 µg/g; Pb : 13,5-17 µg/g, Cd : 0,13 µg/g ;Cr :25-40 µg/g. \(^{(11)}\) Meanwhile according the other research by Hidayati dan Widya yanti Pb content at mud flow was 3,08 ppm and Phenol 1,56 ppm.\(^{(5)}\)

UNDAC reported if the current impact of the physical and chemical characteristics of the mud on human health and environment is expected to be low, mainly due to the current practice of containment of the mud in above-ground basins\(^{(11)}\).But, the continuity of mud flow discharging to Porong river for a long time is predicted would make serious problem in aquatic life, including pond farm activities. In many years, Sidoarjo is known to be region that has high potential commodity of brackish aquaculture included milkfish (Chanos chanos) and Penaeid shrimp. Thus, contamination mud flow to the water body would affect water quality such as increasing turbidity, sulfur, phenol and heavy metal content that would be impact to fish culture productivity. Metals may be toxic because they can replace essential metals in pigments or enzymes disrupting their function\(^{(17)}\).

The one of recommendation from Report Environmental Assesment by UNDAC is: “There is an urgent need to develop a medium term strategy – parallel to the ongoing emergency response - based on a number of options, including a worst-case scenario. The environmental authorities should carry out a full and detailed environmental impact assessment of all options for mud management as soon as possible, involving local expertise and integrating humanitarian and social impacts \(^{(11)}\)"

Phytoremediation is a cost effective, environmental friendly pleasing approach most suitable for developing countries. Phytoremediation using right aquatic plant is predicted beneficial to water quality improvement in water body that contaminated by mud flow. Aquatic plants have been found to play a significant role in removing nutrients and metal content from polluted waters. Plants can break down, or degrade organic pollutants or contain and stabilize metal contaminants by acting as filters or traps. Treating metal contaminants at sites contaminated with metals, plants are used to either stabilize or remove the metals from the soil and ground water through three mechanisms: phytoextraction, rhizofiltration, and phytostabilization.

Water hyacinth (Eichornia crassipes Mart) is the aquatic plants commonly found in tropical were successfully employed in waste-water treatment systems and were found to
remove up to 90% of Nitrogen and 70% of Phosphor, depending on the residence time of wastewater. One gram of water hyacinth could absorbs 100 mg phenol/72 hour and remove 27,52 – 69,49 mg NO₃ \(^{(13)}\) Water hyacinth can trans locate the heavy metal from environment to the root and then transport it to the phloem \(^{(10)}\) suggested if one hectare water hyacinth could absorbs Cd and Hg as much as 1012,5 dan 132,5 g/day. There was explained if water hyacinth can remove Cd 1,24 mg/g dw, Pb 1.93mg/g dw, and Hg 0,98 mg/g dw. \(^{(8)}\)

*Salvinia molesta* is one of a perennial aquatic weed originated from South Eastern Brazil. Due to the morphological characteristic of the weed and high adaptability to the environmental conditions gives advantages for its development. Though extensive literature is available highlighting the potential of several genre *Salvinia* for accumulation or remove of various heavy metals including Ni, Cu, As, Pb, Cr, Zn from wastewaters\(^{(6)}\) The selection of aquatic plant was based on consideration such as high growth rate and tolerance on exposure to high element concentrations. Literature suggests that under ideal growth conditions, *Salvinia* sp. can double its biomass in shorter time span of 2 days \(^{(16)}\)

The aim of this research was to assess the phytoremediation potential of Water hyacinth (*Eichornia crassipes*) and *Salvinia molesta* to the water that contaminated by Sidoarjo mud flow. These treated effluents may be potential to re-use in the aquaculture activity. Thus, it was expected this research would be the one of some possible scenarios for re-use and disposal of the mud.

**MATERIALS AND METHODS**

*Contaminated water and Plant material Preparation*: A Mud water sample was collected from mud flow reservoir. According the preliminary study was known if the sub lethal concentration of mixture mud water and freshwater is at ratio 1:1. So that, all the experimental *Salvinia molesta* were collected from unpolluted water bodies were maintained in cemented pots (~1 m diameter) under natural light conditions. The temperature ranged from 26 to 30\(^{0}\)C.

Phytoremediation treatment: Twenty four plastic containers that filled with 20 liter mixture of freshwater and liquid Sidoarjo mudflow (abbreviation= flsm) with ratio 1:1, were used as culture media for S. *molesta* and *E. crassipes* for one and two weeks. Each container was aerated using aerator continuously. Water samples were taken at the start and every week throughout the experiment and analyzed physic, chemical water quality parameters like salinity, temperature, turbidity, and pH were measured with refractometer,
thermometer, turbidimeter and pH meter respectively. Total nitrates, sulfur were measured following protocols as detailed in APHA (1998). Analyzed for heavy metal content (Cd and Cr) using an atomic absorption spectrophotometer (AAS). The morphological differences of plants before and after exposed in mud water were also observed.

*Statistical analysis:* To know the influence of phytoremediation, data were analyzed statistically using ANOVA with MINITAB (version 14).

**RESULTS AND DISCUSSION**

*Heavy metal analysis:*

All discharged water samples from phytoremediation treatments showed decreasing heavy metal cadmium and chromium content at flsm (figure 1). *S. molest* and *E. Crassipes* could remediate the Cd and Cr because they have phytochelatins (PCs). (7) PCs are capable to bind various metals including Cd, Cu, Zn or As via the sulfhydryl and carboxyl residues. Their biosyntheses are controlled preferentially by the metal Cd or metalloid As. PCs are synthesized from glutathione. The fundamental roles of PCs are metals detoxification. The most effective metal to induce PC synthesis in water fern *Salvinia minima* was Cd, while other metal species were effective to varying extents. (6)(7)

Result of phytoremediation using *S. molest* is better than *E. crassipes*, primarily in Cd removal. Although some reference explained if the *E. crassipes* could absorb Cd 1.24 mg/g dry weight (7) and 1012.5 g Cd/ hectare/day. In these study, *E. crassipes* was not effectively absorb Cd probably caused by the survival of *E. crassipes* at the mud water media lower than *S. molest*. According the morphologically symptoms, the leaves *S. molest* was still consistence as well as control. But, there appear some morphological changes such as discoloration at little part of leaves and leaves enlargement. At microscopic scale, the adaxial surface of leaves in controls showed the presence of egg-beater shaped, hydrophobic, multicellular hairs (Fig. 3), a regular wax coating and sunken stomata. Meanwhile, the treated *S.molest* showed detachment of the tip of the egg-beater shaped hairs(Fig.3). This morphological change was suitable with the previous study by Gupta and Devi (3)
Chromium removal by S. molesta was lower than Cd removal. It probably caused by its sensitivity to Cd. S. molesta was found to be very sensitive to Cd, even at 0.1 ppm, causing growth retardation and lethality. 

Consistency of habitus S. molesta was better than E. crassipes, because the leaves of S. molesta has structure wax coating that make it very hydrophobic. These condition causing the S. molesta difficult to senescence although it exposed by water. Contrary, the E. crassipes leaves from one week treatment had showed the senescence and tend to leach. This condition probably caused by lack of turgor stem pressure that make the leaves lay down and exposed to the solution. Lacking turgor stem should be affected by salinity of media fslm. The one of main carrying capacity in E. crassipes are salinity. E. crassipes is killed in waters that are above 0.2% saline. Meanwhile, the average salinity of fslm at the t0 (time before phyto remediation) is 0.9%. Degeneration of E. crassipes stem would affect the transport physiological role. So that, E. Crassipes less effective to remove the dissolve matter (included Cd and Cr) than in S. molesta. The ANOVA suggested if the Cd and Cr removal at one week treatment by S. molesta higher significantly than E. crassipes. At two weeks treatment plants didn’t show differences, it indicate the plants have saturated by the Cd and Cr. The leaves senescence and discoloration indicated of chlorosis. Salvinia exposed to Cr and Zn exhibited changes in photosynthetic potential. Alteration in levels of photosynthetic pigments affected the photosynthetic performance of Salvinia. The reduction in pigment levels in plants exposed to Cr and Zn rich wastewater could be the due to (i) reduced efficiency of enzymes involved in chlorophyll biosynthesis; (ii) decrease in availability of iron; and (iii) formation of metal substituted chlorophylls. Similar responses of
reduction in photosynthetic pigments on exposure to heavy metals have been reported earlier in *Salvinia* species\(^2\)

![Figure 2](image)

Figure 2. Comparison morphological features of control of *S.molesta* leaves (left) and after phytoremediation treatment. (right). At control there appear egg beater shape hairs (trichome,) meanwhile the treatment leaves showed detachment egg beater shape hairs (trichome,)

**Water Quality improvement**

The figure 3 showed the best increasing water quality after phytoremediation was showed in solution that treated by *S.molesta* at one week. The improvement such as decreasing turbidity and sulfure content potentially to re-used this solution. Turbidity in two week salvinia and E. *crassipes* in all time treatment didn’t significant differences, because at these treatment mostly part of plants has already senescence and tend to leaching. Then these leaves tend to decay by microorganism, and would affect the solution turbidity. The increasing of nitrate content also indicated the increasing of microorganisms activities.. Previous study \(^{14}\) in the same plant object reported if the nitrate removal showed a rather erratic course. It was rapidly removed during the first 12 days by both plants, after which there was a net decrease in nitrate removal, attributed to leaching of the nutrient from the plants before a further increase. Nitrogen, sulfur and iron are the important elements in metabolism of plant and animal that cycled by microorganisms.

Mostly treatment showed a decreasing of total sulfur average number from 0.28 to 0 mg/l. According the UNDAC report is known there medical treatments have been carried out, mainly for people around the mud flow area affected by the release of hydrogen sulphide . Sulfide should be emitted from geochemical source in volcanoes. Meanwhile, soluble sulfides including S*,HS*, and H2S are considered highly toxic to plants These conditions are frequently encountered in anaerobic sediments of eutrophic lakes\(^9\) Macrophytes can protect from sulfides by oxygen release from roots \(^9\)

Decreasing sulphur in the phytoremediated mud water is probably caused by formation of sulphate through sulfur oxidation by sulfur-oxidizing bacteria that live in media. Then it should be trans locate to the plant and through SH-amino acid formation it built for the tissues, or used by microorganism. It reported if water spangle (*S. molesta*) could
removed sulphate efficiently 7.88 mg within three days, and water hyacinth removed sulphate 4.43 mg$^{(14)}$

![Graph showing water quality before and after phytoremediation](image)

Figure 3. Comparison Water Quality of water that contaminated by Sidoarjo mud flow before and after phytoremediation

**Recommendation**

Water body that contaminated by Sidoarjo mud flow is possible to re-use after phytoremediation treatment using *S. molestata*. The best replacement interval of *S. molestata* is one week to avoiding the plant saturation and decaying that tend to re-accumulation pollutant to the environment.

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Lapindo dengan Metode Biofilter Enceng Gondok (Eichornia crassipes (Mart.) Solm.).


O-EC10

REINTRODUCTION AND SURVIVORSHIP OF THE THREATENED PALM Pinanga javana BLUME IN THE GUNUNG HALIMUN SALAK NATIONAL PARK, WEST JAVA

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ABSTRACT

Pinanga javana is one of the Indonesia’s threatened, endemic palms. The significant decline of its populations has become a major concern. The species now only consists of six, scattered populations confined to the sub-mountain areas of Java where extensive land conversions continue. The species recovery depends largely on the type and degree of active management to be applied to existing individual populations. This paper discusses results in reintroducing the palm focusing on its survivorship. A total of 5200 seedlings have been reintroduced to the former habitat in Gunung Halimun in 2005 at 16 different potential sites, covering a wide range of forest and habitat types. The results showed that the palm requires specific environmental conditions for establishment, however, the survivorships and growth conditions varied with habitat types. Based on the tagged individuals after one year planted, the highest survivorships (87%) and the best growth conditions occurred in well-drained sites adjacent to water supplies with moderate to steep slopes, moderate canopy cover (60-70%), clear understorey, and with an average humidity of 79% and soil pH of 6.4. In contrast, high mortality rates and less growth conditions were experienced by individuals planted on barren, degraded sites or under closed-canopy covers. The lowest survivorship recorded was 27%. A number of pests were found to attack the planted individuals, particularly slugs, rhino beetles and grasshoppers. To effectively manage the planted seedlings and conserve the remaining populations, it is crucial to protect the suitable sites in the National Park.

Keywords: Reintroduction, Survivorship, Pinanga javana, Gunung Halimun National Park.

INTRODUCTION

Pinanga javana is an attractive subject for ecological research and reintroduction program, representing a suitable species for implementing the Target 8 of the Global Strategy for Plant Conservation (GSPC). It can link and bridge ex situ protection to in situ conservation. From conservation point of view, this threatened palm is a high priority as it consists of only few remaining small populations confined to an ecosystem that has been
extensively converted into various development projects, particularly large plantations, agriculture, mining operations, and human settlement. This species can be used as a flag species to promote conservation of the Javanese sub-mountain ecosystem. Indeed the occurrence in the remaining Javanese rain forest is a challenging ecological aspect to investigate. This species occurs only in certain sites of the forest and absent from most localities. It is a solitary and slow growing species and appears to require precise environmental conditions for establishment. Soil and habitat characteristics, moisture, interspecific association, light intensity, and nutrient availability seem to influence the establishment and regeneration. The palm may not be able to regenerate until its minimum requirements are met by the habitat quality.

*Pinanga javana* is not only of interest from ecological and conservation points of view, but also from economical significance since it stands to many benefits. It has been used locally and nationally for commercial ornamental purposes. Sometimes the stem is locally used for constructing pondok (local house) and the young stem apex is eaten providing a source for vegetable. Palm is indeed a very important plant group in Indonesia, becoming the most important taxa just after grasses (*Oryza* spp.) in economic importance. By having approximately 570 native palm species, this country undoubtedly possesses the largest number of palms among any other countries in the world (Dransfield 1994). Mogea (1991) provided a list of native Indonesian palms, their uses and their general conservation status, indicating the importance of this plant group for the people. Palm economic importance should be a strong and reasonable justification for making efforts to save as wide a range of species as possible from extinction (Dransfield 1976, Dransfield 1994).

Efforts to reintroduce *P. javana* will become a model to conserve the Indonesian threatened plants and an initial reintroduction project has been started by applying appropriate techniques and procedures to enable the establishment of new individuals. A minimum outcome of any reintroduction program would be the retention of the existing populations (Shaw 1993). Another significant outcome would be the augmentation (restocking) of the declining populations, e.g. being a doubling of present numbers of individuals. Ironically, scientific information about the ecology, biology and habitat quality of many palm species is by far less understood (Enright 1985, Uhl & Dransfield 1987), including their ecological and habitat requirements (for establishment, recruitment, and regeneration), demography and population status, age structure, reproductive biology, and their current and historical distribution (Widyatmoko 2001, Widyatmoko *et al.* 2005). Yet many members of the Arecaceae family have not been clearly identified and understood ecologically, and seem to be no broad generalizations about their ecology, due to the great
morphological diversity which is paralleled by a wide range of their ecological adaptations and behaviour (Uhl & Dransfield 1987).

Studies conducted to gain a better understanding of which species are threatened and what driving factors causing the extinction are therefore needed to provide guidance so that conservation actions, particularly reintroduction and restocking, can be taken before it is too late to save them (BGCI 1995, Barry 2000, Rowland & Maun 2001, Jepson & Whittaker 2002, McDonald 2002 & 2003, Jones 2004).

*Pinanga javana* was classified as “Endangered” by the World Conservation Monitoring Centre (1997). There were 31 palm species (of c.700 Indonesian threatened plants) considered as threatened with extinction. Orchids, timbers, and palms were the top three contributors to the Indonesian threatened plants, comprising 93, 55, and 31 species, respectively (WCMC 1997, IUCN 2007, IUCN 2008). However, the true number of Indonesian threatened palm species is very likely much higher than this figure. According to the IUCN (2000), Malaysia, Indonesia, Brazil, and Sri Lanka were the top four countries with the largest numbers of threatened plant species. The expanding human population and loss of natural vegetation have caused the extinction of approximately 600 plant species since the year 1600, and threaten over 22,000 additional flowering plants with extinction (Smith et al. 1993). It is very urgent, therefore, to prepare comprehensive reintroduction plans for Indonesia.

**Objectives of the Reintroduction**

The objectives of the program were 1) to conduct a reintroduction of *Pinanga javana* to the suitable localities within Gunung Halimun Salak National Park as the former native habitat where the species was locally extinct or believed to be extinct, 2) to carry out a reinforcement of *P. javana* to the target sites within Gunung Halimun Salak National Park where the population(s) still existed but experienced a significant decline by having only very small, vulnerable colonies, and 3) to monitor the planted and remaining populations to ensure the perpetuity and establishment of the provenances and colonies.

The ultimate goal of the reintroduction and reinforcement of *P. javana* is to ensure the perpetuation of the species in the wild and the maintenance of its genetic diversity to allow continuing biological processes. Thus the management objectives for each of the known sites (management units) are different due to their different conditions and characteristics.
The reintroduction of *P. javana* has been developed to be integrated with the existing plans and strategies, both nationally and globally. These include the Strategic Plan of the Center for Plant Conservation - Bogor Botanic Gardens, The Indonesian Agenda 21 (Chapter 16 Biodiversity Conservation), the Indonesia Integrated Biodiversity Strategy and Action Plan (2003), and the Global Strategy for Plant Conservation (Target 8).

**MATERIAL AND METHODS**

**Reintroduction and Reinforcement Sites**

The reintroduction sites were situated in Gunung Kendeng within the Gunung Halimun Salak National Park, stretching from S06°42’53” to S06°45’59.0” and from E 106°34’12.9” to E106°38’08.4”. Administratively the sites belong to Pamijahan Village, Bogor Regency, West Java Province. The reintroduction sites’ topography varied from undulating to steep slopes, ranging from 984 to 1140 m above sea level. The forest types consisted of hill, lower mountain, and mountain forests. Lower mountain forests with slopes ranging from 30 to 70% dominated the land topography. The forest main canopy was mainly dominated by *Schima wallichii, Castanopsis acuminatissima, Altingia excelsa, Ficus ribes, Quercus gemmeliflora,* and *Prunus arborea*. Dominat seedlings and understorey included *Syzygium lineatum, Symlocos sp., Quercus gemmeliflora, Psychotria viridiflora, Euodia latifolia,* and *Macaranga trifoba.*

Gunung Halimun Salak National Park was established in 2003 based on the Minister of Forestry decree No.175/Kpts-II/2003 covering a total area of 113,357 hectares and stretching from 500 to 2,211 m above sea level (Suganda *et al.* 2004). Administratively the National Park belongs to two different provinces: West Java and Banten, belonging to three different regencies: Bogor, Sukabumi, and Lebak (Fig. 1). The National Park includes Gunung Halimun, Gunung Salak, Gunung Endut, and other smaller mounts and forests adjacent to Gunung Halimun. Gunung Halimun Salak National Park was actually the extension of previously Gunung Halimun National Park established in 1992 based on the Minister of Forestry decree No. 282/Kpts-II/Menhut/1992 (covering a total area of 40,000 hectares).
Fig. 1. Map and location of Gunung Halimun Salak National Park where *Pinanga javana* was reintroduced. Source: Gunung Halimun Salak National Park Management Project (2006).

The location of each reintroduction and reinforcement site was recorded using a Garmin Global Positioning System MAP 175. Land slopes were measured using a clinometer (SUUNTO Optical Reading Clinometer PM-5 made in Finlandia), while soil pH and humidity were measured using a soil tester DEMETRA patent no. 193478 Electrode Measuring System, Tokyo, Japan. Prevailing air temperature was measured using a thermo-hygrometer.

**Study Species**

*Pinanga javana* is a robust, erect, always solitary palm. Stem is grayish brown, smooth, with internodes ranging from 10 to 30 cm. The stem height ranges from 5 to 10 m while the stem diameter from 10 to 15 cm. Crownshaft is swollen, purplish brown, usually with 10 leaves in crown, while inflorescence is infrafoliolar. The arrangement of the rachillae is always alternate and distichous and is different from the related species (*P. coronata*) which is small and clustered, and the arrangement of its rachillae is always spiral. Although the
infertescence of *P. javana* is held in an open fan just like *P. coronata*, it is two times as big and solitary. Fruit is ovoid to ellipsoid, 20-25 mm x 11-15 mm in size, pale pinkish yellow when young and turning dark red and black when ripe.

*Pinanga javana* only reproduces sexually, flowers are functionally male and female arranged in triads (one female between two males). Flowering occurs all year round, but the main flowering period seems to be between June and October. Some plants have two or more flowering periods over a year. Seeds are present in October – March, but may persist on the plant for much longer period. Flowering and seed production vary markedly from year to year and heavy flowering does not always mean good seed production as prolonged wet weather during or immediately following the heavy flowering may lead to flowers rotting and falling off the plant. Some flowers seem to be receptive before they are attacked by beetle larvae and weevil. Pollination systems of *P. javana* are not well understood. Based on personal observations, it is very likely predominantly insect-pollinated. Some beetles and weevils seem to be the important pollination agents, while pigeon family (Columbidae) may play a minor role.

*Pinanga javana* is endemic to Java, confined to the submontains of Java. Java has only two species of *Pinanga*: *P. javana* and *P. coronata*, while *P. kuhlii* is considered to be a synonym of *P. coronata*. Since the occurrence of *P. javana* in Gunung Slamet (Central Java) has only been discovered in 2001, it is likely that other sites may be discovered in the future, as it was recently rediscovered in Gunung Wilis (East Java) after a long period of the absence of collection. Previously the last specimen collected from Gunung Wilis was by Backer in 1914. The last specimens collected by Beccari in 1909 from Gunung Tengger (East Java) showed that the palm once occurred in the area. However many field observations conducted after that period found no extant individuals, and the population has been assumed to be extinct from this location. Decline of *P. javana* populations in the wild seems to be obvious, and the present distribution is a significant contraction of the previous range indicated by the existing records. The geographic range of *P. javana* is different from *P. coronata*, while the first is very restricted to the Javanese submontane forest, the later is widely distributed which can be found from Sumatra, Java, to Lesser Sunda Islands, from lowland to montane forest.

Globally *P. javana* consists of small, scaterred populations, and only two of which represent relatively large population sizes. These two populations occur in Gunung Pulosari (Mandalawangi, Banten) in summit mossy forest and in Gunung Salak (Ciapus) on steep rocky slopes. In contrast, Ciomas population (Gunung Salak) only accommodates two
individuals. Gunung Halimun (West Java) and Curug Cipendog (Gunung Slamet) have only a few plants, while Gunung Gede (Cibodas) possesses a small colony.

**Working Stages of the Reintroduction**

Three stages of reintroduction were applied: pre-activities, release stage, and post-release stage. The pre-activities were initiated by investigating the biology of *P. javana* and the ecology of its habitats. The next step was to conduct detail surveys at various locations within Gunung Halimun to search and find any possible remaining populations to ensure the presence/absence of the palm and decide possible conservation actions (i.e. reintroduction and/or reinforcement). Two remaining sub-populations were found in 2003 and seeds were collected from the existing adult plants to ensure the availability of release stock (seedlings). The decline of this palm was very obvious due to serious land destruction. The collected seeds were brought and grown at the Bogor Botanic Gardens nurseries.

Release stage was carried out by reintroducing and restocking (reinforcing) a total of 5200 seedlings (resulted from the seeds collected from Gunung Halimun in 2003) to the former habitats in Gunung Halimun in 2005 at 16 different potential sites. The 16 different reintroduced sites covered a wide range of forest and habitat types in order to represent the ecological characteristics and diverse vegetation of Gunung Halimun. Before transported to the target sites with their planting media (in order to minimize plant stress), all seedlings were checked to ensure the health of individuals (i.e. free from contagious pathogens or parasites). Wherever possible the seedlings were planted near or adjacent to a water shed (stream). Reintroduction was conducted at localities where *P. javana* was locally extinct while reinforcement was carried out at sites where the palm population(s) still existed but experienced a serious/significant decline (i.e. increasing the number of individuals).

A number of 960 seedlings (i.e. 60 individuals for each site, randomly chosen from the 5200 planted seedlings) were tagged for monitoring purposes. Survivorship, growth, and health of the tagged planted individuals were recorded and monitored annually (post-release stage) to ensure the perpetuity of the planted plants. The growth parameters monitored included plant height increase, number of new leaves produced, and plant growth conditions (Fig. 2).
Fig. 2. Monitoring the growth and survivorship of the planted individuals of *Pinanga javana* (left) and a healthy growing individual planted at Cisalada1 site (right).

RESULTS

In Gunung Halimun *Pinanga javana* occurs naturally on very steep hillsides or steep rocky slopes, in moist lower montane forest at altitudes range from 800 to 1700 m above sea level. The species was often found to co-occur with *Pinanga coronata*, *Turpinia sphaerocarpa*, and *Macropanax dispermum*. The local dispersion pattern in Gunung Halimun was clumped. However it was never found on ridgetops during the surveys and planting times. The palm habitat was often inaccessible, under a slightly to moderate canopy cover.

The survivorship of the planted individuals at each locality within Gunung Halimun varied amongst sites as shown in Table 1 and Fig. 3 below. The highest survivorship was recorded at Cisalada1 (87%), followed by that of Pojok Daya (85.7%), and Cigorowek (83.3%), indicating suitable habitat conditions for the species to establish in these three localities. The habitat characteristics at Cisalada1 were moderate to steep slopes, adjacent to a water shed (stream), with a moderate canopy cover (60-70%), representing a relatively clear forest floor, with a relatively neutral soil, situated in a sub-mountain zone, and within a humid environment. Similar habitat characteristics were found at Pojok Daya and Cigorowek sites (Table 2). In comparison with other sites, the Cigorowek mostly consisted of steep slopes and was located at the lowest altitude (984 m). In contrast, high mortality rates and less growth conditions were experienced by individuals planted on barren, degraded sites or under closed-canopy covers. The lowest survivorship was recorded at Kebun Kopi (27.0%) and Pasir Bedil (46.1%). Interestingly the survival rate at Pojok Goong was relatively high
(77.8%) although the site was distant to water (creek) and the forest floor was dominated by dense *Alpinia*.

Table 1. Survivorship of the planted individuals of *Pinanga javana* at various locations within Gunung Halimun Salak National Park in 2006.

<table>
<thead>
<tr>
<th>No</th>
<th>Location (Latitude &amp; Longitude)</th>
<th>No. Planted Individuals</th>
<th>No. Tagged Individuals</th>
<th>Survivorship (%)</th>
</tr>
</thead>
</table>
| 1  | Block Cikuya  
S06°45’59.0" E106°36’53.6’’ | 300 | 60 | 72.4 |
| 2  | Pasir Bedil  
S06°44’59.0” E106°36’51.6’’ | 300 | 60 | 46.1 |
| 3  | Cisalada1  
S06°45’00.1” E106°37’50.8’’ | 350 | 60 | 87.0 |
| 4  | Cisalada2  
S06°44’51.7” E106°34’12.9’’ | 350 | 60 | 66.6 |
| 5  | Pojok Daya  
S06°45’12.5” E106°38’07.1’’ | 400 | 60 | 85.7 |
| 6  | Pojok Goong  
S06°45’17.8” E106°35’57.9’’ | 300 | 60 | 77.8 |
| 7  | Kubang  
S06°45’02.5” E106°37’11.4’’ | 300 | 60 | 66.7 |
| 8  | Kebun Kopi  
S06°44’44.0” E106°37’08.4’’ | 300 | 60 | 27.0 |
| 9  | Block Rawa  
S06°42’53.0” E106°37’21.9’’ | 300 | 60 | 55.5 |
| 10 | Legok Buluh  
S06°45’04.7” E106°37’09.6’’ | 350 | 60 | 67.9 |
| 11 | Pasir Tulang  
S06°45’04.7” E106°37’09.6’’ | 350 | 60 | 63.6 |
Table 2. The habitat characteristics of each location in regard with the survivorship of the planted individuals of *Pinanga javana*.

<table>
<thead>
<tr>
<th>Location</th>
<th>Survivorship (%)</th>
<th>Forest/habitat types</th>
<th>Slope</th>
<th>Distant to water</th>
<th>Canopy cover (%)</th>
<th>Forest floor</th>
<th>Altitude (m asl)</th>
<th>Soil pH</th>
<th>Av. Humid. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Block Cikuya</td>
<td>72.4</td>
<td></td>
<td>Mod - Steep</td>
<td>Adjacent</td>
<td>60-70</td>
<td>Mod</td>
<td>1084</td>
<td>6.1</td>
<td>75</td>
</tr>
<tr>
<td>Pasir Bedil</td>
<td>46.1</td>
<td></td>
<td>Moderate</td>
<td>Distant</td>
<td>30-40</td>
<td>Dense</td>
<td>1080</td>
<td>6.0</td>
<td>68</td>
</tr>
<tr>
<td>Cisalada 1</td>
<td>87.0</td>
<td></td>
<td>Mod - Steep</td>
<td>Adjacent</td>
<td>60-70</td>
<td>Clear</td>
<td>1070</td>
<td>6.4</td>
<td>79</td>
</tr>
<tr>
<td>Cisalada 2</td>
<td>66.6</td>
<td></td>
<td>Mod - Steep</td>
<td>Adjacent</td>
<td>70-80</td>
<td>Mod</td>
<td>1022</td>
<td>6.1</td>
<td>73</td>
</tr>
<tr>
<td>Pojok Daya</td>
<td>85.7</td>
<td></td>
<td>Mod - Steep</td>
<td>Adjacent</td>
<td>70-80</td>
<td>Alpinia</td>
<td>1036</td>
<td>6.3</td>
<td>82</td>
</tr>
<tr>
<td>Pojok Goong</td>
<td>77.8</td>
<td></td>
<td>Moderate</td>
<td>Distant</td>
<td>60-70</td>
<td>Alpinia</td>
<td>1030</td>
<td>6.3</td>
<td>78</td>
</tr>
<tr>
<td>Kubang</td>
<td>66.7</td>
<td></td>
<td>Steep</td>
<td>Adjacent</td>
<td>70-80</td>
<td>Mod</td>
<td>1024</td>
<td>6.9</td>
<td>54</td>
</tr>
<tr>
<td>Kebun Kipi</td>
<td>27.0</td>
<td></td>
<td>Undulating</td>
<td>Distant</td>
<td>10-20</td>
<td>Thick grass</td>
<td>1025</td>
<td>6.3</td>
<td>55</td>
</tr>
<tr>
<td>Location</td>
<td>Survivorship (%)</td>
<td>Location</td>
<td>Survivorship (%)</td>
<td>Location</td>
<td>Survivorship (%)</td>
<td>Location</td>
<td>Survivorship (%)</td>
<td></td>
<td></td>
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<tr>
<td>----------------</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Block Rawa</td>
<td>55.5</td>
<td>Moderate</td>
<td>60-70</td>
<td>Dense</td>
<td>1037</td>
<td>6.9</td>
<td>60</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Legok Buluh</td>
<td>67.9</td>
<td>Steep</td>
<td>Adjacent</td>
<td>Dense</td>
<td>1040</td>
<td>6.7</td>
<td>55</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pasir Tulang</td>
<td>63.6</td>
<td>Step</td>
<td>Adjacent</td>
<td>Mod</td>
<td>1046</td>
<td>6.1</td>
<td>68</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cibadak</td>
<td>58.0</td>
<td>Undulat.</td>
<td>Distant</td>
<td>Dense</td>
<td>1045</td>
<td>6.3</td>
<td>65</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cigorowek</td>
<td>83.3</td>
<td>Step</td>
<td>Adjacent</td>
<td>Clear</td>
<td>984</td>
<td>6.8</td>
<td>63</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tower</td>
<td>55.6</td>
<td>Mod - Steep</td>
<td>Distant</td>
<td>Dense</td>
<td>1140</td>
<td>6.5</td>
<td>68</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bepak</td>
<td>54.6</td>
<td>Mod</td>
<td>Distant</td>
<td>Dense</td>
<td>1120</td>
<td>6.2</td>
<td>70</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ciharang</td>
<td>-</td>
<td>Mod - Steep</td>
<td>Adjacent</td>
<td>Dense</td>
<td>1020</td>
<td>5.9</td>
<td>74</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fig. 3. Survivorship of the planted individuals at various locations within Gunung Halimun observed in 2006. Error bars are the standard errors.
Figure 4 shows the survivorship and growth condition of the surviving individuals of *Pinanga javana* at various sites within Gunung Halimun. A high percentage of good or very good growth conditions was shown by Cisalada1 colony (65.6%) with no dead plants at all. Similarly, a relatively high proportion of good growth was performed by Cigorowek population (58.3%) but with a small proportion of dead plants (8.3%). In contrast, high mortality rates were experienced by populations at Kubang (26.7%), Pasir Tulang (18.1%), and Block Rawa (16.7%).

![Survivorship and growth condition of surviving individuals of Pinanga javana at various sites within Gunung Halimun.](image)

Table 3 shows individual plant growth of planted individuals of *Pinanga javana* at various sites within Gunung Halimun. Growth was measured based on the increase of leaf size and leaf number. The highest annual leaf size increase was shown by Bepak colony (66.8 cm), followed by Pasir Tulang (64.1 cm), and Tower (61.3 cm). Conversely, the lowest annual leaf increment was experienced by Kubang colony (27.8 cm), Block Rawa (40.5 cm), and Legok Buluh (42.7 cm). In terms of leaf number increase, Kubang population showed the highest increase (0.9 leaf year⁻¹). The average increase in leaf number amongst sites was 0.6 leaf per year.
Table 3. Growth of planted individuals of *Pinanga javana* at various sites within Gunung Halimun. Growth was defined as the increase of leaf size and leaf number.

<table>
<thead>
<tr>
<th>Location</th>
<th>Average Survivorship (%)</th>
<th>Average Leaf Size (cm)</th>
<th>Av. Leaf Size Increase (cm)</th>
<th>Av. Leaf No Increase 2005</th>
<th>Av. Leaf No Increase 2006</th>
<th>Average Leaf No Increase 2006</th>
</tr>
</thead>
<tbody>
<tr>
<td>Block Cikuya</td>
<td>72.4</td>
<td>32.2</td>
<td>89.2</td>
<td>57.0</td>
<td>1.0</td>
<td>1.5</td>
</tr>
<tr>
<td>Pasir Bedil</td>
<td>46.1</td>
<td>34.0</td>
<td>78.5</td>
<td>44.5</td>
<td>1.0</td>
<td>1.6</td>
</tr>
<tr>
<td>Cisalada1</td>
<td>87.0</td>
<td>35.1</td>
<td>93.5</td>
<td>58.4</td>
<td>1.2</td>
<td>2.5</td>
</tr>
<tr>
<td>Cisalada2</td>
<td>66.6</td>
<td>31.4</td>
<td>84.7</td>
<td>53.3</td>
<td>1.0</td>
<td>1.6</td>
</tr>
<tr>
<td>Pojok Daya</td>
<td>85.7</td>
<td>34.6</td>
<td>96.6</td>
<td>62.0</td>
<td>1.2</td>
<td>1.8</td>
</tr>
<tr>
<td>Pojok Goong</td>
<td>77.8</td>
<td>32.2</td>
<td>84.0</td>
<td>51.8</td>
<td>1.1</td>
<td>1.6</td>
</tr>
<tr>
<td>Kubang</td>
<td>66.7</td>
<td>33.3</td>
<td>60.3</td>
<td>27.8</td>
<td>1.1</td>
<td>2.0</td>
</tr>
<tr>
<td>Kebun Kopi</td>
<td>27.0</td>
<td>36.2</td>
<td>92.0</td>
<td>55.8</td>
<td>1.2</td>
<td>1.7</td>
</tr>
<tr>
<td>Block Rawa</td>
<td>55.5</td>
<td>36.3</td>
<td>76.8</td>
<td>40.5</td>
<td>1.0</td>
<td>1.5</td>
</tr>
<tr>
<td>Legok Buluh</td>
<td>67.9</td>
<td>34.6</td>
<td>77.3</td>
<td>42.7</td>
<td>1.1</td>
<td>1.7</td>
</tr>
<tr>
<td>Pasir Tulang</td>
<td>63.6</td>
<td>32.7</td>
<td>96.8</td>
<td>64.1</td>
<td>1.0</td>
<td>1.6</td>
</tr>
<tr>
<td>Cibadak</td>
<td>58.0</td>
<td>33.4</td>
<td>83.9</td>
<td>50.5</td>
<td>1.0</td>
<td>1.4</td>
</tr>
<tr>
<td>Cigorowek</td>
<td>83.3</td>
<td>36.1</td>
<td>80.3</td>
<td>44.2</td>
<td>1.0</td>
<td>1.4</td>
</tr>
<tr>
<td>Tower</td>
<td>55.6</td>
<td>31.9</td>
<td>93.2</td>
<td>61.3</td>
<td>1.0</td>
<td>1.3</td>
</tr>
<tr>
<td>Bepak</td>
<td>54.6</td>
<td>35.7</td>
<td>102.5</td>
<td>66.8</td>
<td>1.0</td>
<td>1.3</td>
</tr>
<tr>
<td>Cihang</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>AVERAGE</td>
<td>64.5</td>
<td>34.0</td>
<td>86.0</td>
<td>52.0</td>
<td>1.1</td>
<td>1.6</td>
</tr>
</tbody>
</table>
DISCUSSIONS

The current threats to *Pinanga javana* can be considered at two scales: those common to all sites and those specific to each location (geographic unit). Common threats are particularly represented by its vulnerability to loss due to habitat destruction and modification (such as settlement, large plantation, and crop development) and catastrophic disturbance (such as fires, landslides, and floods). Specific threats to each population vary from site to site.

The decline of *P. javana* is mainly associated with human activities and impacts, particularly habitat destruction and conversion. A large area of the former habitat in Gunung Halimun, northern Gunung Salak, and Gunung Gede (West Java) have been considerably altered by the establishment of large tea plantations and crops as well as human settlement, while many parts of Gunung Slamet (Central Java) have been disturbed particularly due to the development of large pine plantations (especially *Pinus merkusii* and *Agathis borneensis*) and settlement. These locations no longer provide suitable natural habitats and the loss of such a vast area of actual and potential habitats has been creating serious implications for a slow growing species like *P. javana* which is dependent on a narrow altitudinal range and habitat conditions.

Minor threats and effects observed included plant collecting, mammal browsing, and invasive species. Plant collecting for horticultural purposes or for its “umbut” may have reduced slightly some populations. Depredations of deer and monkeys that eat the apical shoot of the young plants have been noted at several sites. Slugs, rhino beetles, grasshopper (*Valanga* sp.) have also been recorded to attack the planted plants at a number of sites within Gunung Halimun.

Two small natural populations occurred in Gunung Halimun are under high level of threats and are very rare: only five adult individuals were known in 2003 and 2004 surveys. These plants occurred on a moist lower montane forest, on a steep hillside vulnerable to floods and landslides (i.e. the sites are very unstable), at an altitude of 1040 m. Active management is required to protect these vulnerable populations as they are under serious threats both from natural and human threats. Detail study and monitoring is also required to provide a scientific basis for the long term management.

Two populations occurred in Gunung Salak, namely Ciomas and Ciapus sites. The Ciomas population was facing a high level of threat and also very rare: only two adult individuals were known. These plants occurred on a moist lower montane forest, on a very
steep hillside vulnerable to landslides (i.e. the sites are very unstable), at an altitude of 1500 m. Active management will be required to maintain this vulnerable colony. The Ciapus population is located at the gorge of Gunung Salak, occurring on a lower montane forest, on steep rocky slopes, at 1000 m in altitude. This is the largest known population of *P. javana*, consisting of approximately 1000 plants in 1971 (J. Dransfield 1758). However a significant decline occurred and now only about 300 plants remained. Active management is required to adequately protect this crucial population. Detail study and research is also needed to provide a scientific basis for the long term management.

Gunung Gede population only consists of one known site, located above Cibodas Botanic Gardens, Cianjur, West Java, where a small number of adult plants occur on a damp montane forest, growing in deep soil, beneath a taller canopy, at an altitude of 1500 m, but not in the ridge top. Active management, in collaboration with the management authority of Gunung Gede Pangrango National Park, is required to maintain this population. The health of existing reproductive mature plants is crucial for the population perpetuity.

Chances for recovery depend largely on the type and degree of active management to be applied to existing individual populations, and on the successful establishment of new individuals and populations in the wild. Current situations indicate that *Pinanga javana* will continue to decline if causal mechanisms continue operating. Without active conservation action (i.e. intervention) it is very likely that the number of sites and its current geographic range will decrease significantly over the next ten years. Although the growth rate is slow and seed production varies significantly from year to year, this palm produces an adequate amount of seed and the percentage of seed germination is relatively high. In conjunction with manipulation of the habitat, the seed potential provides an opportunity to counter causal mechanisms of decline.

Different strategies and objectives for each site indicate the different characteristics of *P. javana* population in each site. However, all sites seem to require active conservation to retain the existing populations. Gunung Salak, Gunung Wilis, and Gunung Slamet (Cipendog Waterfall) should be prioritized for conducting the next reintroduction program, establishing new colonies, and maintaining the current genetic resource. However, the first important step is to conduct detail checks to count the remaining individuals (populations). The strategy used is crucial to determine success of the program and should include the involvement of stakeholders, including local communities. The strategy is the level where various management disciplines (field management, planning, research, and advocacy) are integrated, along with other plans and strategies.
CONCLUSIONS AND RECOMMENDATIONS

The establishment of new *Pinanga javana* colonies should be seen and treated as a complimentary part of the integrated conservation program designated for Gunung Halimun Salak National Park as a whole. Such establishment should aim to mimic or replicate its natural habitat characteristics. As most populations of this palm occur in moist lower mountain forests on steep slopes or hillsides, or even on inaccessible sites, it is therefore crucial to strictly protect such habitats. The protection of its co-occurring species (especially *Pinanga coronata*) will also be important for managing the diversity of the Javanese *Pinanga*. Five geographic units have been ranked in priority order for conducting the next reintroduction program as follows: Mount Salak, Mount Wilis, Mount Slamet, Mount Gede, and Mount Sawal.

A continuing environmental education becomes a crucial process to implement in order to encourage and involve the local communities in the reintroduction activity. The success of this program will indeed depend on their awareness. Active public involvement in the program can be enhanced by involvement in planting and monitoring activities, plant cultivation by private individuals and local societies, involvement of local school groups, and using *P. javana* as a theme plant for special, environmental days. High public awareness of this threatened palm can also be encouraged through the use of the plant for horticultural purposes, while public advocacy package was developed and disseminated through leaflets and publications.

The following management options have been considered for each existing population: 1) conduct ongoing monitoring and this includes field inspections and counting numbers, observing plants health, and conducting detailed measurements that may be carried out every 6 – 12 months, however monitoring alone will not achieve the management goal, 2) collect seeds in order to allow propagation for planting in wild habitats, the establishment of *ex situ* stocks, and the provision of material to horticulturalists, 3) conduct supplementation to provide the opportunity to retain present sites and increase individual numbers, or 4) implement reintroduction to allow new populations to establish in former habitats in which *P. javana* is now extinct.
REFERENCES


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TOWARD THE INDONESIAN REDLIST BOOK:
SPECIES PRIORITY SETTING FOR CONSERVATION OF INDONESIAN THREATENED PLANTS

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ABSTRACT

Indonesia is a megabiodiversity country, but on the other hand Indonesia is also the biggest country in terms of plant endangerment after Malaysia. According to the IUCN, the number of Indonesian threatened plants under the IUCN rating is 386 species, which is unlikely to be conserved all at once. Therefore, species priority setting is very important to be conducted for effective conservation programs in order to reduce plant species extinction and improve plant-use sustainability for human prosperity. For this purpose, Indonesian Botanic Gardens as the competent plant conservation institution has been working on setting priority of these threatened plants for conservation to refine and rank the IUCN categories from the most threatened to the least threatened species. This ongoing project consists of some workshops on species priority assessment using 17 scoring criteria and population study of selected taxa having the largest species number of endangerment. The species priority assessment was carried out by expert panel of selected taxa ensuing 3 categories of priority. For the first workshop in 2009, 4 out of 13 plant families were assessed by expert panel, resulting 100 species for immediate conservation action. We also develop a Delphi-based computer program for assessing conservation priority enables other assessors to consistently apply the scoring criteria for conservation priority. Overall result of this 5-year project will be written in an Indonesian red list book of threatened plants that will significantly contribute to the National plant conservation strategies and policy.

Keywords: threatened plants, setting priority, conservation, scoring criteria, red list

INTRODUCTION

According to the International Union for Conservation of Nature and Natural Resources or IUCN (2008), Indonesian plant species categorized as critically endangered, endangered and vulnerable has reached 386 species. The number has reached 513 species when we include data from WCMC (1997), making Indonesia the leading country in terms of plant species endangerment following Brazil and Malaysia (IUCN 2008). If more intensive studies were done, this number is expected to be larger. Realistically, those threatened species with extinction are unlikely to be conserved all at once, considering limitation on budget allocation, expertise and time. A shortcoming of the IUCN categories is that they do not provide an actual ranking of species from most threatened to least threatened, which
often make difficulty for decision makers to decide species priority especially when dealing with such large number of species. Therefore, species priority setting is very important to develop for effective conservation efforts. Without system of species priority setting, fund allocation and action for conservation could not be done effectively and objectively (Given & Norton, 1993; Colyvan et al., 1999). Priority setting with ranking systems will give meaningful assistance in classifying species by urgency and vitality of each species to be conserved, as well as to deciding how to allocate limited resources to saving the many species that are threatened. These assume that some species are more important than others, in having higher priority for management action. This classification is usually based on a greater perceived threat, and often reflects imminent extinction, as well as some cultural and economic considerations (Given & Norton, 1993).

In conducting setting priority, an estimating or assessment procedure of value statements of given species has to be done (Given & Norton, 1993). Therefore, this system assumes that certain plant species will have more importance than others, depend on their specific conservation’s objectives. If the objective is to prevent extinction, critical (focal) species will get higher priority. If the objective is to conserve genetic diversity, species with many variations will be the first priority to be conserved. When the objective is to promote conservation actions and build public awareness, attractive and charismatic species (flag species) will have the highest priority. When the objective is to monitor certain habitat status, indicator species will be the best option. When the objective is to maintain ecological integrity and other species survivorship, then keystone species will get higher priority.

In conducting their tasks, botanic gardens and other conservation institutions have a number of objectives, not only conservation objective but also research, public education and tourism. Consequently, species priority has been unclear. Up to now, only 18.71% Indonesia’s threatened plant species have been collected in four botanic gardens in Indonesia (Widyatmoko, 2009) On the other hand, in related to botanic gardens’ tasks, target number 8 of the Global Strategy for Plant Conservation or GSPC (CBD, 2003) set up 60% of threatened plants has to be collected ex situ, and 10% among them has to be included in recovery and restoration programs; and more importantly, this target is scheduled for completion in 2010.

Priority setting system in conserving Indonesian threatened plant species with extinction is facing some crucial problems, which are:

1. the absence of species priority setting systems that leads funding and actions for conservation cannot be carried out objectively based on urgency, characteristics, values
and severity of problems faced by given species. Until now, species priority setting for conservation programs has been conducted only based on subjective assessment, mostly focused on limited ecological and biological information, even laid on invalid data, whilst scientific assessment ideally includes factors in distinctiveness (taxonomically and geographic distribution), population status, threats or vulnerability, ex-situ potential propagation and usefulness.

2. Method and procedure to estimate or assess value and importance of species has been unknown or applied yet, resulting the total value of given species selected for conservation program is still unsure.

3. Priority setting in species for Indonesian threatened plant species conservation program is very important to accomplish due to limitation on budget, time and man resources, whereas the number of threatened species is enormous and tend to increase.

The objectives of this ongoing project are to construct a priority setting system in Indonesian threatened plant species *ex-situ* conservation that enables people to use it consistently, to set up priority ranking for conservation of those species, and to create a Red List Data Book for Indonesia, which has never been done before, as a crucial reference to construct conservation program and action in national scale.

To obtain scientific estimation in more objective, accurate, independent and fair assessment to prioritizing Indonesian threatened plant species for conservation programs and action, a series of workshop incorporating expert panel or group specialist as well as population study of given taxa would be the main procedure of the priority setting systems. The result of workshops and fieldwork on population studies would be the primary source or input to construct the system of setting priority for conservation program and action of Indonesian threatened plant species. Besides, the assessment of the expert panel in workshop series would significantly contribute the making of Red List Book of Indonesian threatened plant species.

**MATERIALS AND METHODS**

This 5-year project has been started in 2008 by collecting data of Indonesian threatened plant species under IUCN criteria (IUCN, 2008), WCMC (WCMC, 1997), other hard publication (Mogea *et al.*, 2001; Noerdjito and Maryanto, 2001). Thirteen families were chosen as target taxa due to their contribution to the threatened plant species list issued by
IUCN and WCMC; four families (Arecaceae, Cyatheaceae s.l., Nepenthaceae and Orchidaceae) were assessed in 2009 program, comprising 191 species (Appendix 1).

Fifteen selected experts as specialist group assessed the target taxa using 17 criteria developed by New Zealand Department of Conservation (Molloy and Davis, 1992) with some necessary modifications. This is based on five category types which indicate the distinctiveness (taxonomically and geographical distribution), population features, vulnerability to extinctions, ex-situ propagation potential and cultural values of each species (Table 1). Due to page limitation, full definitions of these criteria are not shown in this paper; one should refer to Molloy and Davis (1992) for complete explanation.

The scoring process was done by using standardized test sheet comprising 17 criteria. Each species in the target list was scored using those criteria, and all scores range between 1 (least threat) and 5 (most threat). Species scores were summed without any weighting to give total scores in order to obtain a linear ranking scheme. There were three approach used in assessment, but we present only the compromised judgment among specialist group. Each group assessed each species in related target taxa altogether for each criterion listed in test sheet. This approach did not deliver individual assessment of assessor but compromised scoring of the group that allows enrichment process among assessors.

Scored species then classified into three categories based on their total scores:

- Category A (total score >50) : species with highest priority (require immediate conservation action)
- Category B (total score 42-50) : species with second priority (their conservation action could be delayed)
- Category C (total score <42) : species with lowest priority (do not need active conservation action yet)

Process and results of the scoring of each expert were entered to the DELPHI-based software we have been designed and developed specifically for scoring and ranking process of the setting priority system. This program has also function as a relational database management system of the Indonesian threatened plant species.

A red list data book of Indonesian threatened plant species would be another outcome of the project. The book will consist of the result of scoring or assessment (including method used) from each workshop showing priority ranking for threatened plant
species conservation program, brief species description and taxonomical account, as well as their current distribution and population status.

RESULTS AND DISCUSSION

The first workshop on species priority assessment for conservation was conducted on 2-3 June 2009. Not all the threatened species on the target species list were assessed by the specialist group because of various reasons. Some are insufficient data, doubtfulness in taxonomical account, and current population distribution. However, some experts involved in the workshop add the list with some species that might have vulnerability to extinction.

Table 1. Criteria used for determining priorities for conservation program (adopted from Molloy and Davis (1992) with some modification).

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<th>Distinctiveness</th>
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<td>1. Taxonomic distinctiveness</td>
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<td>2. Geographical distribution</td>
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<tr>
<th>Population features</th>
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<td>3. Number of populations</td>
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<td>4. Mean population size</td>
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<td>5. Largest population size</td>
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<td>6. Condition of the largest population</td>
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<td>7. Wild population decline rate</td>
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<th>Vulnerability</th>
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<td>8. Legal protection of habitat</td>
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<td>9. Ex-situ protection</td>
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<td>10. Habitat loss rate</td>
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<td>11. Predator/harvest impact</td>
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<td>12. Competition</td>
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<td>13. Habitat specificity and/or nutrient</td>
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<td>14. Reproductive specificity and/or behavior</td>
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<td>15. Other factors affecting survival</td>
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<th>Propagation potential</th>
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<td>16. Ex-situ propagation</td>
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<th>Values</th>
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<td>17. Economic values</td>
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From the scoring result as illustrated in Fig.1, 100 species were classified as the highest priority to conserve (category A), indicated by their total score, whilst Category B and C includes 60 and 19 species respectively. Twelve species were drop from assessment because of insufficient information on most of 17 criteria used in the scoring process. Orchidaceae has the largest species number of the A category, which includes overall orchid species in the species target list. This means that the relevant specialist group agreed that all Indonesian threatened orchid species assessed has the highest priority to be included in immediate conservation programs. However, 9 species in the list were drop during the workshop and replaced by other 9 species considered to have more important values or criteria to be assessed.

Linear ranking of total scores lists species from highest to lowest priority (Table 2). The species with highest priority rank are *Nepenthes adnata* and *Nepenthes campanulata* (Nepenthaceae; each total score of 71), *Nepenthes talangensis* (Nepenthaceae) and *Dendrobium militare* (Orchidaceae; 68) to name some, whilst the lowest rank is *Pinanga rumphiana* (Arecaceae; 33). Although this linear ranking scheme has considerable debates in terms of priority setting application for conservation, the method is still attractive and has some advantages. This method is comprehensive because it covers some meaningful aspects such as distinctiveness in taxonomy and geographic including endemism, current population status, threats including protection management status, vulnerability to extinction, propagation potential and economic values. This method is also simple but comprising quantitative criteria that lead the technique more measurable and easier to use, and more specific because of having separated criteria for plant and animal group.
Fig 1. Summary of categorizing species for conservation priority in workshop 2009

![Bar graph showing conservation priority scores for different species](image)

However, there are some problems has arisen in using this method. Species can have the same total score but for very different reasons, whereas all criteria used have a complex nature of threats species face and of the criteria we use to determine threatened species priority lists. It is revealed that many of these threats and criteria are related, but others are not. This problem can be solved by using multivariate technique in future work, as written by Given and Norton (1993). Although the list given in Table 2 is not the final result, we now have an important reference in deciding how to allocate limited resources to saving the many species that are threatened with extinction in Indonesia. And we must underline that the list is a compromised judgment of relevant expertise using the same standardized criteria.

A DELPHI-based software is still underdeveloped. As a preliminary result, we present a few of display screen of the designed program specific for species priority setting (Fig. 2). The program was successfully designed with user friendly, interactive, simple but comprehensive. This is also one of the main outcomes of the project and will hopefully be protected by intellectual property rights in the future, after some completion, finishing, and several testing as well.
Table 2. List of Scoring Result on Target Species from Workshop 2009

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<tr>
<td>Nepenthes talangensis</td>
<td>68</td>
<td>Nepenthes adnata</td>
<td>71</td>
<td>Nepenthes campanulata</td>
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<td>Dendrobium militare</td>
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<td>mastersianum</td>
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<td>Nepenthes inermis</td>
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<td>Nepenthes clpeata</td>
<td>67</td>
<td>Paphiopedilum niveum</td>
<td>63</td>
<td>Ascocentrum aureum</td>
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<td>Phalaenopsis javanica.</td>
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<td>Paphiopedilum victoria-mariae</td>
<td>63</td>
<td>Dendrobium laxiflorum</td>
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<td>Nepenthes lavicola</td>
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<td>Phalaenopsis inscriptiosinensis</td>
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<td>Dendrobium pseudoconanthum</td>
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<td>Dendrobium ayubii</td>
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<td>Vanda jennae</td>
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<td>Paphiopedilum victoria-regina</td>
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<td>Dendrobium tobaense</td>
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<td>Arachnis hookeriana</td>
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<td>Phalaenopsis gigantea</td>
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<td>Vanda devoglii</td>
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<td>Phalaenopsis venosa</td>
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<td>Paphiopedilum supartidi</td>
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<td>Arenga distincta</td>
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<td>Bulbophyllum phalaenopsis</td>
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<td>Dendrobium capra</td>
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<td>Paraphalaenopsis laycockii</td>
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<td>Nepenthes insignis</td>
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<td>Sommiera leucophylla</td>
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<td>Cyathea magnifolia</td>
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CONCLUSION

Despite some drawbacks of the method used, the list of species priority for conservation from this 2009 workshop held by Bogor Botanic Gardens - LIPI as the national institution for Indonesian ex-situ plant conservation has a crucial role to assist managers in plant conservation-related decision-making. The list could rank the species from the most threatened to least threatened dealing with large numbers of such species for conservation without proposing to compete with the IUCN list. The project results provide an alternative solution to refine the list published by IUCN with current data revealed by relevant expertise, accompanied by a software program to aid people (assessors) assess threat and priority under the standardized criteria. These results have been contributed to the writing of the Indonesian Red List Data Book of threatened plant species for conservation.

ACKNOWLEDGEMENT

This project has been financially supported by LIPI’s DIPA. We greatly appreciate the team member Yayan WC Kusuma for valuable information and discussion and Yoga S. Sudiarsana who develops the software. The significant contribution of steering committee and expert panel in 2009 workshop are also grateful.
**Fig. 2.** The first screen of the designed program for Indonesian threatened plant species priority setting for conservation

**REFERENCES**


Appendix 1. List of Species Target to Species Priority Setting Assessment for 2009 Workshop

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<td>183</td>
<td><em>Phalaenopsis celebensis</em></td>
<td>191</td>
<td><em>Vanda jennae</em></td>
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<tr>
<td>176</td>
<td><em>Paphiopedilum victoria-mariae</em></td>
<td>184</td>
<td><em>Phalaenopsis floresensis</em></td>
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<tr>
<td>177</td>
<td><em>Paphiopedilum victoria-regina</em></td>
<td>185</td>
<td><em>Phalaenopsis gigantea</em></td>
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O-EC12

GERUGIWA (FLORES MONARCH) THEIR DISTRIBUTION AND ABUNDANCE IN RELATING TO POINT OF INTERESTS FOR TOURIST ATTRACTION IN KELIMUTU NATIONAL PARK, ENDE, EAST FLORES.

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ABSTRACT

Flores monarch Monarcha sacerdotum (Gerugiwa local name) is an endemic bird species which exist only in East Flores especially in well known Kelimutu National Park, Ende. This paper determined the trails or patches which will be used by visitors (tourists) for watching that wild endemic bird.

The distance sampling was used for collecting data in zone showed where most visitors activities are allow. The results that average flushing distances are 25 m, and there were 15 points which can be used for perching and laugh singing on the canopy. Among those points there were 7 (seven) points that can be used for tourist birds watching. The relative abundance of these birds in that utilization zone areas are about 1 (one) bird per ha.

Hopefully, this endemic bird (Flores monarch) may have a benefit for improving to the point of interest to the tourist attractions. In addition, the local communities can derive substantial economic benefits from guiding the tourists and additional income for local businesses.

Key words: Flores monarch; Kelimutu National Park; distance sampling; point of interest; zone of utilization.

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INTRODUCTION

Generally, wildlife resources have several values including an aesthetic value, an ecological value, or a recreation value, (Orams, 2000; Reynold and Braithwaite, 2001). The recreation value may be realized by establishing an recreation facilitate such as a points of interest which can be used for watching to the life of wild animal in natural habitat. This action is one part of wildlife tourism program which have already been documented in the strategic planning of Kelimutu National Park. It was an answered to the new conservation management strategies attempted to build local support for conservation initiatives by providing community development projects in the utilization or in the buffer zone adjacent to conservation areas. It hope that this action might have positive impact to the social-economic of the local people which they live in surrounding this national park.

Most wildlife tourism programs have been developed at out of Indonesia, such as in Australia, Africa, USA, and also in China (Nianyong and Zhuge, 2001) and India. Ironically, Indonesia has various wildlife species which can be used as an objects for wildlife tourism, but its almost had never been in touch. Estimated that in 1994 there were between 106 million and 211 million wildlife-related tourists worldwide, although this could have included tourists who took a wildlife or nature-based trip as part of their holiday experience (The Ecotourism Society, 1998; Newsom et al., 2004). For examples, it is a special tour for watching wild animals which they live in marine or in a terrestrial habitat, such as a komodo viewing in Komodo National Park, or a dolphin viewing in Karimunjawa Marine Park, watching bali starling in Bali Barat National Park. Bird watching is very popular hobby and forms a significant part of wildlife tourism (Jones & Buckley, 2001; Sekercioglu, 2002).

Although the wildlife-tourism and ecotourism have a high promising on social economic to the local communities (Burns and Sofield, 2001; Ceballos-Lascurain, 1996; Stecker, 1966; Abdullah and Jamaluddin, 2001; Abdullah, 2001), scientific knowledge and real actions of its implementation in Indonesia is meager. Therefore, this paper had tried for supporting any actions in wildlife tourism activities. In this context, by determining points of interest on bird watching of Gerugiwa might have an significance action in relating to the wildlife tourism purposes. Hopefully, for those people who love to see animals in the wild should much actions in promoting wildlife viewing, conserve biodiversity, foster environmental education and generate economic and financial opportunities based on wildlife resources.
MATERIALS AND METHODS

This study was carried out in the utilization zone of Kelimutu National Park, Ende, East Flores, its geographically position is 8° 43' - 8° 48' South and 121° 44' - 121° 51' East (Review on Management of Kelimutu National Park, 2009) (Fig.1.). One of fauna which most interesting in that areas was Gerugiwa (Flores Monarch) or Monarcha sacerdotum (IUCN, 2009) was a kind of wild endemic bird which has colourful of its plumage, and also produce nice and unique songs.

In order to access the occurrence and abundance of the Gerugiwa, a survey of this bird using distance sampling was carried out (Sutherland, 2000). Two representative strip-transects were established, one along the regular trail and the another one along arboretum trail, the total of 4.2 km long (4200 m). Average distances from bird perching and singing to the observer on either side of the transect of 25 m ( left and right ), Therefore, the area of strip-transect of 4,200 m X (25 m X 2) was 210,000 m² or 21 Ha. Observations on bird singing and perching were recorded from dawn till almost at noon and in the afternoon, each day. In the afternoon the weather was always cloudy and foggy and the bird disappeared. The Bird position when it had perched and sang was mapped using GPS Gamin.....The win velocity was recorded using Beaufort index. Total observations were 10 days, in dry season August of 2009. Observation was equipped with binocular Nikon, 25 X 8.

Fig.1. Study site in the utilization zone of Kelimutu National Park.
RESULTS AND DISCUSSION

Base on direct observation, the characteristic of Gerugiwa has in common is its small size, its size is similar to house sparrow, its has small legs, a short slender bill typifies the insectivorous bird (insect-eater), a short and square tail, a pointed crest on the head. Adult, has a black head as far as the chin, has a conspicuous reddish-orange the throat, a black ring upper breast (collar), greyish upperparts, has brilliant yellowish-green underparts as far as the rump, a grey tail edge with black, and has slight marking on the black wing patch is less conspicuous. This bird enable to produce a wide range of notes, which are extremely varied. The song is very characteristic for this bird, very melodious to the human ear, and thus serves as a valuable aid in identifying this wild aves.

As long as on the regular trail were found a least 12 points where the Gerugiwa mostly have perched and sang at 06.00 until 10.00 a.m. On the arboretum trail at least were found three (3) points. Each point has been occurred one bird only. Among those points there were 7 (seven) points that can be used for tourist birds watching The total estimated number of those birds around of 15. The time for perching and singing of those birds have not been different between in the regular trail and in arboretum trail. They perched on top canopy of trees, mostly on the *Casuarina yunghunia* (Cemara gunung, local name). Gerugiwa have sang very laugh (so strong) and also it have a lot of variation of song, and very melodious. Whenever this bird have been singing, it seem that there were not other bird species produced a voice. Perch site may be important as singing posts for open country passerine without aerial flights (Collin, 1981), and perch site requirements may be quite specific (Marion and Ryder (1975). Surprisingly, the Gerugiwa have been able to sing around of two hours without stop for breaking. Base on that phenomena, perhaps this bird has a certain area which have to be defended or It has a territorialism behaviour. Each birds has a certain areas which can be mapped for determining the territorial for each birds. The imaginer boundary for each birds territorial could be figure out by showing a defence behaviour and making a voice such as a laugh song whenever there was another bird enter in the territorial area. Perhaps the size of bird territories are correlated with the availability and abundance habitat requirements, such as the abundance of the food resources ( Catterall et al., 1982; Manly at al., 1993 ).

The abundance of Gerugiwa along the regular trail and also in arboretum trail, might be evenly distributed. Base on the total area of the stripe-transects, the abundance of this birds as follow; 15 birds in the area of { 4200 m x (25 m x 2) } =
210,000 m² or 15 birds in the area of 21 ha. It was about 0.7 bird per ha or almost one bird per ha. A notes, that 4200 m was the length of the stripe-transect, it be considered as the length of the area sample. The 25 m was the average distances of birds to the observers, on either side of the transect, it be considered as a width of the area sample by multi playing of 2, and the width was 25 m x 2 = 50 m.

CONCLUSION AND RECOMMENDATION

Base on the above discussion can be made several conclusions and recommendations as follow:

1. The distribution and abundance of Gerugiwa in the long of regular and arboretum trail were evenly distributed and estimated one (1) bird per ha respectively. For securing the bird populations, the bird hunting or habitat destruction should be strictly prohibited.

2. There were seven points for using of bird-watching on Gerugiwa in the utilization zone, at Kalimutu National Park. In each points for bird-watching should be developed with an adequate facilities, such as a hide, so the visitors can enter the hide without being seen by the birds. The hide itself provides and effective screen and reduce disturbance, allowing observers to watch birds for a longer period of time.

3. Perch site may be as part of a territory for each bird. As a territory may be used for protecting any significance behaviour of birds such as a breeding site, or a nesting site. This area has to be free from any disturbances. But especially for Gerugiwa, there were evidences of habituation at the existence of observers. Moreover, other studies have shown that habituated birds still show reduced breeding success in high disturbance situation (Keller, 1989).

4. Gerugiwa had been active singing mostly in the morning time, between 06.00 – 10.00 a.m., when the weather was glory. Tourists for going to Bird-watching activity can be scheduled in that time respectively.
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O-EC13

IMPACT OF THE CILIWUNG RIVER ENVIRONMENT ON ITS WATER QUALITY

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The Ciliwung River water from upstream to downstream is heavily polluted by gray water from households, together with discharges from industries, pesticides and fertilizers run-off from agricultural land. The usage of land area in the upstream area of the riverbed has poured copious amount of waste and pollution, which has risen in level, into the river every year. The water quality monitoring of the Ciliwung River was done based on the specific environment alongside the river. Monitoring was done on dry season (2007) and rainy season (2008). Water quality parameters monitored were physical characters (temperature, pH, conductivity, TSS and turbidity) and chemical characters (DO, NH₄⁺, NO₃⁻, Sulfite and Chlor). Nine sampling sides, three at upstream (Puncak), three at midstream (Katulampa, Bogor) and the other three at downstream (Manggarai) were monitored during the research. The results showed that water quality at Puncak is relatively good. The level of conductivity was high at Manggarai, which reach up of 40 at the dry season. The level of turbidity and TSS also tended to be higher at Manggarai, with level of TSS reach on the range of 150-300 ppm. The concentration of DO at all locations was not significantly difference, but its level at dry season was very low, below 1 ppm. The Concentration of ammonium (NH₄⁺) at Manggarai at the dry season was the highest (3-5 ppm), its level was above the standard levels that allowed by Government. The concentration of Sulfite and Chlor was also tend to be higher at Manggarai, at dry season those levels become on the range of 17-28 ppm and 20-30 ppm for Sulfite and Chlor respectively. From all of the data could be concluded that the pollution at downstream (Manggarai) was extremely high. The results of this research my help in giving consideration on creating a policy to regulate the environment, especially land use planning to prevent pollution.

Keywords: Ciliwung River, pollution, water quality, conductivity, sulfite, chlor

not presented
O-EC14

SOIL OF GUNUNG SALAK NATIONAL PARK
AS SOURCE FOR METHANOTROPHIC AND PHOSPHATE SOLUBILIZING
BACTERIA

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The objective of this work was to investigate the ecological role of soil microorganism
in Gunung Salak National Park as methane gas consumer and phosphate solubilizer. The
field studies was located in Gunung Salak, at 1000 m (A), 900 m (B), 800 m (C), and 700 m
asl (D). The D sampling point was tea plantation area. The soil samples were collected
randomly, and transferred to laboratory for further analyses. Microbial community structures
of methanotrophic bacteria were analyzed with culture dependent and non dependent
 technique. Microbial community of methanotrophic bacteria are quite divers which include
alpha sub class Proteobacteria and gamma sub clas of Proteobacteria. All soil has ability to
absorb methane, but soil A has the highest methane absorption capacity, and highest
methanotroph population. Several phosphate solubilizing strain were isolated from forest soil
which indicate the important of forest soil as source for microbial with divers metabolic
function. Those all indicate that forest conservation not only functioning as green house gas
absorber but also important microbial resources.

Key words: methanotrophic, phosphate solubilizing bacteria and Gunung Salak national Park
O-EC15
NUTRIENT INPUT VIA RAINFALL, TROUGHFALL AND STEMFLOW IN THE LOW MONTANE FOREST GN. GEDE PANGRANGRANGO

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ABSTRACT

The nutrient input via rainfall, throughfall and stemflow was carried out in the low montane forest in Gn Gede National Park, West Java. Rainfall, throughfall and stemflow were monitored and sampled in the natural forest in the period of June 2007 – July 2008. From this period of study showed that the precipitation from stemflow was higher compared to that of rainfall and throughfall. Rainfall and throughfall increased during November and December (early of rainy season) and that was followed by decreased pattern until March, and then increased until May 2008. pH of the rainfall was around 5.81-6.67 for rainfall, 6.16-6.78 for throughfall and that was around 6.43-6.89 for stemflow. pH of stemflow was higher than throughfall and rainfall. pH value of stemflow and throughfall was similar on March and May 2008. The input nutrient from the rainfall, throughfall and stemflow was recorded during the research. Nitrite of stemflow was recorded around 0.001-0.02 ppm, that was lower than rainfall and throughfall, was around 0.003 - 0.02 ppm. Amonium of stemflow was on the range of 0.42-1.7 ppm higher than that for rainfall (0.12-0.41 ppm) and throughfall (0.31- 0.46 ppm). For nitrate of throughfall was around 0.08-0.15 ppm higher than that was for rainfall (0.003-0.02 ppm) and stemflow (0.06 – 0.1 ppm).

Keywords: Low Montane Forest, rainfall, throughfall, stemflow and Pangrango National Park.

INTRODUCTION

Litterfall, throughfall and stemflow are important internal pathway in the nutrient cycles of tropical forest by which above-ground organic matter (litter and leachate) reaches the soil surface and nutrient are released by litter decomposition and mineralizations (Wanek et al. 2007, Whitmore 1998). The nutrient transfer to the forest soil depends on the mobility of solute and ions and the nutrients status of the ecosystems, the plant species, tree architecture and season (Parker, 1983). Tree canopies modify raindrop trajectories by partitioning the incident rainfall into throughfall and stemflow. A proportion of the incident rainfall is intercept by, and
retained temporarily on leaf surface, branches and stems. These affect to the differences of nutrient content of the rainfall, throughfall and stemflow, which were flow to the forest floor. Therefore it is interesting to study the nutrient input to the ecosystems. Ammonium, nitrate and nitrite are the nutrient to be examined in this research. There are some ecosystems types in the GN Gede Pangrango National Park, such as montane forest, low montane forest and lowland forest. These ecosystem types different on the structure and species composition, and every ecosystems type specific for transferring nutrient to the ecosystems.

MATERIALS AND METHODS

The nutrient input via rainfall, throughfall and stemflow was carried out in the lowland forest in Gn Gede Pangrango National Park, West Java. One hectare permanen plots with the size of 100 x 100 m² were set up in the lowland forest, with the elevation of 822 m als. in 2006. The ecological study were carried out for longterm period in this permanent plot. About 144 species of plants were recorded, which was belong to 87 Genera dan 49 Family. The dominants tree species were Nauclea lanceolata Bl., Maesopsis eminii Engl., Schima wallichii Ch., Dysoxylum sp. and Pterandra azurea (DC.) Burk. (Alhamd, et al. 2007). Simple equipments made of plastic jars were installed in this forest for trapping the rainfall, which were set ca. 1 m above the ground level in the 5 locations. Five equipments were set up under the canopy for troughfall and 12 set of stemflow traps were set up about 1.3 m above the ground level. Rainfall, throughfall and stemflow were monitored and sampled monthly in the period of June 2007 – June 2009. All sample were analysed for pH, amonium, nitrite and nitrate in the Laboratory of soil and nutrient in Research Center for Biology – Indonesian Institute of Sciences.

RESULTS AND DISCUSSION

From this study showed that stemflow was higher compared to that of rainfall and throughfall (Fig. 1). Rainfall and troughfall increased during November and December (early of rainy season) and that was followed by decreased pattern until March, and then increased until June 2008. That was similar pattern at the following year. If we compared between the dry and rainy seasons, showed that almost no seasonally different among them for rainfall and throughfall, while that was seasonaly different for stemflow.

There was no seasonally different of pH for stemflow, rainfall anf throughfall, but the pattern showed that pH value was low when the precipitation was high. For
example, on August the precipitation was the lowest and that was followed by the highest of pH value. The pH value opposed to that of rainfall, stemflow and throughfall.

![Graph](image1)

**Fig. 1:** Seasonal variation of precipitation (above) and pH (below) in the lowland forest of Gede Pangrango National Park.

Mean annual of rainfall, stemflow and throughfall was significantly different at P < 0.001 for rainfall and stemflow, stemflow and throughfall, while that was significantly different at P < 0.05 for rainfall and throughfall. Annual stemflow was significantly higher than rainfall and throughfall, that was 8727.28 ± 351.64, 4631.04 ± 366.74, and 3338.94 ± 270.65 mm, respectively. The annual stemflow was higher than rainfall and throughfall, this result was not agree to the research of Chuyong et al. (2004) in Africa. They found that stemflow was the lowest compared to rainfall and throughfall, this might be due to the tree architecture was different in the study site. Monthly stemflow (727.27 ± 29.30 mm) was recorded higher than that of rainfall (385.92 ± 30.56 mm) and throughfall (278.25 ± 22.55 mm).
pH value of rainfall, throughfall and stemflow were not significantly different. Mean pH of stemflow was recorded about 7.2 ± 0.08, with the range of 6.9-7.2. Mean pH of rainfall was 7.1 ± 0.05, with the range of about 7.0-7.3. And for throughfall the mean pH was recorded for 7.1 ± 0.06, the range of this value was around 6.9-7.9. This pH value was higher compare to the pH value in the sub tropic forest, which were on the range of 4.3 – 6.8 (Wang, et al. 2004).

Fig. 2: Seasonal variation of ammonium, nitrite, nitrate in the lowland forest of Gede Pangrango National Park.

The input nutrient from rainfall, troughfall and stemflow was recorded during the study period. Nitrite of stemflow was recorded around 0.001-0.02 ppm, that was lower than rainfall and troughfall, with the value was around 0.003 – 0.02 ppm. Ammonium of stemflow was on the range of 0.42-1.7 ppm higher than that for rainfall (0.12-0.41 ppm) and throughfall (0.31- 0.46 ppm). Nitrate content of throughfall was around 0.08-0.15 ppm higher than that was for rainfall (0.003-0.02 ppm) and stemflow (0.06 – 0.1 ppm). Monthly of ammonium, nitrite and nitrate were shown in the Table 1.
Table 1. Monthly ammonium, nitrite and nitrate from rainfall, throughfall and stemflow.

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<th>NO₂ (ppm)</th>
<th>Ammonium (ppm)</th>
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<td>Stemflow</td>
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<td>Rainfall</td>
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<td>Throughfall</td>
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REFERENCES


O-EC16

POTENTIAL OF MOSSES AS ATMOSPHERIC HEAVY METAL POLLUTION INDICATOR AT MOUNT KINABALU, SABAH, MALAYSIA

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This study was conducted to determine the level of atmospheric heavy metal pollution by using local mosses at Mount Kinabalu, Sabah, Malaysia. This study is the first of its kind in South East Asia. The purpose of this paper is to acquaint the readers, especially from Asia region, with bioindicator and biomonitoring based on mosses. Ergo this study produced the pioneer data of atmospheric heavy metals pollution level at Mount Kinabalu, Sabah and hope to become the ‘stepping stone’ for further analysis. The moss species studied were Leucobryum javense (Brid.) Mitt., Leucobryum sumatranum Broth. ex M. Fleisch, Trismegistia calderensis (Sull.) Broth. and Trismegistia panduriformis (C.H. Wright) Broth.. Moss bags techniques were used in this experiments. The moss samples were exposed at three difference zones (roadside, 2 km and 8 km) over a period of 0, 4, 8 and 12 weeks for three months for subsequent analysis of metal concentration (Cd, Cr, Cu, Pb and Zn). The concentrations of heavy metals in the desiccated apical shoots of mosses were determined by atomic absorption spectrophotometry (AAS). Chlorophyll concentrations were also analysed to measure the stress response of the moss. The results showed that mosses would be very effective bioindicators of environmental metal concentrations because the concentration of metal in the moss rapidly and directly reflects the metal concentrations in the ambient air.

Keywords: mosses, moss bags transplants, atmospheric pollution, metals, bioindicators

not presented
O-EC17

STAND STRUCTURE IN PERMANENT PLOT
MT. GEDE PANGRANGO NATIONAL PARK, BODOGOL, WEST JAVA

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Indonesian Institute of Sciences (LIPI)

The current issue of global warming with increasing carbon amount in the atmosphere is a main factor to conduct this research, in which this study is a part of the project to solve the problem. The study of stand structure and its composition of the 1-Ha permanent plot was carried out in the Mt. Gede Pangrango National Park, Bodogol, West Java-Indonesia, in the period of 2006 to 2009. The objectives of this study were to observe the stand structure, to know the relative growth rate of trees (RGR) over 4-year and to estimate the stem biomass and its carbon stock. Species diversity, dominance, species richness, evenness indices of tree structure and tree distribution using Morishita Index (I₀) were enumerated. We recorded a total of 140 species (93 genera and 47 families). Tree mortality rate was highest in 2008-2009 period and the recruitment rate was found only in the 2006-2007 period. The tree density and species richness decreased with increasing the diameter classes of trees indicating that there is still a good regeneration status. The Nauclea lanceolata Blume. (Rubiaceae), Dyssoxylum excelsum (Meliaceae), Maesopsis eminii (Rhamnaceae) and Pterandra azurea (DC.) Burkill (Melastomataceae) were dominant tree species with IVI 16.3; 15.8; 14.1 and 11.7, respectively. For RGR based on basal area of trees for 4-year, Garcinia parvifolia (Clusiaceae) has the fastest RGR, followed by Cinnamomum javanicum (Lauraceae), Memecylon olygeneurum (Melastomataceae) and Actinodaphne procera (Lauraceae), and the RGRs of those trees are inherently showed for juvenile trees. The estimated stem biomass was 94.1 and carbon stock using the stem volume method was to be 42.3 t ha⁻¹. The more detailed information of this study will be discussed in the presentation.

Keywords: Mt. Gede Pangrango, permanent plot, relative growth rate, species richness, and stand structure.

not presented
O-EC18

EFFECT OF ISOLATE COMPOSITIONS AND AERATION RATES ON MERCURY (Hg) BIOREMEDIATION EFFECTIVENESS BY ISOLATES Pseudomonas aeruginosa AND Klebsiella pneumonia

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Abstract

The effectiveness of mercury bioremediation very influenced by many environmental conditions. The purpose of this study is to know influences of some environmental conditions on effectiveness of mercury bioremediation by P. aeruginosa and K. pneumonia. This research mainly examined effects of bacterial compositions and aeration rates on effectivity of mercury bioremediation, based on Atomic Absorption Spectrophotometer measurement. The results showed that, after mercury bioremediation test, the consortium of P. aeruginosa and K. pneumonia were more effective than by single isolat. The effectivity of concorsium isolates was achieved 92.48% in average, compared by single isolat were 80,60%. The aeration rates of 2 vvm improved effectivity of mercury bioremediation, achieved mercury bioremediation rate was 90,19% in average, or equivalent to 13.53 ppm from early level of 15 ppm. The futher research on the effectivity of mercury bioremediation on other parameters, is still required in order to obtain the better quality of wastewater, based on water quality standard.

Keywords: Bioremediation effectiveness, bacterial consortium.

1. Introduction

Many mercury (Hg) was used in some aspects of life, such as for electric component, gold and silver extraction, making amalgam, rusty resistant compound, photography, chemical industry which product chlorine gas and chloride acid, pesticide, the content of thermometer, barometer, aerometer, electric contract, and the other chemist-physic equipments (Goldwater, 1972; Effendi, 2003). Mercury can compose amalgam with most of metals, include gold. Mercury affinity on gold was very strong (Holland, 1980), and was used to separate gold from the other metals (Beard, 1987; Brepohi, 2006).

Mercury belonging of six compounds that most poisonous among the six million substations in nature (Nascimento & Souza, 2003). Its poisonous because had strong affinity to organic compound, that contain sulfur such as enzymes and proteins. This compound was very poisonous for biologys system (Nies, 1999;
Nascimento & Souza, 2003). Mercury could exposed to human through direct contact with skin, respiration, and absorption. The high exposure of this compound, could be hazardous to brain, liver, kidney, lungs, and human antibody system, in all of age (Palar, 2004).

The contaminated environment by the mercury wastewater was very important to overcome as soon as possible, because after it was released to the environment, this compound could stay at there until years of decades (Wagner-Döbler et al., 2000). The contamination of this mercury could be decreased by bioremediation method, because this method proved more efficient and economic than the physical or chemical ways (Beard, 1987; Suhendrayatna, 2001). The bioremediation effectiveness was influenced by the environment factor, include the oxygen availability, humidity, pH, temperature, organic material, contact time, and microbial composition (Anas, 1997; Suhendrayana, 2001; Vidali, 2001).

The result of prior research had been isolated and selected the mercury reducing bacterial from the gold mining area in Kahayan River, Central Kalimantan. There were 8 potential of bacterial isolates which were found, that prove could decrease mercury until average 15 ppm in concentration, from liquid media (Neneng, 2008). This research, used two isolate Gram negative bacterial, from the prior research. Based on the identification result by using Kit Microbact System, known the isolates were Pseudomonas aeruginosa with resemblance with knowing species was 99, 53%, and Klebsiella pneumonia, with resemblance was 80, 58%.

The knowledge about optimal environment condition, was really needed to enhance bioremediation effectiveness, especially if indigenous bacteria isolates which was used different with knowing strain that found before. This research purposed to assay the influence of three environments variables, such as: the variation of isolate compositions and variation of nutrition compositions on effectiveness of mercury bioremediation, by bacterial isolates from Kahayan River, Central Kalimantan.

2. Experimental

2.1 Experimental materials: a) Bacterial Isolates, such as: Pseudomonas aeruginosa and Klebsiella pneumonia. The isolate compositions in experiment treatment were: coding by KHY0 (without isolate), KHY2 (P. aeruginosa), KHY3
(K. pneumonia), and combination isolate between KHY2 + KHY3; b) aerator; c) nutrition composition which tried out in experimental research was mixing between coconut water and sugar in several comparison levels. The election of this nutrition was based on the result of selection in pre-experiment phase, comparing with several kinds of the other nutrition sources. In this research the comparison between coconut water: sugar, is 75%: 5 g/l.

2.2 The preparation of bacterial isolate was done to consider with the treatment condition. The incubation was done in 26°C, as long as 2 x 24 hours. Then, mercury was added into the media until the highest concentrations which could tolerate by the chosen bacterial isolate. The incubation time after mercury addition was done as long as an hour, with purpose there was an enough time for making contact between mercury ions with the bacterial cells.

2.3 Aeration Rates which tried out in pre-experiment level was aeration: 0; 0,5; 1; 1,5; and 2 vvm (volume of air / volume of wastewater /minutes). The aeration supply was done by using rubber-tube which was connected with aerator. The rates of air flowing was controlled in aerator. Based on the result of pre-experiment, was decided two level of aeration rates which used in the experimental research were 0 vvm and 2 vvm.

2.4 Mercury Bioremediation Effectiveness was measured based on percentation (%) the mercury concentration before and after incubated in an hour in liquid culture of bacterial isolates, which used as mercury reducer. The measurement of mercury level was done by using AAS.

2.5 Data Analysis to test the hipotesis by ANAVA Univariat, and continued with LSD test to treatment which had a significant effect.

3. Results and discussion

3.1 Effect of Bacterial Isolates Compositions Treatment on Mercury Bioremediation Effectiveness

Fig. 1 shown, mercury bioremediation effectiveness by isolate consortium treatment (KHY2 and KHY3) more effective than single isolate (KHY2 or KHY3 only). The analisys result of statistics showed that the treatment of bacterial isolate composition has a very significant effect in mercury bioremediation effectiveness.
3.2 Effect of Aeration Rates on Mercury Bioremediation Effectiveness by Bacterial isolates

Aeration rate treatment 2 vvm more effective to enhance mercury bioremediation effectiveness by bacterial isolates comparison with aeration rate 0 vvm. In aeration treatment 2 vvm achieved 90, 19% in average, while in aeration treatment 0 vvm the average was 78, 93% (Fig. 2).

3.3 Interaction Effect of Bacterial Isolates Compositions and Aeration Rates on Mercury Bioremediation Effectiveness

Fig. 3 shown that average of mercury bioremediation effectiveness in treatment combination of isolate and aeration (2vvm) was 90, 19% or 13, 53 ppm. While, in combination treatment of isolate without aeration addition (0 vvm) was
78.93% or 11, 84 ppm. Effect interaction treatment of isolate composition and aeration rates based on the statistic test, is not significant.

![Graph](image)

Fig. 3. Interaction Effect of Bacterial Isolates Compositions and Aeration Rates on Mercury Bioremediation Effectiveness

### 3.4 Discussion

Several members of *Pseudomonas* sp had been used as the mercury reducing bacteria in bioreactor at industry scale in German, and had ability to reduce mercury ion in concentration ≤ 10 ppm (Wagner-Döbler *et al.*, 2003). The *Klebsiella pneumonia* bacterial M426 had been reported by Essa *et al.* (2002), potential to decrease heavy metals pollution in environment, especially cadmium, lead, palladium and mercury through production an restriction agent in form of DMDS (*dimetildisulfida*).

Result of this research supported by Suarsini (2007), which found that the treatment of three species consortium bacteria, that were *Pseudomonas stutzeri, Pseudomonas aeruginosa,* and *Serratia liquefaciens* more effective in degradation liquid wastewater from the household (LCRT), compared with the single isolate treatment. Malatova (2004) also found that application of bacterial consortium of two isolate strains, more effective in increasing the degradation of oil and also lead at the processing of industri organic wastewater. Canstein *et al.* (2002), showed that single isolate sensitive to quick addition of mercury concentration in wastewater, however the consortium of culture biofilms showed high mercury efficiency, which was not effected by mercury addition continually in high concentration.

Based on the result treatment of aeration rates, appeared that mercury reduction activity had been fasted by aeration addition. This result showed that
oxygen was needed in mercury bioremediation process in liquid media. According to Mellor et al. (1996), aerobic bioremediation usually was more chosen, because its ability to degrade pollution 10 until 100 times was faster than anaerobic bioremediation. Cell energy in ATP form also more resulted in aerobe respiration aeration than if it compare with anaerob respiration. This finding was supported by several prior research reports, i.e.: aeration addition 20 l/minutes accelerated phenol degradation (Martani, et al., 2000). *Pseudomonas* bacterial was known could reduction Hg\(^{2+}\) ion become Hg\(^{0}\) element in aerobe condition (Robinson & Tauvinen in Lovley, 1995). The fission of aromatic ring needed oxygen (Atlas & Bartha, 1993). Both of mercury reducing bacterial was used in this research, such as *P. aeruginosa* and *K. pneumonia*, known had aerobe characteristic.

4. Conclusion

4.1. Consortium bacteria such as *Pseudomonas aeruginosa* (KHY2) and *Klebsiella pneumonia* (KHY3), more effective to eliminate mercury ion in water, comparison by using single isolate.

4.2. Aeration rate 2 vvm in the culture media more effective to increase mercury bioremediation, than without aeration (0 vvm).

REFERENCES


O-EC19

EFFORTS OF EX-SITU CONSERVATION OF SOME CULTIVARS OF CLOVE
Syzygium aromaticum (L.) MERRILL ET. PERRY AND ITS RHIZOSPHERE
MICROORGANISMS FROM TERNATE AND SAPARUA, NORTH MALUKU

R. P. Sancayaningsih¹, A. Indriyanto¹) and E. H. Poentyanti²)
¹) Department of Biology GMU, ²) retpeni@yahoo.com Balittro, Deptan.

Syzygium aromaticum (L.) Merrill. Perry cv “afo” is an endemic clove that very productive and may grow for hundred years and almost extinct. It is found in Air Tege-tege, Gamalama, Ternate, North Maluku. While variety of “hari-hari” clove flowers seasonal-independently, and it is only found endemic in Saparua, Maluku. Ecologically, germplasm conservation of endemic species deserves both 2 conservations, in vitro plant propagation and conservation of their rhizosphere mutualistic microorganisms. This study aims to conserve both plant and its mutualistic microorganisms of the rhizosphere.

Shoots, microspores, and epicotyls from varies maturity cannot be used for explant inisiation directly, due to easily contaminated and browning problems. Shoots from seed rejuvenation of afo, and hari-hari were used for explant propagation using MS modified medium. Calli were produced from leave explant of in-vitro seedling of afo and hari-hari. There were 5 isolates of phosphate bacteria isolated from soil samples taken from 12 clove varieties’ rhizospheres. Mycorrhizal colonizations were low in test plant and were unstable during the first and second multiplications, except colonization produced by inoculant taken from soil rhizosphere from radja, sikotok and anak afo that ranged from 20% to 33 %.

Key-words: mycorrhiza, clove cv afo, hari-hari, radja, and sikotok
O-EC20

FEEDING GROUP ZONATION OF EPHEMEROPTERA (INSECTA) AT PLALAR-GREMENG RIVER, GUNUNG SEWU KARST ECOSYSTEM, GUNUNGKIDUL, YOGYAKARTA SPECIAL PROVINCE, INDONESIA

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Abstract

The objectives of this research were to record the changes in composition of the community of Ephemeroptera in Plalar-Gremeng River, Gunung Sewu, Gunungkidul, to examine the causes of these changes, and to discover their significance in the life of the rivers. The ephemeropteran community and abundance of the feeding group (detritivore, gatherer, and scrapper) was compared with the nutrient and water quality of each sampling site in the rivers. It is established that, under site conditions, nutrient status can be regarded as the chief internal factors.

The method was field survey for sampling the substrate at five sites: 1. Up stream of Plalar River; 2. The site before the Plalar Cave; 3. The total dark zone of Plalar-Gremeng Caves; 4. The site after Gremeng Cave; and 5. The down-stream of Gremeng to Beton River. Substrate sampling used Surber benthic sampler (25x25 cm), the sample was containing and labeling. Environmental parameters were measured water temperature; carbon dioxide; velocity; pH; C-organic; P and N substrate; and Ca content. The data was analyzed by description. The results were showed the abundance of Ephemeroptera highest at site-4 (Gremeng River) and the lowest at site-3 (in the dark zone of the cave).

The zonation studies showed that Ephemeroptera play a highly important roles in preserving nutrient status. Ephemeroptera are dominates a subsystem that retard nutrient dynamic, water quality degradation, and thus they play a prominent role in the karst water bodies.

Key-words: feeding-group, Ephemeroptera, Plalar-Gremeng River, karst ecosystem, Gunungkidul
INTRODUCTION

The task of this research which was carried out between October – December 2005 was to study the qualitative and quantitative a composition of the sediment-dwelling genera of ephemeropteran fauna in Plalar-Gremeng River. Factually, based on longitudinal axis both Plalar and Gremeng were one river. But up-stream and down-stream bordered by the cave in small hill. These presentation for the evaluation and zonation of the feeding group. The change was occurring in the nutrient and water quality. A review of literature on the sediment dwelling organisms especially ephemeropterans have already been given (Devai, 1999). This paper aims at providing a summarizing evaluation of previous results. One of the trophic level is detritivore (insect larvae, worms, and crustaceans). Goldman & Horne (1983) explained, that detritivore is the organisms which digest and recycle a portion back into the overlying waters. Kukula (1997) informing about life-cycle of three species Baetis alpinus, B. melanonyx and Rhitrogena iridina at Wolosatka and Tereboviec streams, Bieszczady National Park, southeastern Poland. Baetis alpinus took various courses depending on environment condition. Baetis sp. has 1 – 4.9% average percentage contribution (Jackson, 1978).

MATERIALS AND METHODS

The Ephemeroptera larvae were collected from Plalar and Gremeng river at Gunung Sewu karst ecosystem, Ponjong, Gunungkidul, DIY. The sampling location lies in the eastern part of the Gunung Sewu geological formation and is underline by limestone area. Sampling site of the river (110° 45’ 30” E and 7° 58’ 30” S) about 16.5 km from Wonosari (center of the local Gunungkidul government).

Attention was mainly focused on describing differences along the longitudinal axis of the Plalar-Gremeng River. The sampling design was Stratified Purposive Random Sampling (Devai, 1990). The river in this area was surveyed and could be devided into five section with characteristic condition. About the five sites based on the water current, from up-stream to down-stream of the river here:

Site-1: the up stream of Plalar River;

Site-2: the site before the Plalar Cave;

Site-3. the total dark zone of Plalar-Gremeng Caves;
Site-4. the site after Gremaeng Cave; and

Site-5. The down-stream of Gremaeng to Beton River.

The benthic macro-invertebrate fauna was collected a Surber sampler which sampled an area 25 x 25 cm to a depth 3 cm (Brower et al., 1998). The net bag had and mesh size of 250 µm, the sample was containing and labeling. Weekly sampling was carried out on typical riffle on the river. The total content of the Surber-net was washed into a sample container; the sample were isolated and preserved in 2% formaldehyde (Wetzel & Likens, 1991). In the laboratory, the ephemeropteran were separated from another organisms. Finally the sample was identified in binocular and supported the check-list.

Environmental parameters were measured water temperature; carbon dioxide; velocity; pH; C-organic; P and N substrate; and Ca content. The data was analyzed by description.

RESULTS AND DISCUSSIONS

In Table 1. showed community of ephemeropteran in the Gremaeng River, the one of river in karst ecosystems, Gunungkidul, Yogyakarta Special Province, Indonesia. There was the three main feeding behavior organisms of Order Ephemeroptera. The three feeding behavior are detritivore, gatherer, and scraper; but there is an organism as detritivore, gatherer, and scraper in functional; this is Caenis. Centroptilum, Baetis, and Leptohyphes, all of them as detritivore in trophic level. Gatherer organisms at the sampling site are Atalaphlebia, Siplonurus and one un-identified genus is called Genus A.

Table 1. Members of Ephemeroptera all research

<table>
<thead>
<tr>
<th>Feeding Group</th>
<th>Family</th>
<th>Genera</th>
</tr>
</thead>
<tbody>
<tr>
<td>Detritivore</td>
<td>1. Baetidae</td>
<td>a. Centroptilum</td>
</tr>
<tr>
<td></td>
<td></td>
<td>b. Baetis</td>
</tr>
<tr>
<td></td>
<td>2. Tricorythidae</td>
<td>c. Leptohyphes</td>
</tr>
<tr>
<td>Gatherer</td>
<td>3. Leptophlebiidae</td>
<td>d. Atalaphlebia</td>
</tr>
<tr>
<td></td>
<td></td>
<td>e. Genus A</td>
</tr>
<tr>
<td>Feeding Group</td>
<td>Family</td>
<td>Genera</td>
</tr>
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<td>---------------</td>
<td>----------------</td>
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</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Detritivore</td>
<td>1. Baetida</td>
<td>a. Centroptilum</td>
</tr>
<tr>
<td></td>
<td></td>
<td>b. Baetis</td>
</tr>
<tr>
<td></td>
<td>2. Tricorythida</td>
<td>c. Leptohyphes</td>
</tr>
<tr>
<td>Gatherer</td>
<td>3. Leptophlebiida</td>
<td>d. Atalophlebia</td>
</tr>
<tr>
<td></td>
<td></td>
<td>e. Genus A</td>
</tr>
<tr>
<td></td>
<td>4. Siplonuridae</td>
<td>f. Siphlonurus</td>
</tr>
<tr>
<td>Scrapper</td>
<td>5. Heptagenida</td>
<td>g. Heptagenia</td>
</tr>
<tr>
<td>Detritivore, Gatherer, Scrapper</td>
<td>6. Caenidae</td>
<td>h. Caenis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Total</td>
</tr>
</tbody>
</table>

The abundance of ephemeropteran members recorded in Table 2. show that the feeding group benthic fauna is dominated by gatherer 455 individuals; detritivore 270 individuals; and scraper only 14 individuals. While Caenidae all functional (detrivore, gatherer and scraper) 6 individuals only. The results were showed the abundance of Ephemeroptera highest at site-4 (Gremeng River) and the lowest at site-3 (in the dark zone of the cave).
Percentage of abundance based on sampling site could be show at Figure 1. The biggest percentage of ephemeropteran at site-4: 27.52%, and than site-5: 25.64 %; site-1: 24.70 %; site-2: 21.21 % just smallest at site-3: 0.94 %. Both the site-1 and the site-4 relatively the same in percent maybe because the same condition ie. open water so radiation through to the substrate.

![Figure 1. Abundance (%) of ephemeropteran based on sampling site](image)

- Site-1: up-stream of Plalar river (24.70);
- Site-2: before the mouth of Plalar cave (21.21);
- Site-3: inside cave (0.94);
- Site-4: after the mouth of Gremeng cave (27.52);
- Site-5: main stream, Beton River (25.64)

Figure 2. in the next inform the percentage of abundance based on feeding group showed the biggest percentage of ephemeropteran is gatherer: 55.04%, and than detritivore: 41.86 %; scraper: 2.17 %; and the smallest: 0.93 %. Water temperature is limiting factor for organisms existence. Brittain (1990) cit. Kukula (1997) claim that water temperature is a major factor determining egg development and nymphal growth. *Caenis* (Caenidae) was supposed its very sensitive to the temperature condition because all of site are very shallow becoming rare in abundance.
In Figure 3. detritivores was dominate at site-4 there was 172 individuals especially both genera *Centroptilum* and *Baetis*, while *Leptohypes* dominate at site-2. *Centroptilum* and *Baetis* was the same family – Baeitidae – so similar in the sub-habitat. Another group is getherer was dominate at site-5 there was 181 individuals. The site-5 is the site in Gremeng river near by the main stream Beton River so many nutrient from the up-stream of the watershed. Beside that the scrapper organisms only one genus *Heptagenia* very rare in abundance, the total individuals highest only six individuals. In general discuss the individuals dominate, there was depend on sampling sites the highest is site-4: 205 individuals and than site-5: 191 individuals; site-1: 184 individuals; site-2: 158 individuals; and the rarely site-3: 7 individuals only.

The organisms which more than one type of feeding habits is very rare in this research. This case was supposed there is correlation with substrate or ecosystem type because the water body in limestone condition.
Figure 3. Feeding group zonation of Ephemeroptera in Gremeng River

det–detritivore; gat–gatherer; scr–scrapper; dgs–all of type

axis – sampling site; ordinate – abundance of feeding group (individual)

Table 3. show the data of environmental parameters there was difference scale each the site except pH and water temperatures (minus site-5). Carbon dioxide at site-3 and site-5 was higher than another site because inside of the cave and high in abundance of ephemeropteran. Karst ecosystem developed by limestone so it is normally, pH between 8.39 – 8.70. Ca contents was going-down from up-stream to down-stream (site-1 to site-5) was supposed that limestone erodibility more little at down-stream.

Tabel 3. Enviroment parameters and abundance of ephemeropteran

<table>
<thead>
<tr>
<th>Parameters/Sites</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water temperature (°C)</td>
<td>25.50</td>
<td>25.44</td>
<td>25.00</td>
<td>25.60</td>
<td>28.65</td>
</tr>
<tr>
<td>CO2 (ppm)</td>
<td>5.13</td>
<td>5.63</td>
<td>7.98</td>
<td>4.83</td>
<td>9.28</td>
</tr>
<tr>
<td>Velocity (cm/s)</td>
<td>30.09</td>
<td>11.22</td>
<td>6.20</td>
<td>40.09</td>
<td>5.55</td>
</tr>
<tr>
<td>pH</td>
<td>8.46</td>
<td>8.56</td>
<td>8.55</td>
<td>8.70</td>
<td>8.39</td>
</tr>
<tr>
<td>C-org (%)</td>
<td>0.55</td>
<td>0.73</td>
<td>0.18</td>
<td>0.18</td>
<td>0.18</td>
</tr>
<tr>
<td>N-substrate (ppm)</td>
<td>0.011</td>
<td>0.01</td>
<td>0.007</td>
<td>0.008</td>
<td>0.004</td>
</tr>
</tbody>
</table>
The influence of nutrient (N and P content) is not clear among the five sampling sites. Gradation in substrate makes difference in environmental parameters and finally in community of the benthic organisms. These cases was similar with the characteristic gradient of the sediment quality in the Sempor dam showed different benthic community (Hadisusanto, 2008).

CONCLUSIONS

According to the results there are conclusions:

1. There were six family and eight genera.
2. There was community converted (distribution, abundance, and composition) of the ephemeroptera in each site.
3. The trophic feeding group was dominated by gatherer type: Atalophlebia, Genus A, and Siplonurus.
4. The highest abundance at the site-4, the sampling location between Gremeng Cave and main stream Beton River.
5. There was one genus as detritivore, gatherer and scapper.

ACKNOWLEDGEMENTS

I should like to thank the students who assist the research Ari and Ifa.

REFERENCES


THE CAPACITY OF *Ceratophyllum demersum* L. 
AND *Scirpus grossus* L.F IN ACCUMULATING CHROMIUM FROM WASTE WATER

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Submerged aquatic plant *Ceratophyllum demersum* L. and emergent wetland plant *Scirpus grossus* L.f were treated with waste water containing chromium at concentrations of 0.26 ppm, 0.66 ppm, 2.32 ppm, and 2.64 ppm. The two species were planted in combination of mixed and monoculture. After 14 days of exposure to the waste water, *C. demersum* was able to accumulate chrom as much as 290.72 ± 7.19 mg/kg dw, while *S. grossus* accumulated 145.80 ± 7.49 mg/kg dw, both when the plants grown separately and exposed to 2.64 ppm of chromium. However, highest bioconcentration factor (BCF) was achieved when both plants were exposed to 0.26 ppm of Cr, i.e. 823.29 ± 20.06 for *C. demersum*, and 300.39 ± 30.06 for root of *S. grossus*. The two plants can be categorized as Cr accumulator for phytoremediation of waste water at medium level. Beside that, the two plants seem to accumulate more Cr in their tissues when grown separately in monoculture, compared to that when grown in mixed culture.

Keywords: Ceratophyllum, Scirpus, chromium, phytoremediation
O-EC22

INVASION OF WATER HYACINTH (Eichhornia crassipes) AND GOLDEN APPLE SNAIL (Pomacea canaliculata) IN MANGROVE ECOSYSTEM OF SEGARA ANAKAN, CENTRAL JAVA

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Segara Anakan lagoon is in the process of changing from mangrove ecosystem to freshwater wetlands due to the heavy siltation. At present two third of the lagoon has been filled up by sediment. In the 2002 during the rainy season, the salinity was 0 ppt, and the invasive species, water hyacinth (*Eichhornia crassipes*), was present. In contrast, during dry season, the salinity was between 20 – 32 °/oo. The purpose of this research is to reveal the invasive species in-to this mangrove ecosystem. We conducted the study during rainy and dry seasons of 2007. The water quality of salinity, temperature, pH, DO, NH₄, NO₃, and PO₄ were measured. Samples were taken from upper-lower and upper-middle zones of mangrove using the quadrat plot of 5m x 10m with two replicates in each study site. During the rainy season of May 2007, *E. crassipes* and *Pomacea canaliculata* were present in the lagoon. This indicated that the mangrove ecosystem of Segara Anakan was in the process of changing to freshwater-wetland ecosystem. The salinities during the rainy season were between 0 - 4 °/oo (hypo-haline). In contrast, during dry season especially from September to October 2007, the salinities varied between 39 - 40 °/oo. Thus the variation of salinities was seasonals and not daily. This was a combination of responses between the heavy siltation and the global warming. In the future if this condition persistent, both of these invasive species will threat the indigenous species in the succession processes of freshwater-wetland communities.

Key words: salinity, invasive species, hypo-hyperhaline, brackish estuary, global war

INTRODUCTION

Mangrove ecosystem of Segara Anakan, 24,000 ha, is in the process of change due to heavy sedimentation (Djoahan 2002; Djoahan 2007). Each year this ecosystem received 4.5 million tons of sediment from the disturbed watershed of Citanduy River, and was predicted that this ecosystem will change to other ecosystem (Napitupulu and Ramu 1982). The process of change of this mangrove ecosystem was recognized. Djoahan (2002) reported that during rainy season the water salinities of the Segara Anakan mangrove varied between 0- 4 °/oo. In the brackish water, usually the salinities varied daily between 4-35 °/oo. The sedimentation created a lot of newly formed lands, prograding lands, and shoals in the lagoon. These conditions disturbed the tide periods (Hamidjojo 1982; Sutomo 1982). The changing of this mangrove
ecosystem was characterized by the invasion of mangrove propagules, mangrove and aquatic macrophytes (Djohan 2002).

In the 1997, the mangrove succession in the Segara Anakan lagoon was dictated by the available propagules from adjacent mangrove forest. The Rhizophora was the threatened genus, and primary succession in occurred at mudflats, fringe mudflat (lower zone), and prograding lands. While the secondary succession occurred at mangrove gap canopy. Aquatic macrophyte, Fimbristilis sp. colonized the north-western of the Segara Anakan, which were at Bondan waters and mangrove forest at Tetel (Djohan 2007).

In the 2002, Cyperus digitatus, C. Malaccensis, C. litoralis, and grass of Eriochloa subglabra colonized wide areas and dispersed to the middle of the lagoon and as well as the abandon shrimp ponds. In contrast, in the upper zones of mangrove forest was dominated by 100% of Acanthus ilicifolius and Derris heterophylla. During the rainy season March to April, the salinities were 0 %/oo, and at the northern Bondan-Wates was dominated by Eichhornia crassipes (water hyacinth). But we did not find the Pomacea canaliculata (golden apple snail). In contrast, during the dry season, the salinities varied between 20 – 32 %/oo, and this E. crassipes was absent (Djohan 2002). Kateregga and Sterner (2007); Wunder (2005); Mercado (1979); Julian et al. (1999) reported that E. crassipes was an macrophyte aquatic species and very invasive.

This species thrived in high temperature of 28-30°C full of sun light in the river which had slow current. This thrive population damaged the stream and stop the water drainage. They also disturbed the sails of the canoes and boats in the river. Their origin was from Amazon River, Brazil, Central America. Eichhornia crassipes was introduce species to Indonesia in 1894, as a species collection, through Bogor Botanical Garden. Sulistyawan et al. (2003) reported, around the 1990, this water hyacinth had dispersed to oxbow lakes of Moro watershed in Merauke Papua. In 1995, World Wide Fund (WWF) released insects of Neochetina bruchi dan N. eichorniae, predators, to control the dispersal of this water hyacinth. Results from LANDSAT TM imageries of 10 November 1990, 26 January 1998, and Landsat ETM 25 October 2001 showed the N. bruchi dan N. eichorniae had succeeded in controlling the dispersal of water hyacinth in Moro watershed in Papua and suppressed its present.

E. crassipes grew very well in freshwaters, but in brackish water they only had tolerance to salinity for 0-6 hours. They cannot tolerate the brackish water for long time, and they also cannot lived at higher salinity (Mercado 1979; Julian et al. 1999). In Africa Wilson et al. (2007) reported that E. crassipes was present at the first time on Lake Victoria in 1989, and reached the peak in 1999, where thousand hectares of the lake surface was covered by this aquatic plant population. Eventhough they had released the bio-controlled agent, there was a possibility the population of E. crassipes returned from the deposited seed on the lake sediment. Their growth was also influence by nitrate and phosphate from the agriculture runoff.
In California E. crassipes invaded and threatened the salmon migratory on Sacramento River and delta of San Francisco Bay. Wunder (2005) reported even though this species had wide tolerance to environmental factors, but the salinity limited their distribution on the ebb-tidal streams. The saline mortality for this population was between 6-8 °/oo. Its recovery was high when the population was exposed in short-term to low salinity between 5-7 °/oo in period of 14 days. But if the population was exposed to salinity of 7 °/oo for 21 days, this E. crassipes was not able to recover at all.

**Threat of Golden apple snail (Pomacea canaliculata)**

Golden apple snail (Pomacea canaliculata) was native species of South America, and lived in ponds, marshes, streams and rivers. They dispersed widely in Amazon Inferior Basin and Plata Basin from South Brazilia, Argentina, Bolivia, Paraguay to Uruguay. In North America, it invaded from Texas, Florida to Ohio Tengah (Anon. 2007). Naylor (1996); Teo (2006); dan Halwart (1994) reported that in 1980, this P. canaliculata was introduced intensely into some Asian countries in attempt to increase protein source of local and export commodity. But since then they invaded rice fields and become pest to the young rice. This snails were spread widely through irrigation canals. In 1986, they invaded rice field in Taiwan around 171.425 ha, Japan was 16.196 ha, and Philippines was 400,000 ha. Naylor (1996) reported that economically, this invasion of Golden apple snails caused economic loss around 425 – 1200 millions USD. This was not included the cost of human and ecosystem health due to the molluscide used for controlling this pest. Teo (2006) reported that Cyprinus carpio and African catfish (Clarias gariepinus) were predator potential as bio-control for P. canaliculata in rice field. This fish consumed only the juvenile golden apple snail. The young rice only can tolerate to rice seedling after its over the age of 40 days.

The present of invasive species of freshwater, E. crassipes and P. canaliculata, in Segara Anakan mangrove ecosystem indicated that Segara Anakan mangrove was in the process of change from brackish water to freshwater wetland ecosystem. In this research we questioned: 1. How far was the invasion of both E. crassipes and P. canaliculata in the Segara Anakan mangrove ecosystem? 2. Did the present of E. crassipes and P. canaliculata in this ecosystem was found in in the two seasons, both dry and wet seasons; 4. Did the present of P. canaliculata followed the present of E. crassipes? 5. Did the physico-chemical factors, water salinity, pH, temperature, DO influence the present of these both invasive species? The purpose of this research was to reveal the invasion of E. crassipes and P. canaliculata into the brackish water ecosystem of Segara Anakan mangrove. We hypothesized that the presence of these species, E. crassipes and P. canaliculata, indicated that the Segara Anakan mangrove was in the process of changing ecosystem from tidal-brackish wetland to freshwater wetlands.
METHODS

Time and location of study – This research was conducted during the rainy and dry seasons from April to October 2007, which was carried out on 14-16 April; 18-20 May; 25-26 June, and 9-10 September and 20-21 October 2007. Based on the input of freshwater from Citanduy River into the lagoon, there were 4 locations of the study area. These locations were mangrove forest of: 1. Tetel; 2. Bondan-Wates-Klaces; 3. Motean; and 4. Kali Gatal. The location 4, Kali Gatal, was assumed as non disturbed mangrove areas from tide periods. This location was out-side of the Segara Anakan Lagoon and next to the Kembang Kuning River. The marine water of Indian ocean flows through the south-east Kembang Kuning River (Fig. 1). Mangrove at Kali Gatal was riverine and fringe mangroves. Bondan – Wates located at the northern of Klaces and was very influenced by input freshwater of Citanduy River. The marine water from Indian ocean flows.

Fig. 1. Studied locations at the Segara Anakan Mangrove of Bondan-Wates-Klaces, Motean, and Kali Gatal. The mangrove location at Kali Gatal was out-side of Segara Anakan Lagoon. trough Selok Jero into the lagoon. The mangrove of Bondan-Wates-Klaces was overwashed mangrove. In the 2003 mudflat of Bondan-Wates waters was dredging, but during this studied, this areas was shallow again.

In this study, based on the mangrove zones, each location was separated into two zones: upper lower zone and upper midle zone. On each zone, percentage of coverage of *E. crassipes* (water hyacinth) was sampled using quadrat plot of 5m x 10m with 2 replicates. Then the present of number individual of *Pomacea caniculata* (golden apple snail) was also counted. The water qualities of Bondan, Klaces, and Kali Gatal, which included salinity, DO, temperature, pH were measured. In each location, we used sample composite from two replicates.
RESULTS AND DISCUSSIONS

Results showed that the present of *E. crassipes* and *P. canaliculata* in the mangrove ecosystem of Segara Anakan was found only during rainy season up to June 2007. However at dry season, we did not find at all the *E. crassipes*, and *P. canaliculata*. We only found the dead shell of golden apple snails. The distribution of both these species was influenced by the input of freshwater from the Citanduy River and other streams from Java island. But there were no influence from the rivers of Nusakambangan. This condition was indicated by the domination of *E. crassipes* at the upper lower zones of mangrove forest of Tetel, Bondan-Wates-Klaces. In contrast, at Kali Gatal, the present of *E. crassipes* at Motean dan Kali Gatal mangrove was very low. During rainy season, *E. crassipes* was found abundance at upper-lower zone, but they did not present on either the mudflat or the upper-upper zone. This condition was due to the mudflat area was as opened-land and there was no the seedling of mangrove. Moreover, during high tide, the

![Fig. 2. The distribution of water hyacinth (*Eichornia crassipes*) and golden apple snails (*Pomacea canaliculata*) populations, and the water quality of salinity, dissolved of oxygen, temperature, and pH in the mangrove ecosystem of Segara Anakan, Cilacap. The distribution of both invasive species from mangrove forest of Tetel, Bondan-Wates, Klaces, Motean and Kali Gatal.](image-url)

water depth on the mudflat was higher compared to the upper-lower zone. In contrast, the upper-lower zone was the boundary between mudflat zone and the upper-upper zone. Because the *E.*
The presence of *E. crassipes* was difficult to develop (Fig. 2). In contrast, at the mangrove of Klaces, the coverage of this water hyacinth was around 30%, and at Motean was 15%. Furthermore, we did not find at all this species at mangrove of Kali Gatal. This indicated that mangrove of Kali Gatal had the regular tide periods.

The present of *P. canaliculata* population in the Segara Anakan mangrove followed the present of population of *E. crassipes* with number of individuals per 100 m² in consecutively: at the mangrove of Tetel was 20 individu; Bondan-Wates was 20 individu; Klaces was 10 individu; and Motean was 5 individu. It is interesting to note that in the mangrove Bondan-Wates on the stem of *Achantus ilicifolius* there was the eggs of *P. canaliculata*. This eggs was attached to the stem during May 2007. The present of the eggs of this golden apple snails indicated that during the rainy season the *P. canaliculata* population had reproduced and developed.
Fig. 3. Number of rainfalls and number of rain days at Kawunganten District and Cilacap Regency in 2006 and 2007. The lowest rainfall between June and September 2007. The month of December had the highest rainfalls and the number of rain days (Agriculture and Live Stock Service of Cilacap Regency 2008).

The water quality of Segara Anakan lagoon reveals the high-monthly variations between rainy and dry seasons. During the rainy season on the April – May 2007 the salinities at the mangrove of Tetel, Bondan, Klaces and Motean was 4%o both at high tides and low tides. Thus there were no different between daily salinities, and these conditions were seasonal hypo-haline. Meanwhile the salinities at the mangrove of Kali Gatal was around 8 %o. In contrast during the dry seasons on September, their salinities varied between 39-40 %o both at low and high tides, and also there were no variations of daily salinities. Therefore the water quality during dry seasons both at the lagoon and at the Kali Gatal was on the hyper-haline. In the Segara Anakan lagoon, the salinities varied widely between rainy and dry seasons (Fig. 2). This condition was deteriorated due to marine or freshwater which was trapped by the shoals. On the rainy seasons, during high tide the freshwater from the rivers was trapped on the shoals. This condition deteriorated by the high volume of water from the Citanduy River into the lagoon and the salinity became hypo-haline, 0-5 %o seasonally. In contrast, during the dry season, the debit from Citanduy river was very low, and during the low tide, the marine water was trapped by the shoals. Thus, the global warming decreased the debit of river during the dry season and increased during the rainy season. Furthermore this trapped water evaporated and caused the salinity increased between 39-40 %o. Thus the climate change was worsen the salinity condition at the Segara Anakan Lagoon.

Djohan (2002) reported that in the rainy season, the salinity of Segara Anakan lagoon was 0 %o, she found the present of water hyacinth E. crassipes, but there was no golden apple snail (P. canaliculata). In contrast at the same year, the salinity of the lagoon at the dry season was 20 – 32 %o. This salinity of the lagoon water was in eury-haline condition. She reported that both of these invasive species were not present. There was a very distinctive salinity difference between 2002 and 2007. This differences indicated that there was influenced of global warming through number of rain-days and rainfalls (Fig. 3).

The dispersal of E. crassipes and P. canaliculata into the mangrove of Segara Anakan Lagoon through the outflows of the rivers on Java island. Since there were no daily differences on salinity as a normal brackish estuary, therefore during the rainy season the populations of E. crassipes and P. canaliculata grew thriving on the mangrove of Segara Anakan Lagoon. This circumstance was indicated with the present of the eggs of P. canaliculata, which was attached at the stem of mangrove shrub, Acanthus ilicifolius.

Wunder (2005) reported E. crassipes was capable to grow astonishing, and its population doubled vegetatively in two weeks. Even though they had ability to grow at wide tolerance of light and pH, but the increasing of salinity limited their health and fitness. The salinity of 6-8 %o will killed this population, and at salinity more than 8 %o was no individual will survive. He said that the understanding of combination factors of duration exposure, concentration, and recovery time from mortality were very
important in developing methods to control this population. He reported that E. crassipes which was exposed to the salinity of 7 °/oo for 14 weeks had capability to recover after this plants was treated with freshwater. However, if the exposure was more than 21 weeks, this E. crassipes was not able to recover.

Therefore the present of E. crassipes during rainy season in the mangrove forest of Segara Anakan lagoon indicated that this ecosystem was disturbed as a result of the combination of highly sedimentation, the disturbed tide periods, and was worsen by the global warming debit of freshwater both during rainy and dry seasons, was disturbed too. The flowering of E. crassipes population in the lagoon indicated that the Segara Anakan Mangrove ecosystem was in the process of changing to freshwater wetlands. However, at the end of dry season September to October, the salinity of the lagoon during high and low tide increase up to 40 °/oo, and the condition was hyper-haline. The increasing of water salinity up to 40 °/oo in the Segara Anakan lagoon during dry season was more than the concentration of marine salinity, 36 °/oo.

The present of shoals in the lagoon of the Segara Anakan Mangrove disturbed the tide periods, and this shoals trapped the marine and freshwater either during low tides or high tides. The low debit of freshwater from the streams into the lagoon during dry season caused the trapped marine waters to evaporate and increase the concentration of salinity. Therefore the global warming was worsen the freshwater inflow from the stream into the lagoon. Thus the combination of highly sediment rate and the global warming had deteriorated the Segara Anakan mangrove ecosystem.

The present of E. crassipes and P. canaliculata in the Segara Anakan lagoon were controlled by the water salinity. Thus both the population of E. crassipes and P. canaliculata their present was limited by the concentration of salinity of the lagoon. There were no differences in daily salinity, but the salinity differences were seasonally between rainy and dry seasons. Thus the conditions of salinity in the lagoon was very extreme between rainy and dry seasons, hypo-haline and hyper-haline. In the healthy estuary, the variation of salinity is daily between 4-35 °/oo. The condition of DO was varied between 6,38 – 10 mg l⁻¹ during rainy season, and was between 10,38-11,38 mg l⁻¹ on dry season. While pH was varied between 5,7-5,8 on rainy season. The temperature was 29°C on rainy season and was between 27-29°C during dry season. All these factors was not determine the present of E. crassipes and P. canaliculata in the Segara Anakan lagoon. But the salinity change between rainy and dry season determined the present and absent of E. crassipes and P. canaliculata in the lagoon. In fact this extreme condition of seasonally salinity change will affect the Segara Anakan mangrove communities.

CONCLUSIONS

The invasion of Eichornia crassipes and Pomacea canaliculata in the Segara Anakan lagoon indicated that the Segara Anakan mangrove was in the process of change from mangrove ecosystem to freshwater wetlands. Their present will threat the future freshwater wetland communities. But at the same time the global warming was worsen the process of change, and threatened the mangrove communities. The
salinity in the Segara Anakan lagoon varied seasonal and was not daily. This extreme salinity, hypo-haline during rainy season, and hyper-haline during dry season which exceeded marine water at dry season. This condition was as a result of combination between highly sedimentation rate and global warming through the input of freshwater into the lagoon.

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## TOPIC 3: SYSTEMATICS AND EVOLUTION

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CHLOROPLAST DNA SEQUENCES TO REVEAL PHYLOGENETIC RELATIONSHIP OF INDONESIAN BANANA CULTIVARS

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ABSTRACT

A phylogenetic relationship analysis of Indonesian banana cultivars was conducted using trnL-F intergenic spacer of cpDNA sequences. The ingroup consisted of 28 accessions of banana cultivars and 12 sequences of different taxa of Musa downloaded from GenBank. Two species of Zingiberaceae, Kaempferia sp dan Siphonochilus sp were used as the outgroup. The result showed that sequences of the trnL-F region can undoubtedly differentiate 34 banana accessions having the A-type chloroplast (AA wild; AA; AAA; AAB; and ABB) from those 6 accessions having the B-type chloroplast (BB, AAB, and ABB). Accessions of the A-type chloroplast are most probably originated from female M. acuminata, while accessions of the B-type chloroplast may be derived from female M. balbisiana..

Key words: cpDNA, banana, Indonesia

INTRODUCTION

Cultivated bananas were believed arise from hybridization between wild and seedy Musa acuminata Colla and M. balbisiana Colla (Stover and Simmonds 1987). Based on morphological characters, those cultivars could be divided into AA, AAA, BB, AAB, ABB, BBB, ABBB group genomes (Simmonds and Shepherd 1955; Silayoi and Chomchalow 1987). Therefore, two natural species and a hybrids complex make up the edible bananas in existence (Stover and Simmonds 1987; Espino et al. 1997). M. acuminata and M. balbisiana are recognized in their wild and cultivated forms. A number of AA diploid cultivars was derived from intraspecific hybridization between various subspecies of M. acuminata (Simmonds 1962), while AAA triploids have arisen either from the AA diploid cultivars or subspecies of AA wild by meiotic chromosome restitution. A similar development also occurred to lead appearance of
diploid and triploid *balbisiana* cultivars. On the other hands, the hybrids of *M. x paradisiaca* emerged in different genomic combination and ploidy levels. The AAB and ABB cultivars were originated from interspecific hybridization between *M. acuminata* (AA) and or maybe AAA; and *M. balbisiana* (BB) (Stover and Simmonds 1987; Pillay *et al.* 2004). Hybridization between heterogenic parents also produced a range of genotypes. Crossing between the AAB and a wild diploid parent of AA or BB could produce progeny within various genomic groups (Karamura 1998).

The banana production around the world trends is triploid banana cultivars (Sharrock and Frison 1999). These cultivars are currently threatened by several pests and diseases, so the new resistant cultivars are urgently needed. There is a continuing effort to improve the new resistant cultivars by introducing useful genes from their wild diploid parent, hence the phylogenetic relationships between the wild diploid progenitors and the cultivars need to be elucidated (Carreel *et al.* 2002). The use of molecular genetic analysis may significantly improve breeding efficiency (Tenkouano *et al.* 1999). According to Carreel *et al.* 2002) chloroplast DNA (cpDNA) which is sitoplasmic DNA was considered an essential tool for phylogeny analysis and may suggest a powerful tool to conform hybrid origins of banana cultivars. The transmission monoparental of cpDNA provide an exceptional opportunity for studying maternal lineages. In banana, cpDNA heredity could be used for distinguishing maternal from paternal lineages. According to Gielly and Taberlet (1996), the intergenic spacer of the *trnL*-F is noncoding regions in cpDNA which was suitable for phylogenetic studies due to its small size. The size only ranged from 120 to 350 bp in flowering plant. This region is also uniparentally inherited, not recombining and structurally relatively stable (Barcaccia *et al.* 2007). As compared to coding regions in cpDNA, the *trnL*-F intergenic spacer may develop at rates three times faster than that the coding regions. Therefore, this region may give reasonable resolution within *Musa*, although it was lower than that of a nuclear based phylogeny (Gielly *et al.* 1996).

The main objective of this study is to obtain a phylogenetic inference of cultivated bananas in Indonesia based on molecular approaches. The phylogenetic inference of cultivated bananas was elucidated using the noncoding sequences of cpDNA.
MATERIALS AND METHODS

Plant materials

The ingroup examined consisted of 30 banana accessions collected from various regions in Indonesia and 12 sequences of different taxa of *Musa* downloaded from GenBank (Umali and Nakamura 2003). The ingroup accessions consisted of AA, AAA, AAB, ABB dan BB group genomes. The outgroup consisted of two species, *Kaempferia* sp and *Siphonochilus* from Zingiberaceae. All accessions studied (the ingroup) and the outgroup were presented in Table 1.

Table 1. List of accessions used as the ingroup and the 2 accessions used as the outgroup

<table>
<thead>
<tr>
<th>Accession</th>
<th>Genomic group</th>
<th>Spesies</th>
<th>Region/source/ GenBank Accession number</th>
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DNA extraction

Total DNA was extracted from youngest fresh leaves for each accession by modification of the SDS method described by Dixit (1998). After purification using 10 mg/ml of RNAse free at 37°C for 1 hour, precipitation of DNA was conducted without PEG solution treatment, quantified using spectrophotometer, and then diluted to an estimated concentration of 10 ng μl⁻¹ for PCR template.

DNA amplifications and sequencing
The *trnL-F* intergenic spacer was amplified using primers e and f designed by Taberlet *et al* (1991). PCR reaction mix consisted of 5 µl 10 x PCR buffer with 25 mM MgCl₂, 10 µl 5 x GC-rich solution, 1 µl 10 mM dNTPs, 1 µl 10 µM of each primer, 0.4 µl 5 u µl⁻¹ Taq DNA Polymerase (Roche Applied Science®), and 7.5 µl DNA template (10 ng µl⁻¹) in 50 µl-reaction volume. The amplification was performed for 35 cycles consisting of denaturation at 94 ºC for 30 s, annealing at 52ºC for 30 s, and extension at 72ºC for 1 min. These cycles were headed by a 4 min at 94ºC, and ended by a 10 min final extension at 72ºC. After purification using Millipore system, double stranded PCR products were directly sequenced for two strands using ABI 330 in First Base Laboratories Sdn Bhd, Selangor Darul Ehsan, Malaysia.

**Data analysis**

DNA sequences obtained from the *trnL-F* intergenic spacer were aligned with CLUSTAL X version 3.1 (Thompson *et al*. 1997), and then refined manually in data matrix using Bioedit program. All data sets analyzed by heuristics search methods with tree bisection reconnection (TBR) branch swapping. The analysis was repeated 100 times with the random addition option to minimize problems of multiple of the most parsimonious trees (m.p.t). For phylogenetic inference, all characters were equally weight and unordered, while gaps (insertion and deletion) were treated as missing data. Sequences were analyzed using parsimony algorithm of the software package PAUP version 4 (Swofford 1998).

**RESULTS AND DISCUSSION**

The *trnL-F* intergenic spacer amplifications of the 32 accessions of the ingroup and the 2 accessions of the outgroup produced clear products. Three hundred and seventy one characters of sequences were compared in the *trnL-F* intergenic spacer, of which 317(85.4%) were constant and 31 (8.4%) were parsimony uninformative (autapomorphic) and the remaining 23 characters (6.2%) were potentially informative in parsimony analysis. The parsimony analysis of the *trnL-F* sequences resulted in 200 m.p.t., one of which is presented in Figure 1, with a tree length of 66 steps, a consistency index (CI) excluding uninformative characters of 0.967, a retention index (RI) of 0.974, and a homoplasi index (HI) of 0.0333. The strict consensus tree demonstrated that the phylogenetic tree was recovered in the ingroup with bootstrap support 100% from the outgroup *Kaempferia* and *Siphonochilus*. Therefore, all
banana accessions of the ingroup formed a monophyletic clade supported by a bootstrap value of 100%.

‘Raja Talun’ (AAB) and ‘Siam Paris’ (ABB) formed clade with the bootstrap 68%, while another ingroup accessions formed clade with the bootstrap 66%. The last clade consisted of ‘Pisang Seribu’ (AAB), ‘Raja Lini’ (AAB), and ‘Cardaba’ (ABB) was separated from another accessions containing the A genome alone and the B genome. ‘Lampung’ (AA) and ‘Mas 40 Hari’ (AA) produced clade with the bootstrap 63% and accessions 'Kepok Amerika' (AAB), ‘Brentel’ (AAB), ‘Penjalin’ (AA), ‘Sobo Londo Putih’ (ABB), ‘Jari Buaya’ (AA), ‘Barangan Kuning’ (AAA), ‘Ayam’ (AA), and ‘Kepok Awu’ (ABB) formed one separated clade with the bootstrap 57%.

Cultivated bananas were originated from hybridization between two wild species *M. acuminata* Colla and *M. balbisiana* Colla (Stover dan Simmonds 1987). This result supported the study of Swangpool *et al.* (2007). The present study detected two trends. The first trend showed accessions derived from female of *M. acuminata* and the second trend showed accessions arisen from female of *M. balbisiana*. Thirty four banana accessions which consisted of either pure *acuminata* of AA wild; AA; AA or AAA; and AAA cultivars; or hybrids AAB and ABB cultivars were indicated to contain the A-type chloroplast, while the remaining six banana accessions of BB, AAB and ABB contained the B-type chloroplast (Figure 1).
Figure 1 Slanted cladogram depicting relationships among banana accessions supposed to have the A- and the B-type chloroplast using the trnL-F intergenic spacer of cpDNA.

The result showed that the clades based on sequences of trnL-F intergenic spacer cpDNA described that the wild ancestors of the cultivated bananas because the cpDNA of Musa spp. is maternally transmitted (INIBAP 2002). Banana accessions with the A-type chloroplast were derived from female M. acuminata parent. The group of banana accessions of A-type chloroplast may be derived from female ancestors of M. acuminata subsp. banksii, malaccensis, siamea, or zebrina.
due to the existence of these subspecies in the clade of the A-type chloroplast. The result supported the study by Carreel et al. (2002) describing that *M. acuminata* subsp. *banksii* is related to most cultivars of the starchy bananas through their mitochondrial genomes. It means that the starchy bananas were perhaps originated from male *M. acuminata* subsp. *banksii*.

Banana accessions with the B-type chloroplast were perhaps derived from female *M. balbisiana* parent. Diversity of BB genomic group is less than that of the AA or AAA; and AAA genomic groups (Valmayor et al. 2000). Subspecies of *M. acuminata* contributing their gamet in the triploid accession with the B-type chloroplast were also perhaps limited. As a result the number of accessions with the B-type chloroplast especially triploid AAB are less than those accessions with the A-type chloroplast.

Variation in the *tmL-F* intergenic spacer sequences, especially at the cultivar levels of *Musa* spp. were very low because the regions have to be conserved and non recombined (Gielly et al. 1996). Phylogenetic analysis also could be performed using the other non coding sequences which possessed longer size than the *tmL-F* regions. Small et al. (2004) reported the association between sequence length and the number of phylogenetically informative characters. As sequence length increase, the number of both variable and phylogenetically informative characters also increases. The utility of noncoding chloroplast DNA regions in *Musa* spp. would provide an exceptional opportunity for studying the maternal lineage of clones or cultivars (Carreel et al. 2002). It is hoped that future study will be able to elucidate the exact progenitors of edible bananas (Pillay et al. 2004).

A phylogenetic relationship analysis of Indonesian banana cultivars showed that sequences of the *tmL-F* region can undoubtedly differentiate 34 banana accessions having the A-type chloroplast (AA wild; AA; AAA; AAB; and ABB) from those six accessions having the B-type chloroplast (BB, AAB, and ABB). Accessions of the A-type chloroplast are most probably originated from female *M. acuminata*, while accessions of the B-type chloroplast may be derived from female *M. balbisiana*.

**CONCLUSION**

The data and analysis presented in this study showed that the *tmL-F* intergenic spacer of cpDNA sequences were potential to be used to infer phylogenetic relationships of *Musa* spp. Based on the regions, 34 banana accessions containing the A-type chloroplast consisting of AA wild; AA; AA or AAA; AAA; AAB; and ABB cultivars were clearly distinguished from those 6 accessions of the B-type chloroplast consisting of BB, AAB and ABB cultivars. The A-type chloroplast accessions are most probably derived from female *M. acuminata*, while the B-type chloroplast accessions may be derived from female *M. balbisiana*. 
ACKNOWLEDGEMENT

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O-SE02

GREY HERON (*Ardea cinerea* LINNAEUS, 1758): THE NEW EMERGING VERTEBRATE PEST IN YOGYAKARTA

Bambang Agus Suripto, Desi Kurniawati
O-SE03

THE BIOLOGY OF ASANG FISH (Osteochilus hasselti, (C.V.) : Cyprinidae) IN SINGKARAK AND DIBAWAH LAKE, WEST SUMATRA : Inferred from cytochrome b gen

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Currently, information about the biology of populations and species is gathered through survey and inventory activities as well as research projects. The biology of populations and species is dependent as much or more on historical events as it is on current events, and those historic processes can be inferred from analysis of genetic data. This project compared genetic data in two populations of Asang fish (Osteochilus hasselti, (C.V.): Cyprinidae) in Singkarak and Dibawah lake by using region of mitochondria DNA cytochrome b gene. The important question addressed here is how this diversity varies at those two lakes. Based on nucleotide sequences of a 534 base pair region of the cytochrome b gene were used, both populations showed low level of diversity.

Key words : Fish; Osteochilus hasselti; mtDNA cytochrome b gene
O-SE04

THE COMPARISON OF MORPHOLOGICAL AND MOLECULAR CHARACTERS OF Vanda tricolor Lindl. MERAPI, BALI, EAST JAVA AND WEST JAVA FORMS

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ABSTRACT

Vanda tricolor Lindl. are widely distributed in Sulawesi, Java and Bali. V. tricolor Merapi form is naturally obtained in Merapi. However, the population of this orchid was decreased, recently, because of the pyroclastic flows that is frequently occurred in Mt Merapi. The government has relocated V. tricolor in the slope of Mt, Merapi, but not all of the orchids could adapted to the unique agroclimate of Mt Merapi. The aim of this research is to characterize Vanda tricolor var suavis morphologically and molecularly among the four forms. As the plant materials, V. tricolor were collected from slope of Mt. Merapi, Bali, East Java and West Java. The morphological characters were focused on size and shape of leaf, flower, fruit and smell of flower. The molecular characterization was focused on the character of Phalaenopsis Orchid Homeobox1 (POH1) gene and a specific spacer region of trNL-F chloroplast DNA (cpDNA). The results show that the amplified DNA on the downstream region of POH1 shows West Java form has a single band of different size than that among all forms. Although, the four forms of V. tricolor, Merapi form, Bali, and East java are difficult to be distinguished morphologically. However, West Java form shows smaller size of leaf and flower, but much fragranced than that of the other forms. Molecular analysis of the spacer region of trnL-F cp DNA shows that all of the orchid forms do not show any polymorphic. This data indicate that all forms of V. tricolor might be originally come from the same place, but gradually spread into some places and adapted the new agroclimate that make them different in morphology.

Key words; Vanda tricolor, species form, morphology, molecular character

INTRODUCTION

Indonesian orchids are well known worldwide as natural resources (Irawati, 2002). Although Indonesia is host for thousands of beautiful orchid species, but at present, those are threatened of being decimated and numerous more valuable components may even become extinct in the near future because of forest
destruction and over collecting for trade (Vermeulen, 2002). For all sustainable exploitation, the wild orchid flora needs in situ protection through further explore Indonesia’s orchid flora.

Vanda tricolor Lindl. is one of well known natural orchid that live in the slope of Mount Merapi, although originally, V. tricolor orchids are widely distributed in Sulawesi, Java and Bali. V. tricolor Merapi form is naturally obtained in Merapi. However, the population of this orchid was decreased, recently, because of the pyroclastic flows, that is frequently occurred in Mt Merapi. For solving problem, government has relocated about 200 plants of V. tricolor in the slope of Mt, Merapi, but not all of the orchids could adapt to the unique agroclimate of Mt Merapi. Gardiner (2005) reported that not all of the relocated V.tricolor in Merapi were come from Merapi. Morphological, physiological and molecular characterization of this orchid from some parts of Indonesia is needed for in situ conservation of this orchid, mainly in the slope of Merapi.

In this paper, we report our work on morphological and molecular characterization of V. tricolor that come from four distinct areas: Bali, East Java, West Java and the South slope of Mt. Merapi, which were suspected to be the sources of some relocated V.tricolor plants in Merapi. The morphological characters of leaves, flowers, fruits, seeds and fragrance have been analyzed. It will supported by molecular characterization of the unique sequence of trNL-F (Semiarti et al, 2008a) and the specific region of Phalaenopsis Orchid Homeobox1 (POH1) gene from Phalaenopsis amabilis (Semiarti et al, 2008b) in V tricolor genomes.

MATERIALS AND METHODS

The plant materials and growth condition

Vanda tricolor Lindl. plants from four distinct areas: Mount Merapi-Yogyakarta, Bali, East Java and West Java, that called as V. tricolor Merapi Form, Bali Form, East Java Form and West Java Form, were used as plant materials in this study. Adult plants (two years old plants) were maintained in a glasshouse to produce fruits and fertile seeds. Detail examinations of morphological characters of plant habitus, and the shapes of leaves, flowers, fruits, and seeds were carried out in the the glasshouse. Photographs of plant materials were taken by Digital Camera NIKON Cool Pix 5000 (Nikon, Japan).
**DNA isolation and purification**

Protocorms and leaves of adult plants were used for DNA extraction. DNA(s) were isolated by using a modified CTAB method of Murray and Thomson (Semiarti et al., 2008a, b).

**Polymerase chain reaction**

Genomic DNA(s) of orchids were used as template for the amplification of trnL-F intergenic space of chloroplast DNA using primer C (5' -CGAAATCCGTAGAC- GCTACG) and primer F (5' -ATTGAACTGGTGACACGAG) (Semiarti et al., 2008a). The PCR program was 1 min at 94°C for initial denaturation, 35 cycles of 1 min at 94°C, 1 min at 50°C and 1 min 30s at 72°C. PCR products were purified using PCR purification column (Jet quick, GENOMED GmbH). The second PCR were conducted using POH1 gene specific primers 3'-POH1F1 (5'-GAAGAGCTC- CACGAGGCCAGT) and 3'-POH1R1 (5'-CAAATAGCACCCAACCTTTTC) according to Semiarti et al. (2008b). The same condition for PCR reaction to that of trnL-F amplification was used except the number of cycles for the second round PCR was 30 cycles.

**Genotyping of V. tricolor orchids**

The amplified cpDNA using trnL-F primers and amplified genomic DNA fragments using POH1 primers of V. tricolor plant from each forms, were independently digested with EcoRI restriction enzyme and subjected to fingerprint analysis. The digested DNA was size-fractionated by electrophoresis in 0.7% horizontal agarose gels, stained with ethidium bromide and visualized under UV-transillumination. The genotype of each orchid was decided by the restriction fragment length polymorphism (RFLP) analysis of digested PCR product comparing the RFLP pattern of V. tricolor from four forms.

**RESULTS AND DISCUSSION**

**Morphological Characters**

The result shows that all of orchid forms of V. tricolor Lindl. var suavis have similar morphological characters, except that West Java form has the smallest size of leaves, flowers and fruits, as shown in Table 1 and Figure 1-3.
**Figure 1.** *Vanda tricolor var suavis* Lindl. Orchid plants from four forms. A. Merapi form, B. Bali form, C. West Java form, D. East Java form. Bars: 5 cm.

I.

**Figure 2.** I. The flowers of *V. tricolor* from various forms. A. Bali form, B. Merapi form, C. West Java form, D. East Java form. Bars: 1 cm. II. Labellum of *V. tricolor* from various forms. A. Merapi form, B. Bali form, C. West Java form, D. East Java form. Bars: 1 cm.

Fig. 2I shows various shape and ornamental pattern of flower of *V. tricolor.* From the flatness of flower, Merapi form is the most flat and the roundest than the others. The color of sepals, petals and labellum are vary, in which Bali form and West Java form frequently exhibit pink labellum (Fig 2II). And from the smell, the flower of West Java form much graganced than the others. The size of flowers and fruits of Merapi form and Bali form is not significant difference, the smallest in size of flowers and fruits is West Java form (Fig 3 and Table 1).

**Figure 3.** Fruits of *V. tricolor var suavis* from various forms. A. Merapi form, B. Bali form, C. West Java form (bar 1 cm)
Table 1. Morphological Characters of Vanda tricolor var suavis from various forms.

<table>
<thead>
<tr>
<th>Morphological characters</th>
<th>Size in Various Orchid Forms</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Merapi</td>
</tr>
<tr>
<td>Leaf Length (cm)</td>
<td>31.75 ± 3.50</td>
</tr>
<tr>
<td>Leaf Width (cm)</td>
<td>4.20 ± 0.52</td>
</tr>
<tr>
<td>Leaf thickness (cm)</td>
<td>0.07 ± 0.01</td>
</tr>
<tr>
<td>Flower vertical diameter (cm)</td>
<td>6.17 ± 0.10</td>
</tr>
<tr>
<td>Flower horizontal diameter (cm)</td>
<td>6.24 ± 0.06</td>
</tr>
<tr>
<td>Fruit Length (cm)</td>
<td>14.18 ± 2.01</td>
</tr>
<tr>
<td>Fruit Diameter (cm)</td>
<td>2.81 ± 0.17</td>
</tr>
</tbody>
</table>

± indicates standard deviation that were examined from three samples of independent plants each

Molecular Characters

The molecular analysis revealed that all forms of V. tricolor orchid have the same lengths of PCR product using primer trnL-F, that is about 1.100 bp (Fig 4). Using EcoRI endonuclease restriction enzyme digestion, there were no polymorphic in the trnL-F gene locus. Each fragment was cut into two fragments 700 bp and 400 bp. This data is consistence to the data of Semiarti et al. (2008) that showed no polymorphic, but it should be confirmed using a four base cut restriction enzymes for detail examination and decision of the cp genome.

Figure 4. Amplified cp DNA fragments of trnL-F from V. tricolor orchids. M, Lambda DNA marker/Sty I; lane 1 and 2 PCR products of trnL-F regions, 1) Merapi form, 2) Bali form, Lane 3 and 4, EcoRI digested PCR products of 3) Merapi form; 4) Bali form.

In the case of POH1 gene, at Fig. 5, it is clear that the amplified genomic DNA of the downstream region of V. tricolor, two forms: West Java and Merapi form produced about 500 bp amplified bands, Bali form produced bigger size of bands that about 700 bp, 800 bp, 1.5 kb and 1.9 kb bands. In Merapi form, we could also detect two faint bands of 1.5 kb and 1.9 kb DNA fragments, but not in West Java form. Unfortunately, there was no PCR product from the genomic DNA of V. tricolor east java form yet. From these three forms, the pattern of amplified DNA fragments of the Merapi form is close to West Java form that both forms produced 500 bp DNA
fragments. However, the Bali form distinct to the West Java form but has similarity to Merapi form that produced two large bands: 1.5 kb and 1.9 kb bands.

![Image of DNA gel](image)

**Figure 5.** Amplified genome DNA fragments of *POH1* specific region of *V. tricolor* orchids. Lane 1, Lambda DNA marker/Sty I; lane 2, Merapi Form; lane 3, Bali Form; lane 4, West Java form, and 5 East Java form.

These data indicate that various forms of *Vanda tricolor* sharing very conserved DNA arrangement in cpDNA, at least in the spacer region of trnL-F. Based on the character of spacer region of trnL-F of cpDNA only, Gardiner (2005) reported that reintroduced *V. tricolor* in the slope of Merapi might be come from several areas of Indonesia or other countries. Our data shows that combined the character of chloroplast and nuclear genome, polymorphism among the forms of *V. tricolor* could be detected. It is worth to examine the arrangement of other region in *POH1* gene homologous in *V. tricolor* to find the unique part of *V. tricolor* that distinct to *Phalaenopsis*, and to serve the molecular marker for *V.tricolor* in order to distinguish each forms quickly from molecular view.

From morphological characters, *V. tricolor* West Java form is the smallest in size of plant body among all forms analyzed. But from molecular character in the downstream of *POH1* gene, that all forms of *V. tricolor* showed different character, West Java form is closer to Merapi form and far from Bali form. The data suggests that the morphological characterization only is not sufficient to determine the different between one form to another. It because of morphological character is the results of growth, in which its regulation will be controlled by both internal (growth regulators and other factors within the cell) and external factors (ecological factors such as temperature, light, humidity and other factors from outside the cell). Molecular characterization of some unique regions of the plant genome will help to understand morphology of the plants.

**CONCLUSION**
Four forms of *Vanda tricolor* have very similar morphology of leaves, flowers and fruits, that West Java form is the smallest in size, but much fragranced than the others. Molecular characters at *POH1* gene homologous revealed that all forms have different structure in the downstream region of the *POH1* gene, but it shares similarities in some region of the gene. There are no polymorphic in the spacer region of *trnL*-F cp DNA among four forms of *V. tricolor* examined.

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O-SE05

A PRELIMINARY STUDY OF MOSS FLORA OF MOUNT LUMAKU, SABAH, MALAYSIA.

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An inventory study of mosses from Mount Lumaku, Sipitang, Sabah was carried out for the first time. The field works have been conducted twice; from 29th to 30th July 2008 and on 7th March 2009. The main purpose of this study is to report moss species found in the study area. In this study a total of 51 genera from 28 families of mosses were recorded. This number represents 27.6% of genera and 56% of families out of the 185 genera and 33 families of mosses recorded so far for Borneo. The largest family is Calymperaceae with 6 genera recorded, followed by Dicranaceae (4 genera), Sematophyllaceae (4 genera), Hookeriaceae (3 genera), Meteoriaceae (3 genera), Neckeraeaceae (3 genera), Pterobryaceae (3 genera), Bryaceae (2 genera), Daltoniaceae (2 genera), and Hypopterygiaceae (2 genera). Meanwhile, Bartramiaceae, Diphysiaceae, Fissidentaceae, Garogvagliaceae, Hypnaceae, Hypnodendraceae, Leucobryaceae, Mniaceae, Orthotrichaceae, Plagiotheciacaceae, Polytrichaceae, Pottiaceae, Racopilaceae, Rhizogoniaceae, Schistomitiaceae, Sphagnaceae, Sympyodontaceae, and Thuidiaceae are represented by one genus each. The research also provides identification keys to species within genus and genera within a family, description, illustrations, altitudinal range, habitat, and phytogeography informations for each species.

Keywords: mosses, Sabah, Borneo

not presented
O-SE06

APPLICATION OF DISPERSION AND DIFFERENTIAL CENTRIFUGATION METHOD (DDC) AND SELECTIVE ISOLATION TO UNRAVEL THE DIVERSITY OF STREPTOMYCETES ASSOCIATED WITH THE RHIZOSPHERE OF THE TROPICAL LEGUME, Paraserianthes falcata (L) NIELSEN.

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Large numbers of neutrophilic streptomycetes were isolated from composite environmental samples collected from within and around the root system of the tropical legume, Paraserianthes falcata (L) Nielsen. Streptomycete propagules were extracted from each of the environmental samples using a multistage dispersion and differential centrifugation method (DDC) and a tumble shaking procedure. The resultant suspensions were heat-treated, serially diluted and plated onto two selective isolation media designed to support the growth of taxonomical diverse streptomycetes. The rhizosphere effect was most pronounced on the starch-casein isolation plates with environmental suspensions derived from the various stages of the DDC procedure. A total of 607 isolates were chosen to represent the different colonial types of presumptive streptomycetes isolated from suspensions derived from the two extraction methods. Five hundred and fifty-six isolates obtained using the DDC method were assigned to 37 multi-membered (451 isolates) and 105 single-membered colour groups. In addition, 51 isolates derived from tumble shaking procedure were assigned to 7 multi-membered (42 isolates) and 9 single-membered colour groups. The properties of these multi-membered colour groups were identical to those shown by corresponding colour groups that encompassed strains derived from the DDC procedure. Representatives of 31 out of 37 multi-membered had chemical and morphological properties consistent with their classification in the genus Streptomyces. Therefore, the application of Dispersion and Differential Centrifugation Method along with the selective isolation could provide a strong way to achieve a more representative sampling procedures in order to study microbial diversity within soil samples taken from the environmental habitats.

Key words: dispersion and differential centrifugation, selective isolation, diversity, streptomycetes, rhizosphere, legume, Paraserianthes falcatharia.

Introduction

Innumerable streptomycetes have been isolated from natural environments, including terrestrial, freshwater and marine habitats (Goodfellow & Haynes, 1984; Goodfellow & Simpson, 1987; Korn-Wendisch & Kutzner, 1992). Procedures recommended for the selective isolation of streptomycetes from environmental
samples have been extensively reviewed (Cross, 1982; Williams & Wellington, 1982; Goodfellow & Simpson, 1987; Korn-Wendisch & Kutzner, 1992). Most procedures involve the extraction of propagules from environmental samples, pretreatment of substrates and plating out the treated suspensions onto selective media which are incubated for varying periods under various temperature regimes.

The dispersion and differential centrifugation (DDC) technique is more effective in extracting streptomycete propagules from composite tropical samples than the classical method of shaking soil in physiological saline prior to plating out onto selective media (Milner, 1992; Davenport, 1994; Manfio, 1995). Atalan et al. (2000) showed that the DDC procedure was between three and twelve times as effective in the extraction of streptomycete propagules from non-heat-pretreated soil suspensions than a reciprocal shaking technique. There was also evidence from these preliminary experiments that different types of streptomycetes were isolated at different stages of the extraction procedure possibly reflecting the ease or otherwise of detaching specific types of streptomycete propagules from particulate matter. These observations suggest that the persistent association between soil particles and actinomycete propagules may be major limitations to quantitative and representative sampling of streptomycete populations and that the DDC procedure is effective in breaking down such interactions. In this study, the application of extraction technique in combination with the selective isolation procedures was used to unravel the diversity of streptomycetes associated with the rhizosphere of a tropical legume, *P. falcataria* (L) Nielsen.

**Materials and Methods**

1. **Collection of environmental samples**

   Environmental samples were collected from, and around, the root system of the tropical angiosperm, *P. falcataria* in a reforested area of Cangkringan, near Yogyakarta, Java, Indonesia. Three types of environmental samples were collected at each location, namely, ectorhizosphere, rhizoplane, and non-rhizosphere samples. The ectorhizosphere samples were taken within 20 cm of the root surface whereas soil shaken from the root material was considered to form the rhizoplane sample. Non-rhizosphere soil was taken 5 to 10 m away from root system. Environmental samples (ca.50 g) collected from different locations were thoroughly mixed to give composite samples which were stored in plastic bags.
2. Propagule extraction, selective isolation and enumeration of streptomycetes

Streptomycetes were isolated from the ectorhizosphere, rhizoplane, and non-rhizosphere composite samples using a modification of the DDC procedure (Hopkins et al., 1991), as well as by using a tumble shaking method. This dual approach was followed in order to compare the effectiveness of the two procedures. Each of the supernatant fractions (A, B, C, and D) obtained from DDC procedure were centrifuged at 12,000 x g at 4°C for 20 minutes to produce pellets which were resuspended in 10 ml 1/4 strength Ringer’s solution and heat pretreated at 55°C for 20 minutes in a water bath prior to the preparations of the serial dilutions.

In the corresponding tumble shaking (TS) experiments, one gram of each composite environmental sample was added to 9 ml of 1/4 strength Ringer’s solution in a universal bottle. The bottles plus contents were placed on a Tumble shaker (Model TM1-Tumbler, Luckham Ltd., Victoria Gardens, Burgess Hill, Sussex, England, UK) and shaken at speed setting 4 for 10 minutes at room temperature. The resultant 10⁻¹ dilution were transferred to sterile universal bottles and heat-treated at 55°C in a water bath for 20 minutes then cooled at room temperature prior to the preparation of further serial dilutions down to 10⁻⁶. Each dilution was then spread onto the surface of replicate plates of starch-casein (Küster & Williams, 1964) and raffinose-histidine agar plates (Vickers & Williams, 1984) which had been supplemented with cycloheximide and nystatin each at a concentration of 50 μg/μl. The inoculated plates were incubated at 25°C for 2 weeks when the number of streptomycete-like colonies growing on the starch-casein plates were counted. The results were expressed as the number of colony forming unit (cfu) per gram dry weight soil.

3. Selection, purification and maintenance of isolates

After incubation, the isolation plates were examined both by eye and by using a binocular microscope (Olympus Optical Co., Ltd.). Colonies were isolated, and purified by subculturing them onto modified Bennett’s agar plates (Jones, 1949). The purity of the isolates were then checked by microscopical examination of Gram-stained smears using an Optiphot microscope (Nikon Co., Tokyo, Japan) fitted with long-range working distance objectives. Organisms were tentatively assigned to the genus *Streptomyces* on the basis of colony morphology, notably, aerial spore mass colour, substrate mycelial pigmentation and the colour of any diffusible pigments. Stock cultures were prepared for each strain by transferring the mycelia growth and
spores from each of the purified isolates into cryotubes containing 20% (w/v) sterile glycerol solution (Wellington & Williams, 1978). The frozen glycerol cultures which were stored at -20°C served as a means of long term storage and at the same time provided a convenient source of inoculum.

4. Preliminary characterisation of presumptive streptomycetes

4.1. Colour grouping. The representative isolates (607 isolates) were inoculated onto oatmeal (ISP3; Küster, 1959) and peptone-yeast extract-iron agar plates (ISP6; Shirling & Gottlieb, 1966) which were incubated at 25°C for 14 and 4 days, respectively. The colours were recorded using a National Bureau of Standards (NBS) Colour Name Chart (Kelly, 1958; Anonymus, 1964). The peptone -yeast extract-iron agar plates were examined to determine whether the test strains had produced characteristic dark coloured melanin pigments.

4.2. Morphology. Spore chain morphology and spore surface ornamentation were determined by using a standard procedure for Scanning Electron Microscopy of CAMBRIDGE Stereoscan 240 instrument (Cambridge Instruments Ltd., Cambridge, England, UK); ornaments were assigned either to the categories of Tresner et al. (1961) or to the rugose section of Dietz and Mathews (1971).

4.3. Detection of isomers of diaminopimelic acid. The isomeric forms of A$_{2pm}$ were determined by thin layer chromatography (TLC). The A$_{2pm}$ isomers, which appeared as blue-violet coloured spots after 2 -3 minutes, were identified by comparison with the standard.

Results and Discussion

1. Selective isolation, enumeration and colour grouping

Isolates presumptively assigned to the genus *Streptomyces* were distinguished from other bacterial colonies growing on the raffinose-histidine and starch-casein isolation plates by their characteristic mycelial and pigmentation properties. Large numbers of the target organisms were isolated from the heat treated suspensions of the ectorhizosphere, rhizoplane and non-rhizosphere samples following incubation on the two selective isolation media. Higher presumptive streptomycete counts were consistently found on the starch-casein isolation plates seeded with fractions obtained using the DDC as opposed to the tumble shaking procedure (Table 1.). The highest presumptive streptomycete counts were derived from the supernatant A fractions and the lowest from the supernatant D fractions. The rhizosphere effect
was most pronounced on the starch-casein plates seeded with fractions derived from the various stages of the DDC procedure.

The 556 isolates taken to represent the different kinds of presumptive streptomycetes growing on the isolation plates seeded with fractions obtained from the DDC procedure were assigned to 37 multi-membered (Tables 4) and 105 single-membered colour groups (data not shown). Some multi-membered colour groups were subdivided groups on the basis of small differences of aerial mycelium colour (groups 1, 2, 3, 4 and 7) and by the ability of some strains to produce melanin pigments (groups 9, 13, 16, 29, 30 and 31). In the corresponding experiment involving propagules obtained from the tumble shaking fractions, 51 representative isolates were distributed to 7 multi-membered (42 isolates) and 9 single-membered colour groups (Table 2). Colour groups 2, 3 and 5 were subdivided on the basis of the ability to produce melanin pigments.

Almost all of the colour groups containing strains isolated from suspensions derived from tumble shaking corresponded with colour groups that encompassed strains isolated from the application of the DDC procedure (Table 3). The largest colour group (group 3) consisted of 94 isolates which formed a grayish aerial spore mass which later turned black, a grayish-yellow reverse colour and a yellow diffusible pigment on oatmeal agar; none of these isolates formed melanin pigments on peptone-yeast extract-iron agar.

A. Preliminary characterisation of representative isolates

Some of the cultural and morphological properties of the 46 representatives of the 37 multi-membered colour groups that encompassed isolates obtained using the DDC procedures are shown in Table 4. It is evident that 31 out of the 37 colour groups (84%) contained the LL-A$_{2pm}$ isomer, a result consistent with the assignment of these isolates to the genus *Streptomyces*. The exceptions, the representatives of colour groups 4 (69 isolates), 14 (3 isolates), 18 (4 isolates), 26 (4 isolates), 36 (2 isolates) and 37 (3 isolates) contained meso-A$_{2pm}$; all of the strains which form aerial hyphae that underwent fragmentation contain meso-A$_{2pm}$. Representatives of 31 colour groups (84%) formed chains of spores that were borne on aerial mycelia. It is evident that most of the isolates carried spores in either flexuous (19%) or spiral (32%) chains and that just over half produced spores with smooth surfaces. It is particularly interesting that isolates which were characterised by the presence of rugose-ornamented spores in spiral spore chains were found in colour groups 3, 19 and 27.
Members of most of the larger colour groups were isolated from all three composite environmental samples though most of the isolates from colour groups 7 (84%) and 16 (72%) were from non-rhizosphere soil. In contrast, strains assigned to some of the smaller colour groups were associated with a particular environmental sample, for instance, members of colour groups 22 to 24 were only isolated from the composite rhizoplane sample. Similarly, all three isolates classified in colour group 14 were isolated from the rhizosphere sample. The largest taxon, colour group 3, encompassed roughly the same number of isolates from the ectorhizosphere, rhizoplane and non-rhizosphere samples.

Most of the larger multi-membered colour groups were present in all four DDC supernatants. However, most of the strains assigned to colour group 12 (63%) were derived from supernatant B and most of those classified in colour group 16 were from supernatant A. It is also interesting that all three isolates assigned to colour group 37 were derived from supernatant C.

It can be seen from Table 4 that several different types of streptomycetes were isolated on the two selective media. It is particularly interesting that all 16 representatives of colour group 12 were isolated from the starch-casein isolation plates. In contrast, 10 out of the 11 representatives of colour group 13 were isolated from the raffinose-histidine isolation plates, as were members of several smaller colour groups, namely, 10, 14, 23, 27 and 31.

Table 1. Numbers of presumptive Streptomyces strains (x 10^7 cfu g^-1-dry weight soil) growing on starch-casein isolation plates seeded with either supernatant fractions derived from the application of the DDC or suspensions from TS technique prepared from samples associated with the root system of P. falcataria.

<table>
<thead>
<tr>
<th>Sample source</th>
<th>Extraction technique*</th>
<th>DDC/TS(Times)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>DDC</td>
</tr>
<tr>
<td>Ectorhizosphere sample</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Supernatant A</td>
<td></td>
<td>50.98</td>
</tr>
<tr>
<td>B</td>
<td></td>
<td>20.00</td>
</tr>
<tr>
<td>C</td>
<td></td>
<td>17.80</td>
</tr>
<tr>
<td>D</td>
<td></td>
<td>4.80</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>93.58</td>
</tr>
</tbody>
</table>
Rhizoplane sample

<table>
<thead>
<tr>
<th>Supernatant</th>
<th>Value</th>
<th>Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>18.06</td>
<td>± 1.30</td>
</tr>
<tr>
<td>B</td>
<td>14.65</td>
<td>± 0.46</td>
</tr>
<tr>
<td>C</td>
<td>11.07</td>
<td>± 1.78</td>
</tr>
<tr>
<td>D</td>
<td>1.25</td>
<td>± 0.06</td>
</tr>
</tbody>
</table>

Total: 45.03 ± 3.60

Non-rhizosphere sample

<table>
<thead>
<tr>
<th>Supernatant</th>
<th>Value</th>
<th>Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>12.80</td>
<td>± 1.60</td>
</tr>
<tr>
<td>B</td>
<td>9.40</td>
<td>± 0.98</td>
</tr>
<tr>
<td>C</td>
<td>10.45</td>
<td>± 1.65</td>
</tr>
<tr>
<td>D</td>
<td>0.69</td>
<td>± 0.01</td>
</tr>
</tbody>
</table>

Total: 33.34 ± 4.24

*) SD, standard deviation.

B. A notable feature of the present study was the demonstration that the modified dispersion and differential centrifugation method was between two and three times more effective than the tumble shaking procedure in extracting streptomycetes propagules from the composite rhizosphere and rhizoplane samples collected from the root system of *P. falcatoria* a less pronounced difference was apparent in the corresponding experiments with the composite non-rhizosphere sample. These results are in line with those from previous studies which showed that the multistage DDC method was more effective in extracting actinomycetes propagules from terrestrial environmental samples than classical shaking procedures (Milner, 1992; Davenport, 1994; Manfio, 1995; Atalan *et al.*, 2000). There was also some evidence that members of different colour groups were mainly associated with specific steps in the DDC procedure. A similar observation was made by Atalan *et al.* (2000) with respect to members of three putatively novel streptomycete species isolated from rhizosphere soil.
Table 2. Source of isolates presumptively identified as streptomycetes derived from the composite rhizosphere and non-rhizosphere samples of *Paraserianthes falcatoria*.

<table>
<thead>
<tr>
<th>C. Environmental sample</th>
<th>Tumble shaking</th>
<th>Dispersion and differential centrifugation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Starch-casein agar</td>
<td>Raffinose-histidine agar</td>
</tr>
<tr>
<td>Ectorhizosphere</td>
<td>15</td>
<td>-</td>
</tr>
<tr>
<td>Rhizoplane</td>
<td>16</td>
<td>1</td>
</tr>
<tr>
<td>Non-rhizosphere</td>
<td>13</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>51</td>
<td>556</td>
</tr>
<tr>
<td>Total isolates</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3. Comparable colour groups of presumptive streptomycetes isolated from suspensions derived from the dispersion and differential centrifugation (DDC) and tumble shaking extraction procedures.

<table>
<thead>
<tr>
<th>D. Tumble shaking</th>
<th>E. Number of strains</th>
<th>Dispersion and differential centrifugation (DDC)</th>
<th>Number of strains</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Starch-casein agar</td>
<td>Raffinose-histidine agar</td>
</tr>
<tr>
<td>Multi-membered colour groups</td>
<td>1</td>
<td>13</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>12</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>5</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>4</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>4</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>2</td>
<td>28</td>
</tr>
</tbody>
</table>
The observations outlined above suggest that strong associations between different kinds of streptomycetes and particulate matter in soil maybe major limitations to representative sampling of streptomycete communities in natural habitats. The modified DDC procedure, which involves the use of mild detergent (sodium cholate), buffering (Tris buffer), attenuated physical disruption (mild ultrasonication) and ionic shock (distilled water), maybe effective in breaking down such associations. Further studies are needed to determine the nature of the apparent associations between members of individual streptomycete populations (colour groups) and specific environmental fractions resulting from the application of the DDC procedure.

Table 4. Some cultural, morphological and chemical characteristics of representatives of the multi-membered colour groups that encompassed isolates obtained using the dispersion and differential centrifugation extraction procedure.

<table>
<thead>
<tr>
<th>Colour</th>
<th>Aerial mycelium colour</th>
<th>Reverse mycelium colour</th>
<th>Soluble pigment colour</th>
<th>Number</th>
<th>Represented as</th>
<th>Spore chain morphology</th>
<th>Spore surface ornamentation</th>
<th>Isomer of diaminopimelic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Gray-brown</td>
<td>Brown</td>
<td>Yellow</td>
<td>19</td>
<td>A1P1</td>
<td>Spirals</td>
<td>Warty</td>
<td>LL</td>
</tr>
<tr>
<td>2.</td>
<td>Gray-brown</td>
<td>Brown</td>
<td>Yellow</td>
<td>11</td>
<td>C1P2</td>
<td>Spirals</td>
<td>Smooth</td>
<td>LL</td>
</tr>
<tr>
<td>3.</td>
<td>Gray-brown</td>
<td>Brown</td>
<td>Yellow</td>
<td>5</td>
<td>B10P3</td>
<td>Spirals</td>
<td>Smooth</td>
<td>LL</td>
</tr>
<tr>
<td>4.</td>
<td>Green-white</td>
<td>Green-black</td>
<td>Green</td>
<td>38</td>
<td>B24P1</td>
<td>Flexuous</td>
<td>Warty</td>
<td>LL</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td>Green-white</td>
<td>Green-black</td>
<td>Green</td>
<td>27</td>
<td>D4R2</td>
<td>Flexuous</td>
<td>Warty</td>
<td>LL</td>
</tr>
<tr>
<td>3</td>
<td>Gray-white</td>
<td>Yellow-gray</td>
<td>Yellow</td>
<td>40</td>
<td>A4R2</td>
<td>Spirals</td>
<td>Rugose</td>
<td>LL</td>
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<tr>
<td>3</td>
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<td>Yellow-gray</td>
<td>Yellow</td>
<td>19</td>
<td>A33R1</td>
<td>Spirals</td>
<td>Rugose</td>
<td>LL</td>
</tr>
<tr>
<td>3</td>
<td>Gray-white</td>
<td>Yellow-gray</td>
<td>Yellow</td>
<td>35</td>
<td>B13P3</td>
<td>Spirals</td>
<td>Rugose</td>
<td>LL</td>
</tr>
<tr>
<td>4</td>
<td>White</td>
<td>White</td>
<td>None</td>
<td>42</td>
<td>A13P1</td>
<td>Fragments</td>
<td>Smooth</td>
<td>meso</td>
</tr>
<tr>
<td>4</td>
<td>White-yellow</td>
<td>White-yellow</td>
<td>None</td>
<td>21</td>
<td>D2P2</td>
<td>-</td>
<td>-</td>
<td>meso</td>
</tr>
<tr>
<td>4</td>
<td>White-orange</td>
<td>White-orange</td>
<td>None</td>
<td>6</td>
<td>C28P3</td>
<td>Fragments</td>
<td>Smooth</td>
<td>meso</td>
</tr>
<tr>
<td>5</td>
<td>Blue</td>
<td>Blue</td>
<td>None</td>
<td>6</td>
<td>A23P1</td>
<td>Spirals</td>
<td>Spiny</td>
<td>LL</td>
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<td>None</td>
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<td>Spirals</td>
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<td>LL</td>
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<tr>
<td>7</td>
<td>Gray-light brown to dark brown</td>
<td>Gray-yellow</td>
<td>None</td>
<td>14</td>
<td>B20P1</td>
<td>Flexuous</td>
<td>Smooth</td>
<td>LL</td>
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<tr>
<td>7</td>
<td>Gray-light brown to dark brown</td>
<td>Gray-yellow</td>
<td>None</td>
<td>27</td>
<td>C25P3</td>
<td>Spirals</td>
<td>Smooth</td>
<td>LL</td>
</tr>
<tr>
<td>7</td>
<td>Gray-light brown to dark brown</td>
<td>Gray-yellow</td>
<td>None</td>
<td>9</td>
<td>A13P3</td>
<td>Spirals</td>
<td>Smooth</td>
<td>LL</td>
</tr>
<tr>
<td>8</td>
<td>Gray-white-yellow-green</td>
<td>Brown-yellow</td>
<td>Yellow</td>
<td>2</td>
<td>B2P1</td>
<td>Spirals</td>
<td>Smooth</td>
<td>LL</td>
</tr>
<tr>
<td>9</td>
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<td>Brown</td>
<td>None</td>
<td>3</td>
<td>A21P1</td>
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<td>LL</td>
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<tr>
<td>10</td>
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<td>Yellow</td>
<td>2</td>
<td>A46R1</td>
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<tr>
<td>11</td>
<td>Gray</td>
<td>Gray-yellow</td>
<td>None</td>
<td>2</td>
<td>B13P1</td>
<td>Flexuous</td>
<td>Smooth</td>
<td>LL</td>
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<td>16</td>
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<tr>
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<td>Yellowish pink-white</td>
<td>Brown-yellow-black</td>
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<td>D19P2</td>
<td>Straight</td>
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<td>LL</td>
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<td>White</td>
<td>Violet-orange</td>
<td>Violet</td>
<td>3</td>
<td>B11R1</td>
<td>Fragments</td>
<td>Smooth</td>
<td>meso</td>
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<tr>
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<td>White-yellow</td>
<td>Yellow-brown</td>
<td>Yellow</td>
<td>18</td>
<td>A33P1</td>
<td>Straight</td>
<td>Warty</td>
<td>LL</td>
</tr>
<tr>
<td>17</td>
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<td>White-yellow</td>
<td>None</td>
<td>2</td>
<td>A22P1</td>
<td>Flexuous</td>
<td>Smooth</td>
<td>LL</td>
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<td>White-yellow</td>
<td>Brown-yellow</td>
<td>None</td>
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<td>A50P1</td>
<td>Open</td>
<td>Ridged</td>
<td>meso</td>
</tr>
<tr>
<td>No.</td>
<td>Color</td>
<td>Spore Color</td>
<td>Isolation</td>
<td>Spore Form</td>
<td>Loop Type</td>
<td>Spore Characteristics</td>
<td>Notes</td>
<td></td>
</tr>
<tr>
<td>-----</td>
<td>------------------------</td>
<td>-------------</td>
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<td>---------------</td>
<td>-----------------------</td>
<td>---------------------------</td>
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<td>19</td>
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<td>Gray-yellow</td>
<td>None</td>
<td>7</td>
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<td>Rugose LL</td>
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<td></td>
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<td>Gray</td>
<td>Gray-yellow</td>
<td>None</td>
<td>8</td>
<td>A27P1 Flexuous</td>
<td>Smooth LL</td>
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</tr>
<tr>
<td>21</td>
<td>Gray-white</td>
<td>Gray-yellow</td>
<td>Yellow</td>
<td>2</td>
<td>A13P2 Flexuous</td>
<td>Smooth LL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>Gray-white</td>
<td>Brown-black</td>
<td>Yellow</td>
<td>2</td>
<td>C7P2 Open loops</td>
<td>Smooth LL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>White-gray</td>
<td>White-gray-yellow-black</td>
<td>None</td>
<td>2</td>
<td>A1R2 Spirals</td>
<td>Smooth LL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>Gray-white</td>
<td>Gray-orange</td>
<td>Orange</td>
<td>2</td>
<td>A18P2 Spirals</td>
<td>Knobby LL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>Blue-gray-white</td>
<td>Gray-yellow</td>
<td>None</td>
<td>4</td>
<td>A5P1 Spirals</td>
<td>Spiny LL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>26</td>
<td>White-violet</td>
<td>Yellow-brown</td>
<td>Violet</td>
<td>2</td>
<td>C32P3 Flexuous</td>
<td>Knobby meso</td>
<td></td>
<td></td>
</tr>
<tr>
<td>27</td>
<td>Gray-yellow</td>
<td>White-gray-yellow</td>
<td>Yellow</td>
<td>2</td>
<td>C13R3 Spirals</td>
<td>Rugose LL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>Orange</td>
<td>Orange-yellow</td>
<td>None</td>
<td>6</td>
<td>A14R2 Straight</td>
<td>Knobby LL</td>
<td></td>
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</tr>
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<td>29</td>
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<td>Brown</td>
<td>Yellowish brown</td>
<td>2</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>Greenish gray</td>
<td>Greenish black</td>
<td>Green</td>
<td>4</td>
<td>B25R3 Open loops</td>
<td>Smooth LL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>31</td>
<td>Gray-white-yellow</td>
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<td>Yellow</td>
<td>3</td>
<td>B17R3 -</td>
<td>- LL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>32</td>
<td>Red-white-orange-pink</td>
<td>Red-white-orange-pink</td>
<td>None</td>
<td>4</td>
<td>A37P3 -</td>
<td>- LL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>33</td>
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<td>Red-orange</td>
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Note: ± spores not formed; ± fragmentation spores produced.

Innumerable media formulations have been proposed for the selective isolation of streptomycetes. Many of those have been considered to be successful, but there is a general lack of detailed evaluation. Thus, for example it is possible to obtain cleaner isolation plates of
streptomycetes using chitin agar (Hsu & Lockwood, 1975) or increased sodium chloride concentrations (Williams et al., 1983). Most success has been achieved using computer-formulated selective media, especially raffinose-histidine agar (Vickers et al., 1984; Williams & Vickers, 1988). These workers concluded that there is no such thing as a non-selective medium for all streptomycetes. Ideally several selective media need to be used to gain a more accurate picture of the qualitative nature of streptomycete populations in soil. The value of this approach was exemplified in the present study as members of several colour groups were mainly or exclusively associated with one or the other of the two selective media.

Thirty one out of 37 colour groups that encompassed isolates from the raffinose-histidine and starch-casein agar plates, seeded with the environmental suspensions derived from the modified DDC procedure, contained the LL-isomer of diaminopimelic acid thereby confirming that their original assignment to the genus Streptomyces was correct. In contrast, the representatives of colour groups 14, 18, 15, 36 and 37 contained meso-A$_{2pm}$. These findings are not surprising as it is well known that representatives of several actinomycete genera, notably the genera Actinomadura, Amycolatopsis, Nonomuraea and Saccharopolyspora form colonies that can be difficult to separate from those formed by streptomycetes.

The colour groups which encompass isolates shown to contain LL-A$_{2pm}$ can be seen as taxonomically predictive as many representatives of such groups delineated in earlier studies key out to known species using computer-assisted identification and PyMS procedures (Goodfellow & Haynes, 1984; Williams & Vickers, 1988; Atalan et al., 2000). It is evident, therefore, that many taxonomically different streptomycetes are associated with the root system of P. falcata. Thus, streptomycetes from 20 multi-membered clusters and around 35 single-membered clusters are associated with the ectorhizosphere of this plant; the corresponding numbers for the rhizoplane are 17 multi-membered and around 35 single-membered clusters. Similarly, isolates from 19 colour groups and around 31 single-membered clusters were recovered from the non-rhizosphere composite samples. It is interesting that all these composite environmental samples have a richer streptomycete flora than that found in marine sediments (Goodfellow & Haynes, 1984). These workers assigned over 200 representative isolates to 25 colour groups which contained between 1 and 121 strains.

There is some evidence from the present study that the composition of the streptomycete communities in ectorhizosphere and rhizoplane of P. falcata may
differ both from one another and from surrounding non-rhizosphere soil. Thus, representatives of only 10 out of the 31 colour groups were associated with all three composite environmental samples. This number rose to 12 when the comparison was restricted to isolates from the rhizoplane and ectorhizosphere samples. These data need to be interpreted with care as the apparent specificity may merely be an expression of sampling error. Nonetheless, these findings are in tune with earlier reports which claimed that differences occurred between actinomycete populations in rhizoplane, rhizosphere and non-rhizosphere soil (Vruggink, 1976; Buti, 1978). In contrast, Watson and Williams (1974) found little qualitative different between the population of actinomycetes found in the rhizosphere of the sand dune plants, *Ammophila arenaria* and *Agropyron junceiforme*, and non-rhizosphere soil. They also noted that old, dead roots of *Ammophila* were readily colonised by *Micromonospora, Nocardia, Rhodococcus* and *Streptomyces* strains.

Based on the results of this study, it is reasonable to conclude that the application of DDC extraction method along with the selective isolation could provide a strong way to achieve a more representative sampling procedures in order to unravel streptomycete diversity within soil samples taken from the environmental habitats.

References

Anonymus 1964. *The ISCC-NBS Color-Name Charts Illustrated with Centroid Colors*. Supplement to NBS Circular 553.


O-SE07

GENETIC DIVERSITY ANALYSIS OF INDIGENOUS *Bacillus thuringiensis* ISOLATES PATHOGENIC TO *Crocidolomia binotalis* BY USING MOLECULAR PHYLOGENETIC APPROACH BASED ON 16S RRNA GEN SEQUENCES

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Genetic diversity of indigenous *Bacillus thuringiensis* isolates pathogenic to *Crocidolomia binotalis* was determined by using molecular phylogenetic approach. Six isolates were taken to represent 43 most pathogenic isolates selected from 161 potential isolates. Genomic DNA from the test isolates was prepared by using standard extracted method. Then, 16S rRNA gen were amplified by PCR using five concerned primers. The purified PCR products were sequenced by using an ABI PRISM 310 DNA sequencer. The 16S rDNA sequence data were aligned with corresponding available *B. thuringiensis* sequences retrieved from the NCBI data base by using the CUSTAL X software. Phylogenetic trees were generated by using the PHYLIP software package. The results of the investigation confirmed and extended the value of 16S rDNA sequencing in *B. thuringiensis* genetic diversity studies. The almost complete 16S rDNA sequence data showed that four of the tested isolates (YWKA.1, BAU3-2, BLPPN8.2 and YPPA) formed a distinct center of diversity with *B. thuringiensis* serovar *colmeri* 15A3. Accordingly, the isolate TK9 was found to form a center of diversity with *B. thuringiensis* BAB-B12. However, the isolate LPST-1 seemed to be the most different strain since it solely formed a new center of diversity within the 16S rRNA gene tree. The molecular phylogenetic analysis indicated that most of test isolates belong to novel strain groups within the species of *B. thuringiensis* except the isolate TK-9 which was closely related to *B. thuringiensis* BAB-B1-2. Therefore, it has been demonstrated that molecular phylogenetic analysis provide a powerful way of uncovering genetic diversity of strains within the species *B. thuringiensis*.

**Key words:** genetic diversity, indigenous isolates, *Bacillus thuringiensis*, *Crocidolomia binotalis*, molecular phylogenetic analysis, 16s rRNA gen sequences.
Introduction

*Bacillus thuringiensis* (Bt) is a spore-former bacteria, rods 3 to 5 μm, gram positive, and facultative anaerobic. During sporulation, the bacteria produce parasporal crystal which consists of one or more insecticidal protein in form of crystal complex which is known as ICP (Insecticidal Crystal Protein) or δ-endotoxin (Schneph *et al.*, 1998; Helgason *et al.*, 2000; Bahagiawati, 2002; Bajwa & Kogan, 2001).

Strains of *B.thuringiensis* have been used nowadays as a biological control agent to control pest and disease vector population. Strains of *B.thuringiensis* serovar *kurstaki* have been mostly applied in the agricultural fields (Hoofman & Frudsha, 1993; Zhong *et al.*, 2000; Lone *et al.*, 2001) whereas *B.thuringiensis* serovar *israelensis* have been mainly used health problems (Goldberg & Margalit, 1977). Every strain of *B.thuringiensis* possess a limited spectrum to kill certain group of insects (Bahagiawati, 2002; Wudyastuti *et al.*, 2004).

Development of computer application in the field of microbial systematics has produced numerical systematics which provides capability to carry out classification and identification of microbial strains quantitatively and objectively (Logan *et al.*, 1994; Lecadet, 1999; Sembiring, 2002). Further more, the application of biochemical techniques in microbial systematics by analysing cell wall components, membrane lipids, and proteins have finally developed chemosystematics which is very important for bacterial generic assignment (Brosseau *et al.*, 1993; Obeidat *et al.*, 2004). Finally, revolution of molecular biology has a very important role to provide valuable data for the basis of molecular systematics development which could utilizes genetic information within DNA and RNA molecules in order to classify and identify microbial strains (Stackebrandt & Goebel, 1994). The main objectives of microbial classification is to provide a rigorous basis for identification of microbial strains up to the level of species as a basic unit of taxonomy.

Phylogenetic classification could reflect the degree of evolutionary relationship among microbial strains. Application of phylogenetic classification is performed by construction a phylogenetic tree on the basis of molecular data in form of nucleic acid or protein sequences. Nucleic and protein sequences generally develop with more definitive pattern than morphological and physiological characters. Therefore, nucleic acid sequences are expected to depict a more clear phylogenetic relationship among organisms.
Genetic diversity of bacterial strains could be determined by molecular analysis, namely through the application of molecular systematics based on the analysis of 16S rRNA gene sequence. The result of analysis is formulated in form of phylogenetic tree, nucleotide similarity and nucleotide difference matrix could provide information regarding the genetic diversity among bacterial test strains. This paper, described the effort to unravel genetic diversity of indigenous B. thuringiensis isolates pathogenic to Crocidolomia binotalis by comparing with 30 different known strains, including B. thuringiensis serovar kurstaki which has been widely known to be specifically pathogenic to Lepidopterans and B. thuringiensis serovar israelensis which has been widely known to be specifically pathogenic to Diptera.

Materials and Methods

1. Test Strains.

Six isolates of indigenous B. thuringiensis pathogenic to C. binotalis (BAU3-2, BLPPN8.2, LPST-1, TK9, YPPA, and YWKA.1) were grown in Luria Bertani broth Medium overnight. As musch as 1.0 ml of broth culture was harvested by centrifugation at 13,000 rpm for 5 minutes. Obtained pellet was used as a source of chromosomal DNA.

2. DNA Isolation and Purification.

Bacterial pellet was suspended into 400 µl lysing solution containing 75mM NaCl, 25mM EDTA, 20mM Tris-HCl, pH 7.5. Then, 50 µl of lysozyme solution (100mg/ml) was added, and incubated at 37°C for 1 h. Subsequently, chromosomal DNA was isolated and purified according to standard procedure.


16S rDNA sequence was amplified by PCR using conserved primers (Lane, 1991). The PCR products were purified by a commercial Microclean Kit. The purified PCR products were sequenced by using an automatic sequencing method with Automatic ABI PRISM 310 DNA sequencer.
3.1. Oligonucleotide primers used in PCR amplification and sequencing of 16S rDNA.

Oligonucleotide primers (Lane, 1991) used in the PCR amplification of 16S rDNA were (27f): AGAGTTTGATCCTGGCTCAG and (1529r): CAIAACGACGTGATCC.

3.2. PCR amplification of 16S rDNA.

Polymerase chain reaction of amplifications of the 16S rDNA preparations were carried out in a Thermocycler Gene Cycler (Biorad) using 25 μl PCR solution containing 2-20 ng of DNA template, 2.5μM of both primers, 25 mM dNTPs, 10x of Taq buffers, 2.5 mM of MgCl₂, and 0.0025 U/μl. The PCR amplification was done by one cycle of predenaturation of mixture (96°C; 5 minutes), then followed by 25 cycles with denaturation (95°C; 1 minute), annealing (55°C; 1 minute), ended by final extension (72°C; 10 minutes), and finally cooling to 4°C. Electrophoresis was run at 100 V for 1 h and the size of the amplified 16S rDNA fragments identified by comparison with a molecular size marker (Gene Ruler™ 100 bp DNA Ladder Plus, MBI Fermentas) at the position 1.5 Kb. Purification of PCR product was carried out by a commercial Microclean Kit. Purified PCR products were used for sequencing experiments.

3.3. Sequencing of purified 16S rDNA

The purified 16S rDNA fragments were sequenced by using an Automatic ABI PRISM 310 DNA sequencer by a standardized procedure. The sequencing of the complete length of the 16S rDNA molecule was carried out by using the five different oligonucleotide primers (Lane, 1991) separately, namely (27f): AGAGTTTGATCMTGGCTGAG, (357f): TACGGGAGGCAGCAG, (790f): ATTAGATACCTGGTAG, (981f): CCCGCAACGAGCGACACCC, and (1230f): TACACACGTGCTACAATG.

3.4. Analysis of 16S rDNA sequence data.

The 16S rDNA nucleotide sequence data obtained using the Automatic DNA sequencer were transferred into CHROMAS and Programmer File editor software to
generate a complete sequence of each test strain from each five sequencing primer used. Complete sequence of each test strains were aligned by CLUSTAL X (Thompson et al., 1997) software before using to generate phylogeny tree by using PHYLIP Package (Felsenstein, 1993).

3.5. Alignment of 16S rDNA sequences

The 16S rDNA nucleotide sequences of the test strains were automatically aligned with corresponding available B. thuringiensis strain sequences retrieved from NCBI by using CLUSTAL X (Thompson et al., 1997) software. Two data sets consisting aligned sequences of test strains and reference strains was obtained from alignment process. The first dataset was with PGYLIP format (*.phy) and the second dataset was with GDE format (*.gde). The first data set was subsequently used to generate phylogenetic tree by using PHYLIP Package, and the second dataset was subsequently used to generate nucleotide similarity and difference matrix.

3.6. Construction of Phylogenetic Trees

Evolutionary trees were inferred by using the neighbour-joining (Saitou & Nei, 1987). Evolutionary distance matrix for the neighbour-joining method was generated as described by Jukes and Cantor (1969). A phylogenetic tree based on 16S rDNA sequences was constructed by using PHYLIP Package with the neighbour-joining algorithm (Saitou & Nei, 1987). The root position of the unrooted tree based on the neighbour-joining method was estimated by using Bacillus cereus ATCC 15479T as the outgroup strain. Resultant phylogenetic tree was visualised by TREE VIEW software and then transported into document file (Microsoft WORDS). Finally, the phylogenetic tree was edited and saved as document file and presented as final results.

3.7. Construction of nucleotide similarity and difference matrix.

Aligned sequences of strains contained in the second dataset with GDE format (*.gde) was used to construct nucleotide similarity and difference matrix by using PHYDIT software (Chun, 1999). Resultant matrix was then copied into EXCELL
software and used as a basis to construct the same matrix in document file (Microsoft WORRDS). The final matrix was used to present the final result.

Results and Discussion

Sequencing of 16S rDNA

Comparison of 16S rDNA nucleotide sequences of the B. thuringiensis indigenous strains with the corresponding nucleotide sequences of available various strain representatives of the species B. thuringiensis clearly showed that of strains within the species are very diverse in terms of 16S rRNA gene tree (Figure 1). In fact, all of strains fall into seven outstanding clade. Therefore, this result clearly demonstrated that genetic diversity of strains within the species could clearly be unravelled on the basis of molecular analysis using phylogenetic tree based on 16S rRNA gene sequences.

The first clade was named as Clade finitimus since it contained tree which strains belong to serovar finitimus. The second clade was named as Clade thuringiensis since it contained type strain of B. thuringiensis (ATCC 10792T). The third clade was named as Clade morrisoni since it contained only four strains, including serovar morrisoni (B6069), kurstaki (EU429663.1), dendrolimus (CB02), and serovar galleriae (CB01/T4). The fourth clade was named as Clade colmeri since it contained the only one reference strain in this clade, namely serovar colmeri (15A3) together with four of the test strains. The fifth clade was single-membered clade which contained only serovar aizawai (19198), and therefore it was named as Clade aizawai. The sixth clade was named as Clade kurstaki since it contained a famous commercial strain of serovar kurstaki (Dipel; DQ 683076). Finally, the seventh clade was named as Clade tolworthi-azorensis since it contained only two strains, namely serovar tolworthi IEBC-T09001) and serovar azorensis (BGSC4CB1).

The six test strains were found to be separated into two clades, namely, most of them (four out of six strains (BAU3-2, BLPPN8.2, YPPA, and YWKA.1) fall into the fourth clade, namely Clade colmeri. It means that they are very closely related with B. thuringiensis serovar colmeri 15A3. Therefore, it is important to trace out the database of the strain of serovar colmeri (15A3) in terms information regarding, including the origin and distribution of strain, phenotypic characters ranging from physiological characters to pathogenicity. By doing so, the hypothesis of the similarity
between the test strains and the serovar *colmeri* (15A3) could be tested and verified. Furthermore, by direct comparison between the test strains with the serovar *colmeri* (15A3), the novelties of the indigenous test strains could also be verified and determined exactly.

![Phylogeny tree](image)

**Figure 1.** Neighbour-joining (Saitou & Nei, 1987) Phylogeny tree constructed on the basis of nearly complete of 16S rRNA gene sequences showing relationship amongst the sixth representatives of indigenous *B. thuringiensis* strains pathogenic to Cabbage pest (*Crocidolomia binotalis*) and reference strain of various serovar within the species *Bacillus thuringiensis*. The arrow indicates estimated root
Tabel. 1.16S rDNA similarity values (%) and the number of nucleotide differences found between the test strains (TK9 and LPST-1) and reference strains belong to species *Bacillus thuringiensis* within the Clade *finitimus*.

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Tabel 2. 16S rDNA similarity values (%) and the number of nucleotide differences between four test strains (BAU3.2, BLPPN8.2, YPPA and YWKA-1) and the reference strain of *Bacillus thuringiensis* serovar colmeri 15A3.
Another two test strains, namely TK9 and LPST-1 were found to fell in the biggest clade (first Clade) that was Clade finitimus. However, each of the test strains was relatively separated one another within the Clade finitimus. The strain TK9 was clearly very tightly related with the B. thuringiensis BAB-Bt2. It is therefore very important to search the information from database of B. thuringiensis BAB-B12 in order to carry out direct comparison of strain TK9 with this closest strain. By doing so, the hypotesis of the similarity of the two strains colud be tested and the novelty of the indigenous strain of TK9 could also be verified. Subsequently, the test strain of LPST-1 seemed to be the most different among test strains since it formed a single-membered clade within the Clade finitimus. It means that the strain could be certainly perceived as a novel strain within the species B. thuringiensis. Its novelty was also supported by numerical systematic analysis which showed that the strain was found to be very different with both of reference strains, namelycommersial strain of B. thuringiensis serovar israelensis (Teknar) or B. thuringiensis serovar kurstaki (Dipel). Therefore, in terms of novelty, this strain (LPST-1) could be seen as a new indigenous strain from Indonesia and thus need to be characterized more thoroughly especially regarding the serological test and pathogenicity spectrum.

In terms of nucleotide similarity and nucleotide differences (Table 1 & Table 2) it showed that within the Clade finitimus (Figure 1; Table 1) the strain TK9 was identical with the reference strain of B. thurinensis H3. However this result did not fully in accordance with its phylogenetic relatedness based on phylogeny tree which showed that the closest strain was found to be B. thuringiensis BAB-Bt2. Accordingly, the strain LPST-1 was also found to be identical with the reference strain of B. thuringiensis serovar tenetronion (EU 429671.1) on the basis of nucleotide similarity and the number of nucleotifte difference. Interestingly, this result clearly showed a full congruence with relatedness value based on phylogeney tree analysis.

Regarding another four test strains (BAU3-2, BLPPN8.2, YPPA, and YWKA.1), they were found to be fully congruent between similarity vaues and nucleotidte differences in one hand and phylogenetic analysis, on theother hand (Table 2; Figure 1.). It was also even more interesting that within the clade, the similarity value and the number of nucleotidte difference of strain YPPA was found to be completely congruent with the result of phylogenetic analysis.

Based on the result of the study it is encouraging to conclude that the gentic diversity of strains within the species B. thuringiensis could be clearly uncovered by
using molecular phylogenetic approach based on 16S rRNA gene sequences. Furthermore, on the basis of phylogenetic analysis, it was found that most of the indigenous strains tested in this study were very different with the reference strains which contained various well known strains. Therefore, it is reasonably to propose that the indigenous test strains belong to species B. thuringiensi, pathogenic to cabbage pest (C. binotalis) are most likely novel strains.

References


O-SE08

MOUND CHARACTERISTICS OF ORANGE-FOOTED SCRUFOWL (Megapodius reinwardti) IN RINCA ISLAND, KOMODO NATIONAL PARK.

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Orange-footed Scrubfowl (Megapodius reinwardti) uses heat from the environment by building mounds to incubate its eggs. This study examined mound characteristics of orange-footed scrub fowl in Rinca Island, Komodo National Park. Nests were searched in two locations, i.e. Loh Baru and Loh Buaya, from March to May 2008. Data collected were mound height and diameter, soil texture, soil organic composition, altitude, light intensity, vegetation around mound, distance from the sea shore, temperature inside and outside mound, and humidity outside mound. Sixteen nests were found but only seven nests were accessible for measurement. The average height of the mound ranges 0.48-0.90 m, the diameter at the base ranges 5.11-9.30 m, while diameter at the mouth ranges 2.25-6.05 m. Soil pH ranges 7.5-8, organic content ranges 40.46-59.16%, altitude ranges 4-30 m above sea level and the distance from seashore ranges 300-1100 m. Soil texture is dominated by sand in Loh Baru and by dust in Loh Buaya. Light intensity varied between 18.83 and 238.56 kw/m2, water content varied between 21.42 and 47.06%, temperature outside mound ranges 26.13-30.50°C, and humidity ranges 46.25-92.25%. Temperature inside mound varied in each level of depth, those were 29.25-33.25°C (75 cm), 25.75-33.25°C (50 cm), and 27.75-32.25°C (25 cm).

Keywords: Orange-footed Scrubfowl, Rinca Island, mound characteristics

INTRODUCTION

Orange-footed Scrubfowl (Megapodius reinwardti) belongs to family Megapodiidae which uses heat from its environment to incubate its eggs. The bird constructs incubation mounds made of decomposing plant litter and stones to get appropriate temperature for egg hatching.

The species has wide range of distribution, including Indonesia, Southern New Guinea and Northern Australia (Coates et al 1997). In Indonesia, the distribution of this bird include Lombok, Sumbawa, Komodo, Sumba, Flores, Pantar, Alor, Wetar,
also possible in Timor, east-ward to Romang, Damar and Babar up to Kepulauan Kai, but not present in Kepulauan Tanimbar (Monk et al. 1997). Orange-footed Scrubfowl also recorded in lowland forest in Komodo NP. Orange-footed Scrubfowl occurs in all big islands in Komodo National Park, except Padar Island. The ecological information about Orange-footed Scrubfowl in Komodo Island has been provided by Jessop et al. (2006). In this area, Orange-footed Scrubfowl nests have important value to support not only Orange-footed Scrubfowl breeding but also Komodo dragon. Jessop et al. (2004) reported that more than 70% of komodo females use Orange-footed Scrubfowl nests to bury their eggs. There was less information available on Orange-footed Scrubfowl in the neighboring Rinca Island. This study was done to find out the characteristics of Orange-footed Scrubfowl mound in the neighboring island of Rinca.

METHOD

This research was conducted in Rinca Island, Komodo National Park for ± 3 months, March – May 2008. Data collected consisted of mound dimension, soil texture, density of subsoil-water, soil pH, organic composition of the soil, and altitude from sea level, vegetation around mound, distance from the sea shore, temperature inside and outside the mound, and humidity outside mound.

Nest (mound) measurement was conducted on two kinds of nests, those were active and non-active nests (abandoned nest). Active nests were identified by signs of new pile of organic material, recent digging by Orange-footed Scrubfowl, footprints and recent observation of the bird(s) around nest (Sinclair 2001; Jessop et al. 2004). Mounds with no signs of new pile of organic material, present of plants above nest, soil of nest become hard, were considered as non-active mounds (Jessop et al. 2004).

Vegetation analysis was carried out using the section-line method, each 60 m X 20 m. Temperature inside the mound was measured in various levels of depth (25 cm, 50 cm and 75 cm). Soil texture of each sample of the mound was analyzed in the Soil Laboratory, Faculty of Agriculture, Bogor Agricultural University (IPB). The measurement soil pH was done by reading the Universal indicator paper pH 0-14 MERCK. The value of organic content in the soil comes from burning the substrate, assuming that all organic things can be burnt completely so that the value of organic
content derives from the percentage of former organic weight minus the weight without ash divided by the former weight.

RESULTS AND DISCUSSION

The mounds of Orange-footed Scrubfowl were situated in lowland forest. Loh Baru had five mounds which consist of four active mounds and one non-active mound. Loh Buaya had nine active mounds and two non-active mounds. Most of the mounds in Loh Buaya were shared with komodo.

a. Vegetation around mound

Eighteen plant species were recorded around the mounds in Loh Baru of plants. The most common and dominant species were nita (Grewia sp), kukun (Schoutenia ovata), paci (Voacanga sp), sita (Alstonia spectabilis), nyamplung (Callophylum inophyllum) and asam (Tamarindus indica). Orange-footed Scrubfowl built mounds with one side adhered to the base of big tree. Loh Baru (LBr1) mound associated with tamarind tree whereas LBr2 and LBr3 associated with Ficus tree. This condition related to the supply of leaves, branch, trunk and tree root that were used by Orange-footed Scrubfowl to build a mound. The vegetation around mounds in Loh Buaya were kesambi (Schleichera oleosa), asam, bidara (Zizyphus jujuba), paci and mbiring (Pithecellobium umbelatum). The position of mound was indeed close to big tree, but there was still a distance. The distance helped Orange-footed Scrubfowl to get organic materials from vegetation around nest and while still be able to receive solar heat.

b. Shape and Dimension

Generally, Orange-footed Scrubfowl builds mound that consists of pile of litter and leaves, branches, trunks and sometimes stones. This piling procession happens every day when the birds maintain the nest. The dimension of the mound was influenced by this behavior, such as height and diameter of the mound, and the number of holes of the mound. Mounds in Loh Baru were higher compare to Orange-footed Scrubfowl mounds in Loh Buaya but smaller in diameter.
The hole depth that are used for burying the eggs varied. During observation, first pair of Orange-footed Scrubfowl laid the egg at 75 cm in depth, the second pair laid the egg at 86 cm depth and the third pair laid the egg at 25 cm depth. The differences in depth show us the fact that the bird can bury eggs in varying depths due according to suitable temperature needed for egg hatching.

In case of the number of hole, shared mound had higher number of holes than the mound that only used by Orange-footed Scrubfowl (Table 1).

Table 1. Variation on height, diameter and number of holes of mounds.

<table>
<thead>
<tr>
<th>No.</th>
<th>Nest Code</th>
<th>Height (m)</th>
<th>Diameter (m)</th>
<th>Egg Hole</th>
<th>Deceit Hole</th>
<th>Total Holes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>LBr 1</td>
<td>0.90</td>
<td>0.90</td>
<td>6.63</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>2.</td>
<td>LBr 2</td>
<td>0.84</td>
<td>0.44</td>
<td>8.73</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>3.</td>
<td>LBr 3</td>
<td>1.17</td>
<td>0.61</td>
<td>5.11</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>4.</td>
<td>Lby 1</td>
<td>0.55</td>
<td>0.53</td>
<td>9.30</td>
<td>5</td>
<td>9</td>
</tr>
<tr>
<td>5.</td>
<td>Lby 2</td>
<td>0.77</td>
<td>0.49</td>
<td>6.65</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>6.</td>
<td>Lby Sta 1</td>
<td>1.07</td>
<td>0.64</td>
<td>5.13</td>
<td>2</td>
<td>*</td>
</tr>
<tr>
<td>7.</td>
<td>Lby Sta 2</td>
<td>0.50</td>
<td>0.48</td>
<td>8.70</td>
<td>3</td>
<td>*</td>
</tr>
</tbody>
</table>

Note: LBr: Loh Baru, Lby: Loh Buaya, Lby Sta: Non-active nest in Loh Buaya, T1: Maximum height, T2: Minimum height, Dt: average nest diameter, Dm: average edge nest diameter *: no deceit hole.

c. Physical and Chemical characteristics

Soil acidity expressed from pH value was not different among mounds. Mounds had different level in organic material due to nest and location. Nest in Loh Baru varied in terms of altitude than those in Loh Buaya, although nests in Loh Buaya varied more in distance from seashore than those in Loh Buaya. This condition relate to the topography and the available lowland area in those two locations (Table 2). The topography in Loh Baru is hilly, with narrow lowland that is very differ from Loh Baru which has vast expanse of lowland in Rinca island. The soil substrate in Loh Baru were mostly sandy, resulting most nests adhered to the base of big tree. The roots of the tree hold the soil so the mound will not get washed out by rainwater during the rainy season.
Table 2. The characteristics of nests

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Nest</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LBr 1</td>
</tr>
<tr>
<td>Soil pH</td>
<td>7.5</td>
</tr>
<tr>
<td>Organic material (%)</td>
<td>59.16</td>
</tr>
<tr>
<td>Attitude (masi)</td>
<td>4</td>
</tr>
<tr>
<td>Distance from seashore (m)</td>
<td>300</td>
</tr>
<tr>
<td>Soil Texture (%)</td>
<td></td>
</tr>
<tr>
<td>a. Sand</td>
<td>60.45</td>
</tr>
<tr>
<td>b. Dust</td>
<td>36.19</td>
</tr>
<tr>
<td>Water content (%)</td>
<td>32.77</td>
</tr>
<tr>
<td>Temperature outside (°C)</td>
<td>27.60</td>
</tr>
<tr>
<td>Air humidity (%)</td>
<td>84.92</td>
</tr>
<tr>
<td>Temperature inside nest (°C)</td>
<td></td>
</tr>
<tr>
<td>50 cm</td>
<td>30.06</td>
</tr>
<tr>
<td>25 cm</td>
<td>29.13</td>
</tr>
</tbody>
</table>

The average temperature in active mounds in Loh Baru at depths of 50 cm and 25 cm were 30.56°C and 29.47°C (n = 3). The temperature were lower compared to mound temperature in Loh Buaya at similar depth, which were 31.4°C and 30.71°C, respectively (n = 2). Overall, mound temperature at 50 cm and 25 cm depth were 30.9°C dan 29.96°C, respectively (n = 5). Non-active mounds have lower average temperature which was 27.79°C (25 cm depth, n = 2). The temperature measured in this research fell into the ranges reported by del Hoyo et al (1994) as the temperature range needed for incubation by Orange-footed Scrubfowl, i.e. 28-35°C.

Most of the mounds in Loh Baru obtained heat from microbial respiration. In Loh Buaya, which has opened canopy, mounds obtained heat from solar radiation. The temperature inside tended to increase with depth. Picture 1 shows the differences in temperature for each observation. The temperature at 50 cm depth, tend to be stable compared to those at depth of 25 cm. The temperature of upper layer tend to follow the fluctuation of ambient temperature, while inside layer was more protected and the heat conduction went slowly. The temperature of LBy 1 was
relatively higher than the other mound at 50 cm depth or 25 cm depth. The temperature at 75 cm depth could not be observed constantly. On this depth, the measurable temperature was 27.25-33.25°C in range.

![Temperature graph](image1)

Fig 1. The fluctuation temperature inside nest in two depth (a) 50 cm (b) 25 cm base on time of the day.

The outside temperature of mound was influenced by solar radiation that reached the mound surface. There was difference between temperature and humidity outside the nest. The temperature in Loh Baru was lower than in Loh Buaya but the humidity was higher than in Loh Buaya (Picture 2).

![Humidity and Temperature graph](image2)

Fig 2. Fluctuation of temperature and humidity outside mound (a) per ulangan, (b) per time observed

d. Mound use by komodo

The Orange-footed Scrubfowl mound, were not only use by themselves but also known to be used by komodo dragon. Komodo dragon uses the nest of Orange-footed Scrubfowl for many purposes, such as breeding, sleeping site and cleaning their body from vermin while Orange-footed Scrubfowl uses mound only for breeding.
Active mounds in Loh Buaya were shared between these two species. The tendency of komodo dragon of not using the mound in Loh Baru was related to the position of mound that were far from savanna. Purwandana (2007) reported that komodo prefers using mound nearby savanna so that they can sunbathe while they keep the nest in breeding season.

ACKNOWLEDGEMENT

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LITERATURE CITED


O-SE09

THE DIVERSITY OF FOLIOSE LICHENS IN THE FOREST OF TAHURA R SOERYO, BATU, EAST JAVA

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Lichen is an outstanding successful group of symbiotic organisms, which comprise the strand of algae partner (phycobiont) and fungal partner (mycobiont). According to the morphological forms, there are seven growth-forms of lichen, however, there are five common growth-forms, i.e.: foliose, fruticose, crustose, squamulose, and leprose.

A taxonomic study of the foliose lichens in the forest of Tahura R Soeryo had been conducted based on morphological, anatomical, and chemical characters. In this research involved a method of descriptive explorative and the aim of this research is to study the diversity of foliose lichens in the forest of Tahura R. Soeryo. Tahura R Soeryo is a natural forest located in Tulungrejo, Batu City, East Java. Its altitude is 1000-3000 m, the rainfall is about 2500-4500 mm per year, and the temperature is about 5°C - 10°C. This research was conducted from June - July 2009. Fifteen species of foliose lichen is reported from the forest of Tahura R Soeryo. They are Parmelia soredians, Parmelia crinata, Candelaria concolor, Leptogium azureum, Xanthoria polycarpa, Parmelia sinuosa, Platismatia glauca, Parmelia saxatilis, Lobaria pulmonaria, Phaeophyscia orbicularis, Parmeliopsis aleurites, Parmelia sinuosa, Nephroma parile, Menegazzia terebrata, and Dermatocarpon miniatum.

The taxonomic, the current delimitation and description of the species; and the identification key to its species of the Tahura R Soeryo's foliose lichen are presented in the article.

**Keywords:** diversity, foliose lichen, Forest of Tahura R. Soeryo

**Foreword**

Everyone is familiar with plants as green chlorophyll- containing organisms that manufacture their own food. A lichens is also a plant, but a very special kind, for when we dissect and examine it under a microscope, we find that it is composed of two completely different organisms, microscopic green or blue-green algae and colorless fungal threads called hype. These two components grow together in a harmonious association referred to as symbiosis, or more simply a “living- together”. Lichen symbiosis, however, differs basically from all other kinds in that a new plant
body, the thallus, is formed and this talus has no resemblance at all to either a fungus or alga growing alone (Hale, 1969).

Lichens can be found from extreme low tide level on the sea-shore to the tops of high mountains, and from arctic to tropical regions. The wide distribution is the more remarkable because lichen is a symbiotic association between two quite different organisms: a photosynthetic green alga, or less often a cyan bacterium (‘blue green alga’), and a fungus. The larger lichens in the last two categories grow, on average; are about 5 mm per year in length or rapidly and are usually attached to their substratum by complex fungal strands called rhizome (Nash, 1997).

Growth form means the overall shape and configuration of the lichen talus. There are three major types: foliose, fruticose, and crustoce. A fourth type, the squamulose lichens, may also be recognized. Foliose lichens are flattened, and prostate with an upper surface that is different from the lower. Foliose thallium are either homoiomeric or heteromorous. Foliose lichens develop a great range of talus size and diversity (Richardson, 1992).

Lichens grow relatively slowly and persist for tens or hundreds of years on their substratum. Whether it is tree bark or rock. They are nutrient sources for a wide variety of invertebrates, which are often specialized lichen consumers. These include protozoa, mites, snails, and caterpillars. In some parts of the worlds, the larger ground and tree-dwelling lichens form a food source for various deer, especially reindeer (Richardson, 1975, 1991). Lichens have also evolved very efficient mechanism for accumulating nutrients from the environment in which they live. Some of the metabolic produced by lichens can break down such particles, releasing nutrients which may then be taken up into the cells of the lichens (Richmond, 1992).

Tropical forest has a complex component either flora or fauna. One of the tropical forests in Indonesia is an Arjuno Lalijiwo forest which is more famous with Taman Hutan Raya R.Soeryo (TAHURA). Geographically, Tahura is located in Tulungrejo village, Bumiaji District, East Java. Topography in general has various configurations among superficial, hilly, mountain with 1000-3000 m high from the upper surface of sea shore. Biotic potential of that area is flora condition dominated various kinds of plant (Alexopoulos, et al. 1996).

Lichens are one of the vital diversity, which is had right now that is still neglected. Lichens has not used in maximal way whereas lichens is a very potential ecosystem. Lichens is has function as oxygen supplier. Besides that, Lichens has
function for human life, as bioindicator of pollution. *Lobaria* lichens provide more than 20 percent of mingled nitrogen per year. This sum is out of input nitrogen from other sources, *Usnea barbata*, and *usnea dasypoga* produce antibiotic called usnin acid. Usnin acid is useful against TBC bacterium. In perfume-manufacturing, *Everania prunasti* is used oil essential, *Rocella tinctoria* is used as dye, la spices, flour, soup ingredient, and even frying oil (Alexopoulos, et al. 1996).

Therefore, we had conducted a lichen taxonomical research in purpose to study the diversity of lichens in TAHURA R.SOERYO. By studying and knowing lichens species in Tahura we hope students, college students, and society to know diversity kinds of lichens conserve it.

**Research Method**

This research is descriptive explorative which has purpose to know the diversity of foliose lichens in Tahura R. Soeryo. These researches were conducted from Juny- July 2009, in which involved a method of descriptive explorative technique. For precise identification, it is necessary to examine collected specimens to determine the shape and colour of the lobes, the presence or absence of soredia and isidia and the size and septation of the spores within the fruit bodies and chemical tested. Chemical substances used are P (Phenylenediamin), K (Potassium hydroxide), and C (Calcium hypochlorite). The examination treatment is by dropping chemical substance, then looking at the color changing in medulla.

**Research Result and Discussion**

In this result, we make identification key to easy the characteristic of this lichens. For this identification key we know fourteen eight family i.e. *Parmeliaceae, Candelariaceae, Physciaceae, Lobariaceae, Collemateaceae, Nephromataceae, Teloschistaceae, Verrucariaceaeace*. The Identification key can see in this table identification below:
## Identification key

1. **Lower surface with pores**  
   a. **Lower surface with a darker tomentum**  
   b. **Upper surface green-yellow with white simple rhizine**  

2. **Upper surface grey with black rhizine on the margin of under surface (K+y, P+o)**  
   a. **Menegazzia terebrata**  
   b. **Upper surface green yellow with white simple rhizine**  

3. **Upper surface green-yellow with white line on the under surface (K+r, P+r)**  
   a. **Parmelia saxatilis**  
   b. **Upper surface grayish white to light brown up to 1,6cm broad**  

4. **Thallus is like the bark and has dark brown ostiole on the upper surface with 1mm-7,5mm in size (K+y, P+r)**  
   a. **Dermatocarpon miniatum**  
   b. **The thallus is grayish grey, and has an ostiole on the top of the thallus**  

5. **Has an apothecia that same colour with the thallus, the colour of epithelium is brownish yellow**  
   a. **Xanthoria policarpa**  
   b. **Has an grey isidia and soredia, like the upper thallus**  

6. **Under thallus dark brown with white rhizine(P+r)**  
   a. **Nephroma parile**  
   b. **Under surface is light bluish grey like the upper surface with white soredia**  

7. **The lobes are thin and papery, adnate, 0,6cm broad (P+r)**  
   a. **Leptogium azureum**  
   b. **The lobes have a darker tomentum on the lower surface**  

8. **The lobes are lung-like appaerance and up to 1cm broad with black simple rhizine (K+y, P+r)**  
   a. **Lobaria pulmonaria**  
   b. **The lobes is grayish green and up to 0,2cm broad**
9 a. The thallus has a black squarrose rhizine
(K+violet, P+r)

b. The thallus has white and short simple rhizines

10a. It has narrow lobe, with isidia but does not
has apothecia (P+r)

b. The lobes up to 0.5 cm broad with isidia
margins

11a. Under surface black in the center and lighter
brown in the margin (P+r)

b. Under surface is grayish green like the upper
surface and up to 0.1 cm broad

12a. It has grey and granular soredia in the
margin of the thallus and have long simple rhizine
(P+r)

b. It has brownish yellow soredia on the upper
surface and has a black simple rhizine

13a. The thallus tufted and have a white cracked
on the upper surface(K+y, C+y, P+r)

b. The upper surface is grayish green up to 0.4 cm
broad, and the lower surface is light brown colour

14a. It has narrow hole on the upper surface of
the thallus, and has grayish green soredia (K+y,
P+r)

b. The upper surface is grayish green and up to
0.5 cm broad

15a. under surface is black, and has black simple
rhizines(K+y, P+r)

..........9

..............Phaeophyscia orbicularis

..........10

..............Parmeliopsis aleurites

..........11

..............Parmelia crinita

..........12

..............Candelaria concolor

..........13

..............Platismatia glauca

..........14

..............Parmelia sinousa

..........15

..............Parmelia soredians
**Dermatocarpon miniatum**

A. Upper surface  B. Under surface  C. Sketch (Rahayu, 2009)

DESCRIPTION. This lichen includes Verrucariaceae family, and the common name is Common stippleback or Leather Lichen. Thallus upper surface is grayish-white to light brown up to about 1.6 cm, the under surface tan, with white spot. The talus contains of grey soredia. They do not have a rhizome. They have dark brown ostiole on the upper surface with 10mm-75mm in size. The talus attached on substrate. The talus is like a tree bark. It has perithecia. In Chemical test with C is negative, K is yellow and P is red.

CHEMISTRY. Chemical test in medulla are C-, K+(yellow), P+(red).

**Xanthoria polycarpa**

A. Under surface  B. Upper surface  C. Sketch (Janah, 2009)

DESCRIPTION. This lichen includes Teloschistaceae family. Talus upper surface is grayish grey, up to 1cm across. Under surface of talus has the same color with upper surface. It has a white simple rhizome (not in branched). An apothecia is grayish green, the color of epithelium is brownish yellow. The thallus has an ostiole on the top of talus. The talus is in branched. It does not have isidia or soredia.

CHEMISTRY. Chemical test in medulla are C-, K+(yellow), P-

**Nephroma parile**

A. Upper surface  B. Under surface  C. Sketch (Jannah, 2009)

DESCRIPTION. This lichen includes Nephromataceae family. The upper of talus is grey up to 2 cm across. The under of talus is dark brown. It has isidia and soredia that the color is the same as the upper talus color. It has a white rhizome.

CHEMISTRY. The chemical test in medulla are P+ (red), K+ (White), C-.
**Leptogium azureum**

A. Upper surface  
B. Sketch (Rahayu, 2009)

DESCRIPTION. This lichen include Collemaeaceae family. The color of talus is light bluish grey, adnate, 0.6 cm broad. Under surface is brownish grey. It has white soredia. Rather rare on bark of trees in closed woods. The lobes are thin and papery with a smooth edge.

CHEMISTRY. The chemical test in medulla are C -, K -, P + (red)

**Lobaria Pulmonaria**

A. Upper surface B. Under surface C. Sketch (Rahayu, 2009)

DESCRIPTION. This lichen include Lobariaceae family. This species lung-like appearance was sold as a cure for lung disease. The upper thallus is brownish grey. On the lower surface light tan with a darker tomentum which becomes rubbed off on the raised parts of the talus. The lobes are up to 2.5 cm broad. Isidia form along the ridges on the upper surface. It does not have apothecia. It has a black simple rhizine.

CHEMISTRY. The thallus has white soredia. The chemical test in medulla are C-, K + (yellow), P+ (red).

**Phaeophyscia orbicularis**

A. Upper surface  
B. under surface  
C. Sketch (Mahadi, 2009)

DESCRIPTION. This lichen include Physciaceae family. The upper surface is grayish green and up to 0.2 cm broad. The lower surface is black. It does not have soredia and isidia. It also does not have apothecia. The thallus has a black squarrose rhizines.

CHEMISTRY. The chemical test in medulla are C-, K + (violet), P+ (red).
**Parmelia Saxatilis**

A. upper surface  B. under surface  C. Sketch (Rahayu,2009)

DESCRIPTION. This lichen include Parmeliaceae family. The upper talus green-yellow up to 0.4 cm broad, it has a white pseudoparaphysis. The under surface is brownish yellow and have a white spot. The talus does not have apothecia. It has grey soredia. The rhizome is simple and has a white color. It has narrow lobes. On the upper surface has pseudocyphellae from white reticulum line.

CHEMISTRY. The chemical test in medulla are C -, K +(red), P + (red)

**Parmeliopsis aleurites**

A. Upper surface  B. under surface  C. Sketch (Jannah,2009)

DESCRIPTION. This lichen include Parmeliaceae family. The upper surface is grayish green up to 0.1 mm broad. The lower surface has a same color with upper surface. The thallus has white and short simple rhizomes. It has narrow lobe. It have an isidia and does not have an apothecia.

CHEMISTRY. The chemical test in medulla are C-, K-, P + (red)

**Parmelia crinita**

A. Upper surface  B. under surface  C. Sketch (Mahadi,2009)

DESCRIPTION. This lichen include Parmeliaceae family. The upper of the talus have a grayish green color up to 0.5 cm broad with isidia margin . The under surface black in the centre, with fine, black simple rhizomes, the margins are lighter brown.

CHEMISTRY. The chemical test in medulla are C-, K-, P +(red)
**Candelaria concolor**

A. On the upper surface. B. On the under surface C. Sketch (Mahadi, 2009)

DESCRIPTION. This lichen include Candelariaceae family. The upper of the talus have a grayish color up to 0.1 cm broad. The lower of the talus is grayish green. It has grey and granular soredia in the margin of the talus. It the thallus have a black and long simple rhizine. It have rare an apothecia. The apothecia are grayish green with a brown epitheilium.

CHEMISTRY. The chemical test in medulla are C-, P+(red), K-.

**Platismatia glauca**

A. on the upper surface. B. On the under surface C. Sketch (Rahayu, 2009)

DESCRIPTION. This lichen include the Parmeliaceae family. The upper of the talus is having a grayish green up to 0.5 cm broad. The under surface of the thallus is black. The thallus tufted. The upper surface has a white cracked. It has brownish yellow soredia. The thallus has a black simple rhizome. The margin of thallus is not flat.

CHEMISTRY. The chemical test in medulla are K+(yellow), C+(Yellow), P+(red)

**Parmelia sinuosa**

A. on the upper surface. B. On the under surface C. Sketch (Rahayu, 2009)

DESCRIPTION. This lichen include the Parmeliaceae family. The upper of talus is grayish green up to 0.4 cm broad. The lower surface has a light brown color. It has a narrow hole on the upper surface of thallus. The thallus has black simple rhizomes. It also has grayish green soredia.
CHEMISTRY. The chemical test in medulla are C-, K+(yellow), P+(red)

Menegazzia terebrata

A. Upper surface B. Under surface C. Sketch (Rahayu,2009)

DESCRIPTION. This lichen include the Parmeliaceae family. The upper surface is grey color and perforated by small holes which are often soredia, while on the under surface brown color with light spot. It has black rhizines located on the top under surface thalus.

CHEMISTRY. The chemical test in medulla are K+(yellow), C-, P+(orange)

Parmelia soredians

A. Upper surface B. Under surface C. Sketch (Rahayu,2009)

DESCRIPTION. This lichen include the Parmeliaceae family. The upper of thalus is grenish green color and up to 0.5 cm abroad. The under surface of thalus is black color. It has a black and simple rhizines.

CHEMISTRY. The chemical test in medulla are K+(yellow), C-, P+(red)

Conclusion

There are fourteen eight family i.e. Parmeliaceae, Candelariaceae, Physciaceae, Lobariaceae, Collemateceae, Nephromataceae, Teloschistaceae, Verrucariaceaeace. There are seven spesies Parmeliaceae i.e. Parmelia soredians, Parmelia crinita, Parmelia sinuosa, Parmelia saxatilis, Menegazia terebrata, Platismatia glauca, Parmeliopsis aleurites. Except seven other family we found such as one species Candelaria concolor from Candelariaceae family, Phaeophyscia orbicularis from Physciaceae family, Lobaria pulmonarya from Lobariaceae family, Leptogium azureum from Collemaeteceae family, Nephroma parile from Nephromataceae family, Xanthoria polycarpa from Teloschistaceae family, And Dermatocarpon miniantum from Verrucariaceae family.
Reference


O-SE10

SPECIES DIVERSITY AND DOMINANCE IN Shorea lumutensis-STAND IN PANGKOR ISLAND, PERAK, MALAYSIA

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High Conservation Value Forest (HCVF) stand of Shorea lumutensis, which is one of the rare and endemic dipterocarps in Peninsular Malaysia, was established in Pangkor Island, Perak, Malaysia in an effort to conserve the species. A research was carried out in the HCVF stand in an effort to identify species dominance and social behavior for future ex-site rehabilitation effort.

A total of six (6) phytosociological relevés (each with the size of 50x50m) was established in the HCVF stand. The diversity analysis (richness, heterogeneity and evenness) based on five canopy layers, i.e. emergent or super tree (ST: above 30m in total height), dominant (T1: 15–30m), co-dominant and suppressed (T2: 8–15m), shrub (S: 2–8m) and herb (H: lower than 2m) were analyzed by the principle component analysis (PCA).

The result showed that the H-layer recorded higher richness for Plot 1 (P1), P2 and P3 with 69.141, 65.178 and 83.135, respectively. The layer also attained mean evenness value for species distribution of above 0.8 with the and high level of heterogeneity. On the other hand, the S, T2 and T1 layers showed species domination, of which the S layer is dominated by Diospyros subrhomboidea (Plot 3 – P3), Aporosa frutescens (P5) and Fordia unifoliata, Vatica pauciflora, Teijsmanniodendron coriaceum (P6). The T2 layer of P2 is dominated by Fordia unifoliata, and Plot 6 by Aporosa frutescens, Teijsmanniodendron coriaceum and Xanthophyllum affine. Meanwhile, Shorea maxwelliana, Vatica pauciflora and Hopea latifolia are dominating the T1 layer in Plot 3, and Swintonia floribunda dominates Plot 6. The ST layer recorded the lowest values for richness, evenness and heterogeneity. Only two individuals of Shorea lumutensis are found in the T1 layer. The study suggests that bigger HCVF is needed to protect Shorea lumutensis.

Key words: Diversity, endemic species, Shorea lumutensis, HCVF

not presented
O-SE11

PHYLOGENETIC RELATIONSHIP OF THE GENUS TRICHOTOSIA SPECIES IN SABAH, MALAYSIA

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This study was conducted to determine the genetic variation among the species of Trichotosia (Orchidaceae) in Borneo based on ribosomal Internal Transcribed Spacer (ITS) DNA sequence data. Samples were collected from eight localities within Sabah, namely Mount Kinabalu, Mount Trus Madi, Mount Lumaku, Mount Tambuyukon, Mount Alab, Minduk Sirung, and Poring. DNA was extracted from fresh leaves of Trichotosia using the cetyltrimethyl-ammonium bromide (CTAB) method. DNA sequences from eight samples Trichotosia have been obtained and analyse to examine for phylogenetic status of the genus.

Keyword: Genetic variation, Trichotosia, ITS, Orchidaceae

not presented
O-SE12

TUBER MORPHOLOGY VARIATION AND CLASSIFICATION OF YOGYAKARTA WATER YAM (Dioscorea alata L.) KULTIVAR

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Water yam (Dioscorea alata L.) was species which have morphological variation of vegetative organ especially on stems, leaves, and tubers. Morphological variation of stem and leaf was already observed, and the result show that water yam cultivar could be classified base on it's characters, especially based on leaf shape and stem, wing stem, and leaf color (Araki et al., 1983; Purnomo et al., 2008). Water yam cultivars have variation on tuber shape and color, but it has hesitate to created the identification key. The objective of the research was to determined the tuber morphological characters, which can be used as a marker to classified cultivars of water yam. Samples of tuber were collected from some places in Yogyakarta region, and it's was planted in the collection garden. Morphological characters of tuber were observed i.e. shape, size, skin color, tuber color, fibrous root, and fiber (Schiwaki et al., 2000; Hasan et al., 2006). Data were analyzed descriptively and comparative table of each cultivar (cv.) to created the parallel identification key concerned to water yam cultivars. The research result showed that water yam tuber have four basic shape, cylindrical (cv. Ulo, Jingking, and Luyung), ovals (cv. Beras and Legi), obovate (cv. Ungu, and Kendil), and ramified (cv. Bangkulit and Kuning). Cultivar Ulo is the largest tuber size compare with the other water yam cultivars. There were three tuber basic colors i.e. white (cv. Beras. Legi, ulo, jingking, and kendil), violet (cv. Ungu, dan luyung ungu), white with violet edge (cv. Bangkulit), and yellowish white (cv. Kuning). Hasan et al. (2006) was used tuber color characters to classified the Malaysian water yam cultivars rather than tuber shape. Fibrous root and fiber of tuber weren’t characteristic on water yam cultivar, and according to Satoru et al. (2008) their existences were influenced by means soil fertility. Based on character analyzed discussion, the identification key was arranged consecutively from tuber shape, color, size, fibrous root, skin color, and fiber characters.

Key word: Tuber morphology, water yam (D. alata L.), Yogyakarta.
O-SE13

SPECIES DIVERSITY OF RATTANS IN THE GENUS CALAMUS IN MUNA REGENCY, SOUTHEAST SULAWESI, INDONESIA

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Rattans are spiny climbing plants in the Areaceae family which comprise about 600 species in 14 genera. There are eight genera of rattans reported in Indonesia, in which Calamus being the largest genus. Muna Regency in Southeast Sulawesi is one of the regions in Indonesia where Calamus is found the forest areas. Given that rattans is the most non-timber forest product in the region, the information on their species diversity and local distribution is essential to recognize the potential of this important natural recourses. The study aimed at revealing species diversity of rattans in the genus Calamus in Muna Regency, and mapping their local distribution. Samples collection was done during 2007 in six localities representing various forest habitats with altitudinal level ranging from 10 to 320 m above sea level. Species identification was carried out based on 75 morphological characters from stem, leaves, flower, fruit, and seeds. The identification key from Dransfield & Manokaran (1996) was used as reference. Results indicated that there are eight rattan species found in the study areas, from which six have been identified, i.e. C. koordersianus, C. leiocaulis, C. ornatus, C. leptostachys, C. inops and C. zollingeri. The two unidentified samples were those without flowers, and known with their local names as Kale and Batu rattans. Based on the morphological characters examined in this study, the main distinguishing characters of these rattans species are type of climbing organs, leaves arrangement on the stem, shape of leaflets, and arrangement of spines on the leaf sheath.

Keywords: Calamus, rattans, species diversity, morphology, Muna Regency
O-SE14

LEPIDOPTERAN DIVERSITY IN KARST ECOSYSTEM, LUWENG JOMBLANG, SEMANU, GUNUNG KIDUL

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Luweng Jomblang is an Kars Ecosystem sited in Semanu, Gunung Kidul. The preliminary study in this area indicated there were highly diversity of plant species which are as producen in the ecosystem. The physical factors such as temperature, humidity and the light intensity and light periodicity support the continuity of the whole trophic level in the ecosystem. One of the second trophic level in the area were lepidopteran which consumes nectar and polen. Assumng they have important role as a polinator. The objective of the research was inventory the species belong to Odro Lepidoptera in Luweng Jomblang.

The research was conducted in Month March and April 2009 by obsevations method. There are 11 species butterfly identified, Catopsilia pomona, Catopsilia pyranthe, Eurema hecabe, Parantica vitrina, Hebomoia glaucippe, Phaedyma columella, Hypolimnas bolina, Phalanta phalanta, Papilio memnon, Graphium sarpedon, dan Graphium doson. These species’ belong to Family Papilionidae, Nymphalidae, dan Pieridae.

Keywords: Butterflies and Karst Ecosystem

not presented
MORPHOLOGY OF WHITE OYSTER MUSHROOM
(Pleurotus ostreatus Jacq. ex. Fr. Kummer) ON MODIFICATION MEDIA OF MUSHROOM CULTIVATION

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White oyster mushroom (Pleurotus ostreatus Jacq. ex. Fr. Kummer) is an edible mushroom, which contains high proteins, vitamins and minerals. Besides their high nutritional values, the morphology of fruit body of P. ostreatus has important economic values on market. The morphology of mushroom fruit bodies can be influenced by the growth media used during cultivation. The aims of this research were to study the morphology of P. ostreatus on different modification media of mushroom cultivation. There were three main media used in this research: wood sawing dust, cardboard and banana leaves. Each media were divided into three combination of mixed which were (1) mixed of 15% bran and 2% CaCO3, (2) mixed of 5% pound corn, 1% CaCO3 and 1%TSP fertilizer, (3) mixed of 10% bran, 2% CaCO3, 5% tapioca flour and 1% TSP fertilizer. Result showed that the morphology of P. ostreatus on different cultivation media were white fruiting bodies; plane or depressed cap shapes; smooth, striate or wavy surface types; incurved, straight or recurved margin types; adnate attachment of gill surface to stem; smooth, wavy or ragged edge types; distance, close or crowded space types; ventricose or equal stem shapes; and cylindrical of spore shape with verrucose ornamentation. The modification of cultivation media of P. ostreatus influenced the morphology of P. ostreatus on the shape, surface, and margin type of caps, the attachment of gill surfaces, lamella space types, and stem shapes. The best morphology of P. ostreatus fruit body was achieved by using modification medium of cardboard, followed by sawing dust and then banana leaves.

Keywords: mushroom, morphology, cultivation, media

INTRODUCTION

White oyster mushroom (Pleurotus ostreatus Jacq. ex. Fr. Kummer) cultivation has increased tremendously throughout the world during the last few decades. Oyster mushroom accounted for 14.2 % of the total world production of edible mushroom in 1997 [3]. As an edible mushroom, P. ostreatus are considered to be healthy food because they contain large amounts of qualitatively good protein (27%), karbohidrat (58%), a low fat content (0.8%) [6], and also vitamin (B1,B2,C) and a good source of minerals. Oyster mushroom can be cultivated in any type of
ligno cellulose material like straw, sawdust, rice hull, etc. The basic substrate formulation for oyster mushroom is 80-95% sawdust, about 5-10% of supplements and 1-2% CaCO₃ [5], however, the composition is varied in several parts of the world. Shah et al. [9] found that sawdust gave the maximum yield of the oyster mushroom cultivation on different substrates. Wood sawdust is normally used for *P. ostreatus* because it composed of high cellulose contents such as 40-60% cellulose, 15-30% hemicellulose, 20-30% lignin and 1-2% minerals [8]. Oei [5] stated that substrate materials such as barley or wheat straw, wood chips, sawdust, coffee pulp, corn cobs, cotton waste, bean straw, cotton straw, coconut soil, and used paper, banana leaves could be used as substrate materials for *Pleurotus* spp. Presently, sawdust is commonly used and is the preferred medium at commercial scale. Supplements can be added to increase yields by providing specific nutrients for the growth of the mycelium such as urea, bran, grain, or molasses [10]. The effect of different substrates for *P. ostreatus* cultivation on yield has been studied thoroughly, however, the morphology of the fruit bodies which has important values for commercial scale, has not been reported. The aim of this research was to study the morphology of *P. ostreatus* on different modification media of mushroom cultivation. Wood sawing dust, cardboard and banana leaves were used as the main substrates with an addition of other supplements for *P. ostreatus* cultivation.

**MATERIALS AND METHODS**

**Substrate materials and supplements**

There were three main media used in this research: wood sawing dust, cardboard and banana leaves. Each media was divided into three combinations of mixture, which were (1) mixture of 15% bran and 2% CaCO₃, (2) mixture of 5% pound corn, 1% CaCO₃ and 1% TSP fertilizer, (3) mixture of 10% bran, 2% CaCO₃, 5% tapioca flour and 1% TSP fertilizer.
Table 1. Composition of modification media for *P. ostreatus* cultivation

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Code</th>
<th>Main substrate</th>
<th>Bran</th>
<th>CaCO₃</th>
<th>Tapioca flour</th>
<th>Pound corn</th>
<th>TSP fertilizer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wood sawing dust</td>
<td>GA</td>
<td>100</td>
<td>15</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>GB</td>
<td>100</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>GC</td>
<td>100</td>
<td>10</td>
<td>2</td>
<td>5</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>KA</td>
<td>100</td>
<td>15</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cardboard</td>
<td>KB</td>
<td>100</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>KC</td>
<td>100</td>
<td>10</td>
<td>2</td>
<td>5</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Banana leaves</td>
<td>PA</td>
<td>100</td>
<td>15</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>PB</td>
<td>100</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>PC</td>
<td>100</td>
<td>10</td>
<td>2</td>
<td>5</td>
<td>-</td>
<td>1</td>
</tr>
</tbody>
</table>

**Preparation of substrate materials and measurements**

For composting, sawdust was soaked in water for a week to moisten them thoroughly. Cardboards were cut about 5-10 cm and immersed in water for six days. The banana leaves used in this study were chopped to 1-2 cm, and then dried for three days. All substrate materials fermented for five days by covering the polythene sheet before filling the bags. Each substrate was filled in 1.5 kg polythene bag and their mouths were plugged by inserting water absorbing cotton with the help of plastic rings. Each of nine treatments was repeated three times. All bags were then closed with plastic bag for four days. The bags were autoclaved at 121°C for eight hours and allowed to cool. After sterilization, next day the bags were inoculated with spawn of *P. ostreatus* at the rate of 5% per bag according to the dry weight of substrates, all substrates were inoculated in the same day. The bags were kept on 23-28 °C for spawn running, and 13-15 °C for fructification with moisture about 90-96 % and water content of 35-45%. Bags were watered each day to maintain water content. Days of completion of spawn running and days of fructification (pinhead and fruiting bodies formation) were measured.

**Observation of the morphology of fruiting bodies**

The morphology of fruit body were evaluated at different stages (pin head-1st day, harvest time-3rd day and mature-5th day of growth) on the color of the fruit bodies, cap shapes, surface and margins, gill attachment to stem, gill margin, gill
spacing, stem shapes and spore shape and ornamentation based on criteria of Bougher and Syme [2].

RESULTS AND DISCUSSION

There are three important phases for oyster mushroom cultivation, which are spawn running, pinheads formation and fruiting bodies. It is evident from Figure 1 that spawn running took 17 to 27 days after inoculation, with banana leaves showed shorter day for completion of spawn run. The lignin content of banana leaves which was 26-28% less than wood showing dust and cardboard might influence the spawn running. Tan [12] reported also that the spawn running on different substrates took 17 to 20 days after inoculation, the spawn running required proper humidity and temperature. Tan [12] reported that temperature of 25°C showed good results for spawn running.

![Graph](image)

**Fig. 1.** Days for completion of spawn running of *P. ostreatus* cultivation at different substrates

The pinheads formation is the second stage of mycelial growth during cultivation of *P. ostreatus*. Small pinheads like structures were observed, these pinheads were formed 6-12 days after the spawn running. Tan [12] noted that pinheads formation of *P. ostreatus* cultivation on different substrates were 6-7 days. Cardboard substrates with an addition of 15% bran and 2% of CaCO₃ (KA) produced pinheads earlier at 6 days after spawn running, as well as GB than other treatments (8-12 days) (Fig. 2).
Based on the morphological observations, it was found that *P. ostreatus* was white in color at all development stages. Table 2 showed that cap shapes of fruit bodies of *P. ostreatus* were influenced by different modification media. It was shown that the cap shapes of pin head mostly was different from the shapes at harvest time or when in maturity. Oei [5] stated that the shapes of mushroom cap changed when the mushroom was mature, because the water content decreased. Depressed is the ideal cap shapes for commercial scale, and this was found on modification media of GA, GC, KA, KC, PB, and PC (Fig. 3). Ainsworth and Bisby’s [1] stated that the cap shape of *P. ostreatus* was plane to depressed. The cap surface showed smooth and striate, some of them showed wavy surface at maturity as mentioned also by Ainsworth and Bisby’s [1]. The cap surface changed with the age because the cap became dry at maturity [6]. The results showed that the cap margins of *P. ostreatus* on different modification media were incurved-straight-recurved as mentioned by Ainsworth and Bisby’s [1]. Generally, the cap margin sold in the market was straight, which in this study was found at harvest time on almost all of treatments except for KB and PA (Table 2).
Table 2. The morphology of cap shapes, surface and margin at different modification media of *P. ostreatus* cultivation

<table>
<thead>
<tr>
<th>Code</th>
<th>Pin harvest time</th>
<th>Mature</th>
<th>Pin harvest time</th>
<th>Mature</th>
<th>Pin harvest time</th>
<th>Mature</th>
</tr>
</thead>
<tbody>
<tr>
<td>GA</td>
<td>plane depressed</td>
<td>depressed</td>
<td>smooth smooth striate</td>
<td>incurved straight</td>
<td>recurved</td>
<td></td>
</tr>
<tr>
<td>GB</td>
<td>plane convex depressed</td>
<td>depressed</td>
<td>smooth smooth striate</td>
<td>incurved straight</td>
<td>straight</td>
<td></td>
</tr>
<tr>
<td>GC</td>
<td>plane depressed</td>
<td>depressed</td>
<td>smooth striate</td>
<td>wavy</td>
<td>incurved</td>
<td>straight</td>
</tr>
<tr>
<td>KA</td>
<td>plane depressed</td>
<td>infundibuliform</td>
<td>smooth striate</td>
<td>wavy</td>
<td>incurved</td>
<td>straight</td>
</tr>
<tr>
<td>KB</td>
<td>plane plane depressed</td>
<td>depressed</td>
<td>smooth smooth striate</td>
<td>incurved</td>
<td>incurved</td>
<td>straight</td>
</tr>
<tr>
<td>KC</td>
<td>plane depressed</td>
<td>infundibuliform</td>
<td>smooth striate</td>
<td>wavy</td>
<td>incurved</td>
<td>straight</td>
</tr>
<tr>
<td>PA</td>
<td>plane plane Plane</td>
<td>depressed</td>
<td>smooth smooth smooth</td>
<td>incurved</td>
<td>incurved</td>
<td>incurved</td>
</tr>
<tr>
<td>PB</td>
<td>plane depressed</td>
<td>infundibuliform</td>
<td>smooth striate</td>
<td>wavy</td>
<td>incurved</td>
<td>straight</td>
</tr>
<tr>
<td>PC</td>
<td>plane depressed</td>
<td>depressed</td>
<td>smooth striate</td>
<td>striate</td>
<td>incurved</td>
<td>straight</td>
</tr>
</tbody>
</table>

Gill attachment to stem was mostly adnate with few exception of adnexed on PB when mature. These results were in contradiction with characters stated by Ainsworth and Bisby’s [1], which found that the gill attachment of *P. ostreatus* was adnexed-sinuate-decurrent. *P. ostreatus* showed smooth to wavy gill margins when they were still in pinhead and at harvest time, however, it was then changed to ragged, crenate and some of them were still smooth or wavy (PA) (Table 3). The gill margin on PA was not normal in their development of the fruit body as stated by Oei [5] that banana leaves were not good for substrate materials of mushroom cultivation because they lack of cellulose and lignin.
Fig. 3. Morphology of fruit bodies of *P. ostreatus* at different media of cultivation at harvest time. 

\[ a = \text{GA, } b = \text{GB, } c = \text{GC, } d = \text{KA, } e = \text{KB, } f = \text{KC, } g = \text{PA, } h = \text{PB, } i = \text{PC} \]

<table>
<thead>
<tr>
<th>Code</th>
<th>Gill attachment to stem</th>
<th>Gill margin</th>
<th>Gill spacing</th>
</tr>
</thead>
<tbody>
<tr>
<td>GA</td>
<td>adnate adnate adnate</td>
<td>smooth wavy ragged distance close crowded</td>
<td></td>
</tr>
<tr>
<td>GB</td>
<td>adnate adnate adnate</td>
<td>smooth wavy crenate distance close crowded</td>
<td></td>
</tr>
<tr>
<td>GC</td>
<td>adnate adnate adnate</td>
<td>smooth wavy ragged distance close crowded</td>
<td></td>
</tr>
<tr>
<td>KA</td>
<td>adnate adnate adnate</td>
<td>smooth wavy ragged distance close crowded</td>
<td></td>
</tr>
<tr>
<td>KB</td>
<td>adnate adnate adnate</td>
<td>smooth smooth wavy distance distance crowded</td>
<td></td>
</tr>
<tr>
<td>KC</td>
<td>adnate adnate adnate</td>
<td>smooth wavy wavy distance close crowded</td>
<td></td>
</tr>
<tr>
<td>PA</td>
<td>adnate adnate adnate</td>
<td>smooth smooth smooth distance distance distance</td>
<td></td>
</tr>
<tr>
<td>PB</td>
<td>adnate adnate adnate</td>
<td>smooth wavy ragged distance close crowded</td>
<td></td>
</tr>
<tr>
<td>PC</td>
<td>adnate adnate adnate</td>
<td>smooth wavy wavy distance crowded crowded</td>
<td></td>
</tr>
</tbody>
</table>

Table 3. The morphology of gill attachment to stem, gill margin and gill spacing at different modification media of *P. ostreatus* cultivation.
Table 3 showed that the gill spacing of *P. ostreatus* was close at harvest time and lean to crowd in maturity because of age [4]. At day four to five, the spores started to fall down indicated that the fruit body turned to mature stage. The stem shape of *P. ostreatus* was ventricose, where the stem was swollen in the middle, except for PA because its growth was not optimal (Table 4). According to Zadrazil [13], stem of *P. ostreatus* was short and stick to the edge of cap. The spore of *P. ostreatus* was cylindrical and showed verrucose ornamentation with width was 2.5 and length 7.5-10 μm (Table 4). Stamets and Chilton [11] stated that the spores of *P. ostreatus* were bigger in cylindrical shape with the size of 8-11 × 3-4 μm. The strain of *P. ostreatus* may influence the spore size. Spores were not found in the pin head form because the cap was still young.

Table 4. The morphology of stem shapes, spore shapes and spore ornamentation at different modification media of *P. ostreatus* cultivation

<table>
<thead>
<tr>
<th>Cod</th>
<th>Pin head</th>
<th>Harvest time</th>
<th>Mature</th>
<th>Spore shapes</th>
<th>Spore ornamentation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pin head</td>
<td>Harvest time</td>
<td>Mature</td>
<td>Pin head</td>
<td>Harvest time</td>
</tr>
<tr>
<td>GA</td>
<td>ventricose</td>
<td>ventricose</td>
<td>ventricose</td>
<td>nf</td>
<td>cylindrical</td>
</tr>
<tr>
<td>GB</td>
<td>ventricose</td>
<td>ventricose</td>
<td>ventricose</td>
<td>nf</td>
<td>cylindrical</td>
</tr>
<tr>
<td>GC</td>
<td>ventricose</td>
<td>ventricose</td>
<td>ventricose</td>
<td>nf</td>
<td>cylindrical</td>
</tr>
<tr>
<td>KA</td>
<td>ventricose</td>
<td>ventricose</td>
<td>ventricose</td>
<td>nf</td>
<td>cylindrical</td>
</tr>
<tr>
<td>KB</td>
<td>ventricose</td>
<td>ventricose</td>
<td>ventricose</td>
<td>nf</td>
<td>cylindrical</td>
</tr>
<tr>
<td>KC</td>
<td>ventricose</td>
<td>ventricose</td>
<td>ventricose</td>
<td>nf</td>
<td>cylindrical</td>
</tr>
<tr>
<td>PA</td>
<td>equal</td>
<td>equal</td>
<td>equal</td>
<td>nf</td>
<td>nf</td>
</tr>
<tr>
<td>PB</td>
<td>equal</td>
<td>ventricose</td>
<td>ventricose</td>
<td>nf</td>
<td>nf</td>
</tr>
<tr>
<td>PC</td>
<td>equal</td>
<td>ventricose</td>
<td>ventricose</td>
<td>nf</td>
<td>nf</td>
</tr>
</tbody>
</table>

nf = spores were not found

From those results, it can be summarized that the morphology of *P. ostreatus* on different cultivation media were white fruiting bodies; plane or depressed cap
shapes; smooth, striate or wavy surface types; incurved, straight or recurved margin types; adnate attachment of gill surface to stem; smooth, wavy or ragged edge types; distance, close or crowded space types; ventricose or equal stem shapes; and cylindrical of spore shape with verrucose ornamentation. The modification of cultivation media for *P. ostreatus* influenced the morphology of *P. ostreatus* on the shape, surface, and margin type of caps, the attachment of gill surfaces, lamella space types, and stem shapes. Cardboard which is known as synthetic material from woods, is cheaper and showed good growth of *P. ostreatus* than wood sawdust. Cardboard was also shown to produce more fruit bodies for *P. ostreatus* cultivation than wood sawdust and banana leaves (Fig.3). Therefore, it is suggested that the substrate materials for *P. ostreatus* can be replaced to cardboard rather than wood sawdust which is difficult to obtain and more expensive. Banana leaves are not recommended for growers as a substrate for *P. ostreatus* cultivation, because the morphology of *P. ostreatus* was not developed to the ideal morphology for commercial scale. Oei [5] found that the use of dead banana leaves which were still hanging on the plants for mushroom cultivation, was better than dried leaves. It can be concluded that the best morphology of *P. ostreatus* fruit body was achieved by using substrate materials of cardboard, followed by wood sawing dust and then banana leaves. The best media composition of *P. ostreatus* cultivation was cardboard with an addition of 15% bran and 2% CaCO₃.

REFERENCES


O-SE16

MORPHOLOGICAL VARIATION OF Cibotium barometz
FROM WEST SUMATRA

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ABSTRACT

Cibotium barometz is one tree fern of the family Cyatheaceae that is usually recognized as ‘chain fern’ in the medicinal plants trade. The distribution of the species in Indonesia is limited. At present, Sumatra is the only mainland where the population is abundances. Morphological comparison study of the population in West Sumatra has been carried out. Ninety one collection numbers from sixteen localities have been examined by using worksheet paper containing thirty characters. Three groups are recognized based on the character combinations: hair colors, the existent of hairs on stipe and costae, lamina incision, segment distant and the branching of veinlets. The possibilities of the systematic treatments are discussed.

Keywords: West Sumatra, Cibotium barometz, morphological variation.

INTRODUCTION

Cibotium barometz (L.) J. Sm. is one of tree fern belongs to the family Cyatheaceae (Holttum 1963, 1966). The species easily recognize because of the gold yellowish-brown smooth and shining hairs covered on its rhizome and basal stipe. The plant habitat usually prostrate and the rhizome rarely find more than 1 m high. There are 12 species of Cibotium recorded in the world (Hassler & Swale 2002), but only two species occurring in Indonesia, namely Cibotium barometz (L.) J. Sm and C. arachnoideum (C. CHR.) Holttum (Holttum 1963).

In China, Europe, and Thailand, the species has important value for medicinal purpose, which is known in medicinal trade as ‘gou ji’ or ‘chain fern’ (Anonimous 2008). In Indonesia the species has several local names such as: penawar jambi, penghawar jambi, paku simpai or bulu jambe (Jambi), pakis simpe, poong simpe (Bengkulu) (Rugayah & Praptosuwiryoy 2005), pakis kijang, bulu kijang, kapuk kijang (West Sumatra) (Praptosuwiryoy & Atikah 2008).
The gold yellowish-brown hairs at its rhizome and stipes has been used in China, S.E. Asia as a styptic for a bleeding wounds. The extract of the rhizome ('gouji') is also used by Chinese and Japanese as an antirheumatic, to stimulate the liver and kidney, to strengthen the spinal, to expel wind and dampness, and as a prostatic remedy. Several medicinal factories in S.E. Asia sold the dry rhizome as raw material. A single French factory is known to process 100 kg of "pili cibotii" per year. Dried rhizome parts, 'rhizoma cibotii', are offered for sale by companies selling herbas (Praptosuwiryo, 2003). In Bengkulu and West Sumatra the hairs have also been used to stuff pillows and cushions but the hairs easily break when they become dry and they can irritate the skin and lungs when they pass through the pillows case (Rugayah & Praptosuwiryo, 2005).

*Cibotium barometz* is distributed from NE. India to S. China and Taiwan, southwards to Malesian regio. In Malay Peninsula, Sumatera and Java, this species usually grow on steep ground in mountain forest, from near the sea level up to 1600 m dpl. (Holtum 1963). Population of the species in several countries becomes decrease rapidly because of over exploitation. Local people usually cut off the apex of rhizome, not pile the hairs ('pili'), that is high risk for its regeneration. Recently reseach in Indonesia revealed that population record of this species from Java is still absent (Puspitaningtyas dkk 2006, Praptosuwiryo 2008) and Sumatra is the only mainland where the population can be found (Budianti dkk 2005, Praptosuwiryo & Atikah 2008, Praptosuwiryo & Wardani 2008, Praptosuwiryo dkk 2006, Rugayah & Praptosuwiryo 2005, 2006a, 2006b). Moreover, the species now being included in appendix II of the Convention on International Trade in Endangered Species (CITES) since 1976. It is means that no export is allowed without prior permit issued by the Indonesian management authority, which recommended by the scientific authority.

LIPI as a scientific authority has a responsibility to control the wild population of the species, in order to utilize it in sustainable use. Biological aspect is one of the important information that is needed to be known, including the potential distribution map and its morphological variation to determine the annual quotas. To obtain those data, inventories and explorations have been conducted in nine localities in Bengkulu (Praptosuwiryo & Wardani 2008, Rugayah & Praptosuwiryo 2005, 2006a, 2006b,) and ten localities in West Sumatra (Praptosuwiryo & Atikah 2008).

Species have been recognized morphologically, behaviorally, genetically and ecologically. Species descriptions constitute scientific hypothesis that can be testet and falsefied, and such hypothesis are fundamental units in systematic biology.
Once a species has been diagnosed, it represents a working hyphothesis that can be tested through the application of new evidence or analytical methods (Haufler et al 2000.). This paper presents a preliminary study on morphological variation of Cibotium barometz in West Sumatra.

MATERIALS AND METHODS

Inventory and exploration of C. barometz from sixteen locations (Hutan Manggilang, Bukit Sakti, Bukit Salang, Bukit Temenggung, Bukit Bungsu, Bukit Sarang Nio, Bukit Runcing, Bukit Barisan, Hutan Sekunder Palangki Tangan, Hutan Sekunder Pua Data, Bukit Sitaba, Bukit Taeh, Hutan Sekunder Mangunai, Bukit Rambut Tulang, Bukit Alang Lauik) in West Sumatra have been conducted. Ninety one herbarium collection numbers have been collected. At present the herbarium specimens are still handled and deposited in the herbarium of Bogor Botanic Gardens (BOHB). The duplicated specimens will be housed in the Herbarium Bogoriense (BO).

Metodological preparation of dried herbarium collections were carried out by using standart metodology for collecting the tree ferns specimens (Janssen 2006). The basal cutting of stipe, 1-2 pairs of the largest pinnae, and the apex of lamina were taken for herbarium specimens. The others morphological data, such as rhizome size (length and diameter), the size (length and diameter) and color of stipe, size and colour of hairs, size (length ang broad) and colour of lamina, the number or pinnae and the colour of indusia, were examined directly in the field. Five to ten collections number have been were taken on the elevation intervals of 50 m a.s.l. for each locality and then recorded and measured based the main character as follow (Table 1.).
### Tabel 1. Worksheet Paper Containing 30 Characters for Morphological Variation Observation on *Cibotium barometz* in West Sumatra

<table>
<thead>
<tr>
<th>Morphological characterization</th>
<th><em>Cibotium barometz</em></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>No. Coll. :</td>
<td>Collector :</td>
<td>Sub District :</td>
</tr>
<tr>
<td>Collector :</td>
<td>Locality :</td>
<td>District :</td>
</tr>
<tr>
<td>Locality :</td>
<td>Altitude :</td>
<td>Province : West Sumatra</td>
</tr>
<tr>
<td>Sub District :</td>
<td>Latitude :</td>
<td>Date :</td>
</tr>
</tbody>
</table>

#### Rhizome
- Habit
  1. Prostrate
  2. Erect V
- Length : ... cm
- Diameter : ... cm

#### Pinnae
- Position
  1) Opposite
  2) Sub opposite
  2) Alternate
- Length of stalk : ... cm
- Form
- Apex
  1. Acute
  2. Blunt

#### Hairs
- Colour
  1. Yellow
  2. Lanceolate
  3. Narrowly ovate
- Size (the largest)
- Length : ... cm
- Wide : ... cm

#### Segment
- Size
- Length : mm
- Wide : mm
- Apex
  1. Acute
  2. Acuminate V
  3. Attenuate
- Number of pinnulae : ... pairs

#### Frond
- Stipe
  - Length : ... cm
  - Diameter : ... cm
  - Colour : dark green (upper)
- Form
- Apex
  1. Acute
  2. Blunt

#### Veins
- Form
  1. Linear
  2. Lanceolate
- Once forked
- Twice forked
<table>
<thead>
<tr>
<th>Lamina</th>
<th>Sori per segment</th>
</tr>
</thead>
<tbody>
<tr>
<td>- outline form</td>
<td>- size (the largest)</td>
</tr>
<tr>
<td>1. Ovate</td>
<td>- length ... cm</td>
</tr>
<tr>
<td>2. Elliptical</td>
<td>- wide ... cm</td>
</tr>
<tr>
<td>- Length : cm</td>
<td>- basal</td>
</tr>
<tr>
<td>- Broad : cm</td>
<td>1. cuneate</td>
</tr>
<tr>
<td>- number of pinnae: ... pairs</td>
<td>2. truncate</td>
</tr>
<tr>
<td></td>
<td>3. obtuse</td>
</tr>
<tr>
<td></td>
<td>- apice</td>
</tr>
<tr>
<td></td>
<td>1. Acute</td>
</tr>
<tr>
<td></td>
<td>2. Acuminate V</td>
</tr>
<tr>
<td></td>
<td>- number of free</td>
</tr>
<tr>
<td></td>
<td>tertiary leaflets : .... pairs</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Notes:</th>
</tr>
</thead>
<tbody>
<tr>
<td>RESULTS AND DISCUSSION</td>
</tr>
<tr>
<td>-------------------------------------------------------------------</td>
</tr>
<tr>
<td>Morphological comparison study of the population in West Sumatra has been carried out.</td>
</tr>
<tr>
<td>Ninety one collection numbers from sixteen localities at Lima Puluh Kota (Hutan Manggilang, Bukit Sakti, Bukit Salang, Bukit Temenggung, Bukit Bungsu, Bukit Sarang Nio, Bukit Runcing, Bukit Barisan, Hutan Sekunder Palangki Tangan, Hutan Sekunder Pua Data, Bukit Sitaba, Bukit Taeh, Hutan Sekunder Mangunai, Bukit Rambut Tulang, Bukit Alang Lau) have been examined. Three groups are recognized based on the character combinations: hair colors, the existent of hairs on stipe and costae, lamina incision, segment distant, the branching of veinlets and the numbers of sori (Table 2, Fig. 1.).</td>
</tr>
</tbody>
</table>
| Varian 1. It has widely variation especially on its size and colour of all chararters examined. The size of the rhizome: 9-46 x 9-32 cm, hairs colour from pale brown to dark brown, gold yellowish to yellow reddish. Stipe: 74 – 270 cm long,
and (1.8-) 2 - 5 cm diam., dark green, dark green, green brownish to purplish (color of stipe showing a gradation from the basal to the end part, usually green from basal and more brown or more purple to the upper; hairs on the basal generally longer and more dark in comparison to those of the rhizome, while on the end part glabrous or with very short hairs, as like as minute scales, easy fallen off, dirty white in general.

Lamina: light green or green yellowish on upper surface, light glaucoe to rather green bluish on lower part, ovate or lanceolate in outline, 127-294 cm length, 90 – 93 cm broad, pinnae 12 – 22 pairs. Pinnae oblong ovate to lanceolate, 16 – 41 pairs, some specimens showing tertiary leaflets but with imperfect incisison. But these characters are not supported by dense hairs on the costa and costule as on the Varian 3.

**Varian 2.** This varian is only found in one locality at Bukit Sarang Nio (Harau Village, Harau Sub District, Harau District). Several individuals of living collection in the field are generally still young, therefore only two individual mature specimen are successfully examined.

Rhizome: ca. 15 cm long, hairs whitish, brownish or pale brown. Stipe less than 75 cm long, 1.5 cm diam., green-purplish, hairs on the basal usually longer and more pale in comparison with those on the rhizome. While hairs at the end of stipe shorter as like as minute scales, easily fallen off dirty white in general.

Lamina: ovate or lanceolate in outline, 125 cm long and 80 cm broad, pinnae 10 pairs, light green on upper surface, light glaucoe to bluish on lower surface. Pinnae lanceolate to oblong ovate.

**Varian 3.** Varian 3 is found in one locality at Bukit Temenggung, and only one individual was examined. Rhizome 23 x 13 cm, hairs gold yellowish to yellow reddish. Stipe: 194 cm long, 2 cm diam., green; hairs on the basal usually longer and reddish in comparison with those on the rhizome, while hairs at the end part as like as minute scales but longer and more thick compare to the other varians, easily fallen off, pinkish in general. The pinkish hair colours only found in this varian

Lamina: ovate in outline, 262 cm long and 170 cm broad, pinnae 15 pairs, green on upper surface, light glaucoe to grayish on the lower ones. Pinnae narrowly oblong, 33 pairs and showing tertiary leaflets especially at the basal part. Cotulae and vein of the lower surface lamina is hairy as like as hairs on its costae but white colour not pinkish. This character is specific for this varian.
Table 2 indicated that the three variants can be easily identify. The existence and colour of hairs is the main character to distinguish them. Varian 2 quite distinct with varian 1 and 3 because of rhizome hair colour, and varian 3 differ from varian 1 and 2 because of hairs colour on costae, costulae and the existence hairs on lamina veins.

Table 2. Morphological Comparison among the Three Varian on Cibotium barometz of West Sumatra

<table>
<thead>
<tr>
<th>Character</th>
<th>Varian 1</th>
<th>Varian 2</th>
<th>Varian 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hair color</td>
<td>Yellow gold, redish, brown</td>
<td>White-brownish</td>
<td>Yellow gold, brown-reddish</td>
</tr>
<tr>
<td>Hair existence</td>
<td>Hair on the rachis thin or absent; hair on the lower veins absent; dirty white</td>
<td>Hair on the rachis thin; hair on the lower veins absent; dirty white</td>
<td>Hair on the rachis and lower veins relatively more thick and longer; pinkish</td>
</tr>
<tr>
<td>Tertiary leaflets</td>
<td>Absent, if present has not divided perfectly or only one well divided segment on the basal</td>
<td>Absent</td>
<td>Present on secondary leaflet mainly on the upper basal</td>
</tr>
<tr>
<td>Segment distance</td>
<td>Wide</td>
<td>Between Varian 1 and Varian 3</td>
<td>Narrow</td>
</tr>
<tr>
<td>Veinlets branching</td>
<td>1-2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Indusium and sorus</td>
<td>(1-) 2; 3-6; light green – brownish</td>
<td>1-3(-5); pale green- brownish</td>
<td>1-2; olive –green-purple - redish</td>
</tr>
</tbody>
</table>

Compare to the other species (C. arachnoideum) occurring in Indonesia (found in central and north Sumatra), it is indicated that varian 3 has similarity to this species on some characters such as the colour of sori and pairs number of sori on each lamina segment and hairs colour on its lower surface costae, costulae and lamina veins. C. arachnoideum has 1-2 pairs of sori and pinkish hairs also found on its costae and costulae as well as the existence hairs on lamina veins. This similarities characters give a suggestion that the varian 3 is a putative hyrid.

Hybridization in pteridophytes is of frequent and has been recorded in every one of major groups (Psilotum, Lycopodium, Isoetes, Selaginella, Equisetum, and the ferns) (Walker 1984). Many putative hybrids of the ferns have been confirmed cytologically, such as Adiantum villosum x A. lucidum in Trinidad (Walker 1979),
introgressive hybridization in *Pteris* in Sri Lanka (Walker 1984), *Plagogyria adnata* x *P.yakushimensis* (Nakato & Kato 2002). Although natural hybridization in pteridophytes is of frequent, however, it is not easily concluded that varian 3 as the hybrid between *C. barometz* and *C. arachnoideum*. It necessary to make further extensive study on both morphological camparison and cytotaxonomic research to investigate the systematic of the three varians.

Fig. 1.: Morphological variation of *C. barometz* : a. Varian 1; b. Varian 2; c. Varian 3. a1., b1, and c1. A part pinna with two fertile pieces of basal pinnulae. a2 and b2. Rhizome with young crozier frond and basal stipes covered with yellowish gold – pale brown – dark brown shiny hairs. a3 and b3 transversal section of stipe showing the vascular bundle character of the two varians (Varian 1 and 2) are similar. C2. Basal stipe of Varian 3.

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[Diakses 11 Juli 2008]


Pengetahuan Indonesia, Pusat Penelitian dan Pengembangan Biologi, Bidang Botani.


O-Se17

MORPHOLOGICAL VARIATION OF BENTHIC FORAMINIFERA ON BIDADARI ISLAND OF SERIBU ISLANDS

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The benthic foraminifera research was transpire on Bidadari Islands of Seribu Islands as long as three days on May 21st to 23rd 2008. It was recognized to 99 species belonging to sub-order Milioliina, Rotaliina and Textulariina from sixteen sites extended over the island include northern, western, eastern and southern part; on depth 11 – 14.5 m below sea level. Based on the multiple linear regression test, their abundance is exceedingly dependent upon the abiotic factors include depth, salinity, pH and turbidity. Collected samples from the dwelling sediment lead to morphological variation on type of several species. Thus, shell of Amphistegina lessonii shapped more flat than normal; and chamber development of Ammonia beccarii became abnormal. Shell of several species are head for smaller than usual include Ammonia beccarii, Asterorotalia trispinosa, Quinqueloculina spp. In addition, Asterorotalia trispinosa is sprout out by tree spines attached to a suture and center part of the chamber.

Keyword: Morphological variation, benthic foraminifera, Bidadari Island, Seribu Islands

not presented
O-SE18

THE DISTRIBUTION OF WEST MALESIAN FERN GENUS DIPLAZIUM (WOODSIACEAE) INSIDE AND OUTSIDE MALESIA

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ABSTRACT

The fern genus Diplazium is an important component of tropical rainforest of the Old World and the New World that consisting of about 400 species. Study on the distribution of West Malesian Diplazium inside and outside Malesia has been carried out by examining specimens deposited at BO and SING and conducting field work in Java, Sumatra, and Borneo. Sixty nine species of Diplazium are distributed in West Malesia. The total number of species for each main island are 40, 30, 29, 28 for Borneo, Java, Sumatra, and Malay Peninsula, respectively. The West Malesian species can be divided into three types of geographical distribution: (1) very wide distribution species (19 species), (2) Malesian species, species that having wide distribution range in Malesian region only (26), and (3) locally endemic species (24). The highest number of endemic species is occurring in Borneo (9 species). Whereas the lowest number of endemic species is in Malay Peninsula (2 species). Java and Sumatra have six and seven endemic species, respectively.

Keywords: West Malesia, diplazioid fern, Diplazium, distribution

INTRODUCTION

The fern genus Diplazium is an important component of tropical rainforest of the Old World and the New World. It is a terrestrial ferns which is commonly found in the moist ground at the humus rich soil both in the primary and secondary forest at 20-3400 m altitude. Most of species grow in shadowed place and fond of moist humus rich soil. Some species are locally abundant by stream in the mountains. Only a few species are found in limestone areas and in rheophitic areas.
Since 1801 *Diplazium* have been the subject of numerous taxonomic studies conducted, including morphological, anatomical, cytological and molecular investigations. It is estimated that the genus consisting of about 400 species (Ching 1964, Copeland 1947). Roos (1995) estimated that 300 species of its are found in Malesian region. Few species are found in continental Africa (Kramer et al 1990). In the Neotropics there are nearly 100 species (Pacheco 2004).

Distributional data are important in answering many questions about polyploidy and speciation (Baack 2004). Usually, increasing in ploidy level is associated with the origin of novel adaptations (Levin 2002). Polyploid often occupy different habitats from those of their diploid parents (Soltis & Soltis 2000).

Study of the biogeographical distribution of organism are also very useful in inferring the monophyly of a taxon. The elementary questions of historical biogeography concern areas of endemism and their relationships (Nelson & Platnick 1981). By analogy to phylogenetic systematics, where species or higher taxa are grouped, in cladistic biogeography the units grouped are areas of endemism (Linder 2001). For example, study on molecular phylogenetic and historical biogeography of Hawaiian *Dryopteris* (*Dryopteridaceae*) (Geiger & Ranker 2005) indicate that Hawaiian *Dryopteris* is not monophyletic, and there were at least five separate colonizations of the Hawaiian Islands by different species of dryopteroid ferns, with most of the five groups having closest relatives in SE Asia.

This paper presents a preliminary compilation of Western Malesian *Diplazium* distribution and their inferred center of diversity in Malesia based on specimens examined and direct observation in the field and their distribution outside West Malesia that obtained from literature studies. In the light of current concerns on the loss biodiversity it is also useful to highlight the areas with high species numbers and endemicity. The aims of the research are map the distribution of Western Malesian *Diplazium* and determine the endemic species.

**MATERIAL AND METHODS**

The determination of species distribution patterns was studied by examining specimens deposited at BO and SING, conducting field work in Java, Sumatra, and Borneo. Beside that many literature were also studied for determining the range area distribution of each species.
Following those conducted by Parris (2003) in presenting the distribution of Grammitidaceae in the world, for the purpoing of examining the distribution of Western Malesian Diplazium, it is convenient to divide the world into five regions: (1) New World (North, Central and South America, the West Indies and the islands of the Galapagos, Falklands and South Georgia); (2) Africa (continental Africa, Madagascar and the islands of the Azores, Canaries, Ascension, St. Helena, Tristan da Cunha group, Seychelles, Comoros, Mauritius, Reunion, Marion, Crozets and Kerguelen); (3) Asia excluding Malesia (India, Sri Lanka, Nepal, Thailand, Cambodia, Laos, Vitenam, China, Taiwan and Japan); (4) Malesia (Malaysia, Singapore, Brunei Darussalam, Indonesia, Philippines and Papua New Guinea), and (5) Pacific (Australia, New Zealand and the islands of Micronesia, Melanesia and Polynesia).

RESULTS AND DISCUSSION

A large number of Diplazium specimens (1051 collections number) deposited at BO and SING and new collections obtained from the field have been examined. Sixty nine species of Diplazium are recorded within West Malesian region. Diplazium of Malay Peninsula, Borneo, Sumatra and Java (including Bali) comprises of 28, 40, 29, and 30 species, respectively. The distribution for each species inside and outside West Malesia are presented in Table 1.

Revision on Bornean and Sumatran species has not been conducted since van Alderwereld van Rosenburgh (1908). For Bornean species, formerly author who reported the check list of this genus locally were lwatsuki & Kato (1984), Tagawa (1972), Kato et al (1991), and Parris et al 1992). For Sumatran species, the check list provided is only those reported by Mitsuta (1985) of West Sumatra. Thus this study was the first account of Diplazium throughout Borneo and Sumatra. Beside that three and four species are now recognized as new species for Borneo and Sumatra, respectively Table 1.).

For Javanese species the recent account after Backer & Posthumus (1939) was Praptosuwiryo (1999). The first author described 17 species, three species of them have been included in other genera. Meanwhile second author described 22 species and 4 varieties. Now, eight species were added for Java and seven species of them are proposed as new species (Table 1.).
Holttum (1940, 1966) reported 27 species of Malay Peninsula that include *D. heterophlebium*, *D. curtisii* and *D. amplissimum*. As stated in Chapter 9, the first two species are included in the doubtful names. The latest species was included in *Cornopteris* as *C. atroviridis* (v.A.v.R.) M. Kato (Kato 1979). *D. aequibasale*, *D. christii*, and *D. fraxinifolium* are new record for Malay Peninsula.

Based on the range of its geographical distribution, the West Malesian species can be divided into three types: (1) very wide distribution species, (2) Malesian species, and (3) endemic species to island in West Malesia. The three types of the geographical distribution are discussed below.

1. Very Wide Distribution Species

A conception of very wide species adopted here is referred for species that is distributed at least two region or more of regional division following those adopted by Paris (2003). Of the sixty nine species of *Diplazium* from West Malesia, 19 of them distribute very wide (Table 1.), in the New World. They are *D. accedens*, *D. bantamense*, *D. cordifolium*, *D. crenatoserratum*, *D. dilatatum*, *D. donianum*, *D. esculentum*, *D. fuliginosum*, *D. malaccense*, *D. pallidum*, *D. polypodioides*, *D. prescottianum*, *D. riparium*, *D. silvaticum*, *D. simplicivemen*, *D. sorzogonense*, *D. subserratum*, *D. tomentosum* and *D. xiphophyllum*. Three species of them are distributing across four main region (Africa, Asia, Malesia, Pacific), viz. *D. accedens*, *D. dilatatum*, and *D. esculentum*.

Some species that widely distributed are varying in morphology and genetic (Praptosuwiroyo 2008). Therefore some varieties are found in these species. Kato (1995) recorded 2 varieties in *D. donianum* and *D. dilatatum*. In this study, *D. accedens*, and *D. cordifolium* are recognized to have three varieties. Meanwhile in *D. pallidum* and *D. silvaticum* are recorded two varieties.
Table 1. Distribution of West Malesian *Diplazium* Inside and Outside Malesia

(PM = Peninsular Malaysia, B = Borneo, S = Sumatra, Pa = Palawan, J = Java, B = Bali, Philippines, C = Celebes, M = Moluccas, LSI = Lesser Sunda Islands, IJ = Irian Jaya, PNG = Papua New Guinea.

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<th>Outside Malesia</th>
<th>Geographical Distribution Range</th>
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<td>2.</td>
<td><em>D. acuminatum</em> Blume</td>
<td>S</td>
<td></td>
<td>Endemic</td>
</tr>
<tr>
<td>3.</td>
<td><em>D. aequibasale</em> (Baker) C.Chr.</td>
<td>P, B, S, J</td>
<td></td>
<td>Malesia</td>
</tr>
<tr>
<td>4.</td>
<td><em>D. albidosquamatum</em> Alderw.</td>
<td></td>
<td></td>
<td>Endemic</td>
</tr>
<tr>
<td>5.</td>
<td><em>D. angustipinna</em> Holtt. (Holtt.)</td>
<td>PM, B</td>
<td></td>
<td>Malesian</td>
</tr>
<tr>
<td>6.</td>
<td><em>D. asymmetricum</em> Praptosuwiryo, sp. nov.</td>
<td>J</td>
<td></td>
<td>Endemic</td>
</tr>
<tr>
<td>7.</td>
<td><em>D. atrosquamosum</em> Copel.) C.Chr. &amp; Holtt.</td>
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<td></td>
<td>Endemic</td>
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<tr>
<td>8.</td>
<td><em>D. bantamense</em> Blume</td>
<td>PM, B, S, J, Ph, M</td>
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<td>Very wide</td>
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<td>9.</td>
<td><em>D. barbatum</em> C.Chr. in C.Chr. &amp; Holtt.</td>
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<td></td>
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<td>10.</td>
<td><em>D. batuyauense</em> Praptosuwiryo, sp. nov.</td>
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<td>11.</td>
<td><em>D. beamani</em> M.G. Price</td>
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<td>12.</td>
<td><em>D. betimusense</em> Alderw.</td>
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<td>13.</td>
<td><em>D. christii</em> C. Chr.</td>
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<td>15.</td>
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<td>17.</td>
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<td>18.</td>
<td><em>D. crinitum</em> (Baker) C.Chr.</td>
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<td>19.</td>
<td><em>D. cumingii</em> (Presl) C.Chr.</td>
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<td>20.</td>
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<td></td>
<td>Endemic</td>
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<td>22.</td>
<td><em>D. dolichosorum</em> Copel.</td>
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<td>Malesia</td>
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<td>23.</td>
<td><em>D. donianum</em> (Mett.) Tardieu</td>
<td>S, J, PNG</td>
<td>Japan, Taiwan, S. China, Indochina, Thailand and India</td>
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<td>25.</td>
<td><em>D. fraxinifolium</em> Presl</td>
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<td>Malesia</td>
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<td>26.</td>
<td><em>D. fulliginosum</em> (Hook.) M.G. Price</td>
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<td>Malesia</td>
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<td>27.</td>
<td><em>D. halimunense</em> Praptosuwiryo, sp. nov.</td>
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<td>28.</td>
<td><em>D. hewittii</em> (Copel.) C.Chr</td>
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<td><em>D. hottae</em> Tagawa</td>
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<td>31.</td>
<td><em>D. kunstleri</em> Holtt.</td>
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<td>32.</td>
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<td>34.</td>
<td><em>D. lobbianum</em> Moore</td>
<td>J, Ph, IJ, PNG</td>
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<td>35.</td>
<td><em>D. lomariaceum</em> (Christ.) Price</td>
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<td>36.</td>
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<td>37.</td>
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<td>Distribution</td>
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<td>38</td>
<td>D. megasimplicifolium Praptosuwiryo, sp. vov.</td>
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<td>Diplazium meijeri Praptosuwiryo</td>
<td>S</td>
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<td>40</td>
<td>D. melanolepis Alderw.</td>
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<td>41</td>
<td>D. moultonii (Copel.) Tagawa</td>
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<tr>
<td>42</td>
<td>D. pallidum (Blume) Moore</td>
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<td>43</td>
<td>D. parallevenium Praptosuwiryo, sp. nov.</td>
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<td>D. petiolare C. Presl.</td>
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<td>D. poense C. Chr. in C.Chr. &amp; Holtt.</td>
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<td>46</td>
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<td>Thailand, Sri Langka, S, India, Himalaya, Indochina, Taiwan. Very wide</td>
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<td>D. porphyrorachis (Baker) Diels</td>
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<td>48</td>
<td>D. prescottianum (Wall.) Moore</td>
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<tr>
<td>49</td>
<td>D. procumbens Holtt.</td>
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<td>Malesia</td>
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<td>50</td>
<td>D. profluen Praptosuwiryo, sp. nov.</td>
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<td>Endemic</td>
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<tr>
<td>51</td>
<td>D. riparium Holtt.</td>
<td>PM, B, S, J, Ph, M</td>
<td>Thailand</td>
<td>Malesia</td>
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<td>52</td>
<td>D. silvaticum (Bory) Swartz</td>
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<td>Malesia</td>
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<td>53</td>
<td>D. simplicivenium Holtt.</td>
<td>PM, J</td>
<td>Malesia</td>
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<td>54</td>
<td>D. sorzogonense C. Presl.</td>
<td>PM, S, J, M</td>
<td>Thailand, Vietnam, Indo-China</td>
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<tr>
<td>55</td>
<td>D. speciosum Blume</td>
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<td>Malesia</td>
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</tr>
<tr>
<td>56</td>
<td>D. spiniferum Alderw.</td>
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<td>57</td>
<td>D. squarrasum K. Iwats. &amp; M Kato</td>
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<td>Endemic</td>
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</tr>
<tr>
<td>58</td>
<td>D. subintegrum Holtt.</td>
<td>PM, S</td>
<td>Malesia</td>
<td></td>
</tr>
<tr>
<td>59</td>
<td>D. subalternisegmentum Praptosuwiryo, sp. nov.</td>
<td>B</td>
<td>Endemic</td>
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</tr>
<tr>
<td>60</td>
<td>D. subpolypodioides Alderw.</td>
<td>S, J</td>
<td>Malesia</td>
<td></td>
</tr>
<tr>
<td>61</td>
<td>D. subserratum (Blume) Moore</td>
<td>PM, S, J</td>
<td>Malesia</td>
<td></td>
</tr>
</tbody>
</table>
62. *D. subvirescens* Praptosuwiryo, sp. nov. J Endemic

63. *D. tomentosum* Blume PM, B, S, J, M Very wide

64. *D. tricholepis* C. Chr. B Endemic

65. *D. umbrosum* (Smith) Beddome J Endemic

66. *D. velutinum* Holtt. PM Endemic

67. *D. vestitum* C. Presl. B, Ph, M Malesia

68. *D. wahuense* Kato, Darnaedi et K. Iwatsuki B Endemic

69. *D. xiphophyllum* (Baker) C. Chr. PM, B, S, J, M Thailand Very wide

The very wide species in general are also showing the long distance gradient distribution. They are commonly growing in lowlands or medium altitude of mountain forest. For example, *D. accedens*, *D. polypodioides*, and *D. pallidum* are found at 80-1400 m, 200-1900 m, and 600-1500 m, respectively.

2. **Malesian Species**

Malesian species denote for species that having wide distribution range in Malesia region only. These species may be only found in West Malesia or distributed throughout Malesia. Most of species are distributed widely in Malesian region. Some species, such as *D. aequibasale*, *D. angustipinna*, *D. kunstleri*, *D. latisquamatum*, *D. procumbens*, *D. subintegrum* and *D. subpolypodioides* are only found in West Malesia.

3. **Endemic Species to Island in West Malesia**

Endemic species concept adopted here refer to a species that restricted to a particular local or island. The endemic species of *Diplazium* are showed in Table 1. Endemic species criteria was determined by inspection of distribution maps cited from literatures. The total number of local endemic species of West Malesian species recorded in this study are 23 species. This number are high enough. Smith (1972) and Wagner (1972) have discussed the high proportion of pteridophytes on oceanic islands. Smith (1972) compiled data from several islands and showed that ferns have much lower of endemism than angiosperm at both the genus and species levels. On Hawaii, about 16% of angiosperms genera are endemic vs. 6.7% of
pteridophytes genera. Ranker et al (1994) give interpretation for this fact in two fold: first, ferns are more capable of long-distance dispersal and establishment, second, continued gene flow from mainland sources may slow speciation rates for ferns, thereby constraining the evolution of endemic taxa.

Most of endemic species are found mountain forest at 900-1600 m above sea level. It is evidence that the lower and intermediate elevation of mountain forest is also the center of endemism areas. Some endemic species have a relative strict distribution based on elevation. D. betimusense was found at 300-400 m. D. atrosquamsosum and D. lobbianum were only found at 1500-2100 and 1600-1800 m, respectively. One species that has longer distant gradient distribution is D. tricholepis, 100-2100 m.

Some endemic species are found in certain habitats. D. wahauense only grows at the reophytic areas of Borneo. D. albidosquamatum and D. crinitum grow in shady places of limestone area. D. betimusense and D. squarrossum were found growing at shady forest near streams. This endemic statements, however, can change in accordance with the science advances. The new exploration and the change of the taxon delimitation would give new data for a taxon. Consequently taxa that in the past stated as endemic species would be non endemic species.

REFERENCES


### TOPIC 4: PHYSIOLOGY AND DEVELOPMENTAL BIOLOGY

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<td>The carrying Capacity of Guabana (Annona muricata L.) to the Fifth Instar of Atakas (Attacus atlas L.) Larvae in the Field</td>
<td>O-PD20</td>
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<td>Takatoshi Kiba and Hitoshi Sakakibara</td>
<td>How do plants respond to nitrogen-shortage by regulating nitrate uptake?</td>
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<td>Theresia Tri Suharni and Ludmilla Fitri Untari</td>
<td>The Growth of Chlorella pyrenoidosa in wastewater of molasses ethanol fermentation (vinase)</td>
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<td>O-PD23</td>
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</tbody>
</table>
O-PD01

DIMETHYL SULFOXIDE'S EFFECT ON SPERM QUALITY OF GORAMY FISH, Osphronemus goramy LACEPEDE, 1801 TWENTY FOUR HOURS POST-CRYOPRESERVATION

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The effect of dimethyl suloxide (DMSO) in various concentration of 0%, 5%, 7%, 10%, 13%, 15%, and 17%, respectively, on sperm quality of Osphronemus goramy Lacepede, 1801 twenty four hours post-cryopreservation has been studied. Sperm was collected by hand-stripping method, and was put on 2ml of cryotube, and then was diluted by combination of DMSO, and 189M extender. The ratio of sperm and diluent was 1 : 4 according to Horton & Otto (1976). Sample (sperm + diluent) was equilibrated at 4°C for 45 minutes, and was vaporated for 10 minutes, and was then frozen in Liquid Nitrogen for 24 hours. Thawing was carried out at 40°C for 30 seconds. According to Tukey test (P>0.05), 13% of DMSO was showed the highest post-thawed sperm motility (68,58%) and sperm viability (63,5%), and also was showed the lowest post-thawed sperm abnormality (29%), respectively.

Keywords: DMSO, cryopreservation, goramy, sperm quality

INTRODUCTION

Indonesia is one of the two megabiodiversity countries in the world, besides Brazil. About 44 out of 360 species of fresh water fish are endemic in Indonesia (World Conservation Monitoring Centre, 1992). Indonesian giant goramy (Osphronemus goramy, Lacepede 1801) is an indigenous species in Indonesian fresh water which also has very important economic value (Sunarma et al. 2007). Those of endemic (local) species are getting extinct if exploited in uncontrolable. There were two strategies in order to protect those of local (endemic) species, either by in situ (re-stocking) or by ex situ conservation (cryopreservation). Cryopreservation is a process to maintain genetic material in subzero freezing. The expectation result of the cryo- preserved materials were still performed in good physiological function. The successful of cryopreservation were influenced by cryoprotectant and extender. The methodologies, development and application of cryopreservation of fish spermatozoa were reported some for species: carp (Withl-
1982; Harvey, 1983; Horvath et al, 2003), rainbow trout (Stoss & Donaldson, 1983) and other salmonids (Harvey & Ashwood-Smith, 1982). This communication presents the effect of dimethyl sulfoxide (DMSO) in various concentration of 0%, 5%, 7%, 10%, 13%, 15%, and 17%, respectively, on sperm quality of Osphronemus goramy Lacepede, 1801 twenty four hours post-cryopreservation.

MATERIALS AND METHODS

Collection of ejaculated semen

Mature male goramies obtained from a private commercial hatchery were brought into laboratory. The ejaculates from a total of six male goramies were collected by hand stripping, 12—15 hours after injected intra-muscularly with Ovaprim at a dose of 0.2 ml/kg body weight according to modification method of Sunarma et al. (2007).

Semen dilution

The ejaculated semen were diluted with the solvent (189M extender + dimethyl sulfoxide (DMSO) in various concentration of 0%, 5%, 7%, 10%, 13%, 15%, and 17%, respectively; 1 : 4) according to Withler and Morley (see Horton & Otto 1976) and modification method of Sunarma et al. (2007).

Equilibration and freezing

Samples were stored in 2 ml cryogenic tube, equilibrated at temperature 4 °C for 45 minutes according to the modification method of He & Woods (2003). Samples were then vaporated by put the cryogenic tubes 3 cm from the surface of Liquid Nitrogen, and then were frozen (cryopreserved) in Liquid Nitrogen for 24 hours.

Post-thawed parameters examined

After thawing by immersing the cryogenic tubes in a waterbath at 30 °C for 30 sec., each sample was then evaluated for the following parameters using a light microscope with the aid of a digital eye-piece connected to the computer (image driving software; Scopephoto 2.0.4): the percentage of spermatozoa motility, viability, abnormality, and the spermatozoa per milliliter ejaculate (spermatozoa concentration). Some physical and chemical characteristics were also observed, such as: semen (sperm) color, volume, and pH.
RESULTS AND DISCUSSION

Fresh semen were milky white, pH 8—8.5, and 0.7—1.5 ml of volume per ejaculate. The viable or motile sperm showed green color (transparent) on the sperm head, while the non-viable sperm showed pink or red color on the sperm head (Fig. 1). Most of the abnormal sperm has bigger head (Fig. 2). The percentage of spermatozoa motility, viability, abnormality, and spermatozoa concentration of fresh semen were: 82.4±4.93%, 64.5±10.15%, 34.75±9.32%, and (8.51±3.76) x 10^10 cell/ml, respectively (Table 1). While post-thawed spermatozoa motility in control (0%) and in various concentration of 5%, 7%, 10%, 13%, 15%, and 17% of DMSO, were: 31.55±10.11%, 55.13±8.71%, 62±15.60%, 47.625±3.87%, 68.58±17.98%, 62.83±15.06%, and 68.4±7.67%, respectively. Post-thawed spermatozoa viability in control (0%) and in various concentration of 5%, 7%, 10%, 13%, 15%, and 17% of DMSO, were: 27.25±13.96%, 58.5±10.85%, 41±5.23%, 55±6.28%, 63.5±4.93%, 47.5±3.87%, and 51.5±12.58%, respectively. On the other hand, post-thawed spermatozoa abnormality in control (0%) and in various concentration of 5%, 7%, 10%, 13%, 15%, and 17% of DMSO, were: 39.25±5.19%, 44±7.44%, 45.75±14.73%, 30.75±5.91%, 29±2.31%, 41.25±6.45%, and 43.25±8.46%, respectively. While post-thawed spermatozoa concentration in control (0%) and in various concentration of 5%, 7%, 10%, 13%, 15%, and 17% of DMSO, were: (3.01±1.12) x 10^8 cell/ml, (2.83±1.36) x 10^8 cell/ml, (4.24±0.70) x 10^8 cell/ml, (57.56±1.23) x 10^8 cell/ml, (58.64±2.10) x 10^8 cell/ml, (5.59±2.27) x 10^8 cell/ml, and (54.7±2.19) x 10^8 cell/ml respectively.

All of the percentage of spermatozoa motility, viability, abnormality, and spermatozoa concentration were shown in Table 2. According to the Kruskal-Wallis test, the highest post-thawed spermatozoa motility were shown by 13% of DMSO, while the lowest one were shown by 0% of DMSO (control). Most treatment groups (5%, 7%, 10%, 13%, 15%, and 17% of DMSO) were shown relative higher and significant different compared to the control. The highest post-thawed spermatozoa viability were also shown by 13% of DMSO. Post-thawed spermatozoa viability were relatively higher and were shown significant different also compared to the control. On the other hand, the lowest post-thawed spermatozoa abnormality were shown by 13% of DMSO. While another treatment groups (5%, 7%, 10%, 15%, and 17% of DMSO) were not significant different in post-thawed spermatozoa abnormality compared to the control. Accordingly, 13% of DMSO was the optimum concentration that could protect spermatozoa during cryopreservation for 24 hours.
Fig. 1: Viable and non-viable spermatozoa (Eosin staining; 10x40)

Fig. 2: Normal and abnormal spermatozoa (Eosin & Giemsa staining; 10x40)
### Table 1. Fresh semen (sperm) profile

<table>
<thead>
<tr>
<th>n</th>
<th>Vol. (ml)</th>
<th>pH</th>
<th>Color</th>
<th>Motility (%)</th>
<th>Viability (%)</th>
<th>Abnormality (%)</th>
<th>Conc. (10^9)</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.8</td>
<td>8</td>
<td>Milky white</td>
<td>87.2</td>
<td>58</td>
<td>47</td>
<td>5.41</td>
<td>Semen were pooled from 6 males.</td>
</tr>
<tr>
<td>2</td>
<td>0.7</td>
<td>8</td>
<td>Milky white</td>
<td>84.6</td>
<td>57</td>
<td>37</td>
<td>5.16</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.8</td>
<td>8.5</td>
<td>Milky white</td>
<td>82.1</td>
<td>79</td>
<td>28</td>
<td>11.17</td>
<td>Semen volume range: 0.1—0.2 ml.</td>
</tr>
<tr>
<td>4</td>
<td>1.5</td>
<td>8</td>
<td>Milky white</td>
<td>75.7</td>
<td>64</td>
<td>27</td>
<td>12.29</td>
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</tr>
<tr>
<td></td>
<td>X (avg)</td>
<td></td>
<td></td>
<td>82.4</td>
<td>64.5</td>
<td>34.75</td>
<td>8.51</td>
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</tr>
<tr>
<td></td>
<td>SD</td>
<td></td>
<td></td>
<td>4.93</td>
<td>10.15</td>
<td>9.32</td>
<td>3.76</td>
<td></td>
</tr>
</tbody>
</table>

### Table 2. Post-thawed semen (sperm) profile

<table>
<thead>
<tr>
<th>Control</th>
<th>5% DMSO</th>
<th>7% DMSO</th>
<th>10% DMSO</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>Vol. (ml)</td>
<td>pH</td>
<td>Color</td>
</tr>
<tr>
<td>1</td>
<td>0.125</td>
<td>10</td>
<td>Yellow</td>
</tr>
<tr>
<td>2</td>
<td>0.125</td>
<td>10</td>
<td>Yellow</td>
</tr>
<tr>
<td>3</td>
<td>0.125</td>
<td>10</td>
<td>Yellow</td>
</tr>
<tr>
<td>4</td>
<td>0.125</td>
<td>10</td>
<td>Yellow</td>
</tr>
<tr>
<td></td>
<td>X (avg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.125</td>
<td>10</td>
<td>Yellow</td>
</tr>
<tr>
<td>2</td>
<td>0.125</td>
<td>10</td>
<td>Yellow</td>
</tr>
<tr>
<td>3</td>
<td>0.125</td>
<td>10</td>
<td>Yellow</td>
</tr>
<tr>
<td>4</td>
<td>0.125</td>
<td>10</td>
<td>Yellow</td>
</tr>
</tbody>
</table>

X (avg) | 62 | 41 | 45.75 | 4.24 | X (avg) | 47.625 | 30.75 | 55 | 57.56 |
SD       | 15.60| 5.23| 14.73 | 0.70 | SD       | 3.87 | 5.91 | 6.28 | 1.23 |
Table 2. Post-thawed semen (sperm) profile (continue)

<table>
<thead>
<tr>
<th>n</th>
<th>Vol. (ml)</th>
<th>pH</th>
<th>color</th>
<th>Mo (%)</th>
<th>Vbl (%)</th>
<th>Abn (%)</th>
<th>Conc. (10^8)</th>
<th>pH</th>
<th>color</th>
<th>Vbl (%)</th>
<th>Abn (%)</th>
<th>Conc. (10^8)</th>
</tr>
</thead>
<tbody>
<tr>
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<td>0.125</td>
<td>10</td>
<td>Yellow</td>
<td>64.2</td>
<td>69</td>
<td>27</td>
<td>61.6</td>
<td>10</td>
<td>Yellow</td>
<td>56.1</td>
<td>43</td>
<td>46</td>
</tr>
<tr>
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<td>0.125</td>
<td>10</td>
<td>Yellow</td>
<td>92.3</td>
<td>58</td>
<td>31</td>
<td>58</td>
<td>10</td>
<td>Yellow</td>
<td>46.7</td>
<td>32</td>
<td>53</td>
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<td>10</td>
<td>Yellow</td>
<td>48.9</td>
<td>61</td>
<td>31</td>
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<td>10</td>
<td>Yellow</td>
<td>66.7</td>
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<td>44</td>
</tr>
<tr>
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<td>0.125</td>
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<td>Yellow</td>
<td>68.9</td>
<td>66</td>
<td>27</td>
<td>56.65</td>
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<td>Yellow</td>
<td>81.8</td>
<td>47</td>
<td>47</td>
</tr>
<tr>
<td></td>
<td>X (avg)</td>
<td></td>
<td></td>
<td>68.58</td>
<td>63.5</td>
<td>29</td>
<td>58.64</td>
<td></td>
<td>X (avg)</td>
<td>62.83</td>
<td>41.25</td>
<td>47.5</td>
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<tr>
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<td>SD</td>
<td></td>
<td></td>
<td>17.98</td>
<td>4.93</td>
<td>4.93</td>
<td>2.10</td>
<td></td>
<td>SD</td>
<td>15.06</td>
<td>6.45</td>
<td>3.87</td>
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<table>
<thead>
<tr>
<th>n</th>
<th>Vol. (ml)</th>
<th>pH</th>
<th>color</th>
<th>Mo (%)</th>
<th>Vbl (%)</th>
<th>Abn (%)</th>
<th>Conc. (10^8)</th>
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<td>74.6</td>
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<td>53.45</td>
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<td>Yellow</td>
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<td>36</td>
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<td>59.5</td>
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<td>56.05</td>
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<tr>
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<td>X (avg)</td>
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<td>68.4</td>
<td>43.25</td>
<td>51.5</td>
<td>54.7</td>
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<td></td>
<td>7.67</td>
<td>8.46</td>
<td>12.58</td>
<td>2.19</td>
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</table>
Fig. 3: Histogram of Post-thawed spermatozoa motility

Significant different among treatments were shown by different notation (P > 0.05)

Fig. 3: Histogram of Post-thawed spermatozoa viability

Significant different among treatments were shown by different notation (P > 0.05)
Fig. 3: Histogram of Post-thawed spermatozoa abnormality

REFERENCES


O-PD02

STRESS INDUCED MICROSPORE EMBRYOGENESIS IN *Mimulus aurantiacus*

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*Mimulus aurantiacus* is one of the important ornamental plants in Europe this plant is clonally propagated by cutting. Doubled haploid line and F1 hybrids could improve the quality of this plant. The most efficient method in creating doubled haploids plant is via microspore embryogenesis. Stress is known to play an important role in inducing microspore embryogenesis in both the monocots and dicots. The objectives of this research is to induced embryogenic microspores by stress and to promote further development of microspore derived embryo into plants.

Anthers containing microspore at late uninucleate stage were cultured in A2 medium containing various levels of sugars and auxins at 4, 25 and 34°C for 2-10 days in the dark. Microspores were isolated by stirring the anthers then cultured in A2 medium free of auxin at 25°C in the dark.

Result shown that, subjection of excised anthers to heat shock in high level of sugar caused morphological in the microspores change that induce the embryogenic microspores. Some of viable and embryogenic microspore were observed when anthers were pre cultured in A2 medium containing 0,5M maltose + 2 mg/l 2,4-D at 34°C for 4 days. Most of them develop into multicellular structures in the embryogenesis medium A2.

We show here that heat shock combine with high level of maltose and 2,4-D pulse induced embryogenic microspores and further development of microspore derived embryos in mimulus.

**Keywords:** anther, heat shock, maltose, auxin, microspore, embryogenic, multicellular structure, *Mimulus aurantiacus*
O-PD03

THE ROLE OF DUNG BEETLES AS THE SECOND SEED DISPERSER

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Dung beetles were captured in the Nikko National Park, Tochigi Prefecture, Japan, to investigate the number and species of Scarabaeid during seasonal change, and to clarify the role of tunneller type of dung beetles as second seed disperser. From the 1,049 dung beetles captured using traps during May to November 2007, there were 10 species as generalist (8 species of tunnellers and 2 species of dweller). There were 404 individuals of Caccobius jessoensis and 327 individuals of Phelotrupes auratus collected. Caccobius nikkoensis and Onthophagus ater were also collected, which were 143 and 107 individuals, respectively. Phelotrupes laevistriatus, Aphodius eccoptus, Aphodius pratensis, Onthophags artipennis, Liatongus minutus and Capris pecuarius were also collected in a few number of individuals.

The body size of dung beetle was an important factor when a seed was transported into the soil beneath the dung. There was a strong possibility that Phelotrupes auratus acts as a second seed disperser because its body size was the largest beetle in their habitat. They brought seed that content of dung into the deep of soil, then save the seed from predator at the moment. They also made the seed soil bank for the next forest generation and within the suitable condition the seed could germinate well. It was clearly analyzed that the role of the dung beetle was as the second seed disperser in the warm and cool temperate deciduous forest ecosystem.

**Key Words**: Dung beetle, seed disperser, Phelotrupes auratus, tunneller, Nikko National Park.
O-PD04

MECHANISTIC STUDIES EXPLORING THE EFFECT OF ABSCISIC ACID ON GIBBERELLIN CONTENTS

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The effects of Abscisic acid (ABA) on the expression levels of mRNAs for gibberellin (GA) biosynthesis and GA metabolism have been evaluated. Total RNAs were extracted from scutella of germinated barley grains treated with or without ABA (5 M), followed by cDNA synthesis, and Q-PCR was then performed on a Rotor-gene 2000 Real-Time Cycler. The relative expression level of GA 20-oxidase mRNAs, GA 3-oxidase mRNAs, and GA 2-oxidase mRNAs were calculated from the Ct values and the PCR amplification efficiencies of samples, and normalised using the mouse gene CYP2C38. For metabolism experiments, [14C]GA₃₃ or [14C]GA₁₉ was fed to germinated grains with or without ABA being present. There were three replicates for each treatment. The germinated grains were extracted and samples were chromatographed on reverse phase C₁₈ HPLC for GA analysis. The radioactivity in the metabolites was determined by liquid scintillation counting.

The results of Q-PCR experiments showed that ABA caused reductions to about 20% and 40% in the relative expression levels of Hv20ox₁ and Hv3ox₂ mRNAs respectively, and it repressed the expression of Hv2ox₃ mRNA. When [14C]GA₃₃ was fed to germinated grains with or without ABA being present, considerable metabolism to [14C]GA₄₄ and [14C]GA₁₉ was observed. There was no consistent difference between control and ABA-treated grains in metabolism to GA₄₄ and GA₁₉, but there was a reduction in metabolism to GA₂₀. Feeding with [14C]GA₁₉ showed that the conversion to [14C]GA₂₀ was less in ABA-treated grains than in controls. These results suggest that the decline in endogenous GA₁ content in ABA-treated grains is mainly due to a reduction in the activity of the GA 20-oxidase which catalyses the conversion of GA₁₉ to GA₂₀.

Key words: abscisic acid (ABA), gibberellin (GA), GA metabolism.

INTRODUCTION

An increased ABA content is commonly associated with stress conditions, such as drought, cold or high salinity (reviewed by Chandler and Robertson, 1994; Rock, 2000). Zhang et al. (2005) compared the effect of ABA (50 μM) on two different genotypes of wheat (dry climate or wet climate origins), and found that ABA caused a significant reduction in stem height, total biomass, total grain mass, total leaf area, and leaf area/mass ratio, especially in the dry climate genotype. Similar
findings have been reported in which ABA accumulation due to water deficit inhibited leaf growth of cassava (Manihot esculenta Crantz) and reduced the transpiration rate. Upon rewatering, ABA contents in the leaves declined to control levels, corresponding with a rapid recovery of leaf growth rates (Alves and Setter, 2000). It has been shown that ABA interferes with GA signalling (Gubler et al., 2002). In germinated barley, both shoot and root growth was reduced in the presence of ABA. Relative to controls, the contents of GA1 (active), GA1 precursors, such as GA44 and GA20, and GA1 catabolite all decreased in germinated grains exposed to 2 µM or 5 µM ABA. It was noted that the content of GA53 increased slightly (less than 2-fold compared to control) in germinated grains treated with either 2 µM or 5 µM ABA. This finding is of interest in terms of the possibility that ABA has an effect on the expression and/or activity of GA 20-oxidase enzymes that catalyze the late steps of GA biosynthesis (GA53 to GA20 via GA44 and GA19), or of GA 2-oxidase enzymes that catalyse the catabolism of GA1 and its precursors. The elevated content of ABA imposed by dehydration also caused a reduction in the content of GA1 precursors, particularly GA44 and GA20, but GA19 was not altered by accumulation of ABA, and this was also observed in germinated grains treated with 2 µM ABA. This result suggests that the conversion of GA19 to GA20 is probably inhibited in the presence of accumulated ABA. A slight increase in GA53 content was found in germinated barley grains that had been subjected to 2 days dehydration (Dewi and Chandler, 2007). The effect of ABA on the expression of GA 20-oxidase has been examined in sorghum. Under control conditions, it was shown that in the embryo of seeds with low dormancy the expression of SbGA20ox mRNA increased progressively during imbibition, whereas in the embryo of highly dormant seed it increased from 0 – 40 hours and then slightly decreased. When ABA (50 µM) was applied to embryos of low or high dormancy seeds, the expression of SbGA20ox mRNA was suppressed, and it was suggested that ABA had a direct effect on GA biosynthesis (Perez-Flores, 2003).

It is important to unravel the mechanism(s) by which ABA leads to lower GA1 contents, since antagonism between ABA and GA is already documented in different assay systems. The experiments described here were aimed at investigating two possible mechanism(s) by which ABA results in lower GA contents: (1) Does ABA cause any change in the expression of mRNAs that encode GA biosynthetic and/or catabolic genes? and (2) Does ABA treatment affect the activity of the enzymes that catalyse the biosynthesis and/or catabolism of GAs?
The effects of ABA on the expression levels of mRNAs that encode GA biosynthetic and catabolic genes were evaluated by Q-PCR. Q-PCR is the method of choice now for quantitation of transcripts. This method is more sensitive than other methods such as RNA (northern) blotting and in situ hybridisation (Giuletti et al., 2001), and more reliable for detecting expression of individual members of a gene family. As the pathway of GA biosynthesis is known, and radio-labelled GA intermediates are available, metabolism studies were also employed to indicate which step(s) are actually affected by ABA treatment.

MATERIALS AND METHODS

1. Expression levels of GA biosynthetic and catabolic genes

Barley grains were surface sterilized, placed in filter envelopes moistened with 1mM potassium phosphate buffer with or without ABA (5 μM), stratified (4°C in dark) for 3 days then transferred to a constant 20°C growth room. Germinated grains were harvested on 1, 3 and 5 days of growth at 20°C. The scutella were isolated under a binocular microscope and used for RNA extraction. Samples were ground into a powder with a mortar and pestle in liquid nitrogen. Total RNA was extracted using PCI (Phenol : Chloroform : Isoamylalcohol = 50 : 48 : 2). An aliquot of 10 μg total RNA from each sample was purified from any DNA contamination before reverse transcription was carried out, using a DNA-free kit (Ambion, Austin, TX) according to the manufacturer’s instructions. RNA integrity and concentration were checked on formaldehyde agarose gels stained with ethidium bromide. For each sample, 2 μg RNA was spiked with 20 ng mouse liver RNA as an external standard (Takara Bio), and reverse-transcribed using Super Script III (Invitrogen, Australia) according to the manufacturer’s instruction. The cDNA was diluted 5-fold, and 4 μL used in a 20 μL Q-PCR with QuantiTect™ SYBR® Green PCR Master Mix (Qiagen), including specific primers, template and water. The sequences of the forward and reverse primers were listed in Table 1. Q-PCR was performed on a Rotor-Gene 2000 or Rotor-Gene 3000 Real-Time Cycler (Corbett Research, Sydney). The relative expression levels of GA 20-oxidase mRNAs (Hv20ox1, Hv20ox2, Hv20ox3, and Hv20ox4), GA 3-oxidase mRNA (Hv3ox2), GA 2-oxidase mRNAs (Hv2ox1, Hv2ox3, Hv2ox4, Hv2ox5), as well as HvKO1 and HvKAO1 mRNAs were calculated from the Ct values and the PCR amplification efficiencies of samples, and normalised using the mouse gene CYP2C38. The expression level of the standard gene was stable both within and across
experiments. The Q-PCR assay was repeated two or three times and for any claimed effects of the treatment the result was confirmed in one further independent experiment.

Table 1. Primers used in Q-PCR to amplify a reference gene, GA biosynthetic genes, and GA catabolic genes

<table>
<thead>
<tr>
<th>Gene name</th>
<th>Primer name</th>
<th>Sequence (5' – 3')</th>
<th>TM</th>
<th>% GC</th>
<th>Product length (bps)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytochrome P₄₅₀ (Mus musculus) MLR4</td>
<td>MLR4 F</td>
<td>ACAGGCAAAACCACATCGAACA</td>
<td>67.6</td>
<td>47.62</td>
<td>193</td>
</tr>
<tr>
<td></td>
<td>MLR4 R</td>
<td>GCTACGGTGCTATCAAACCAC</td>
<td>63.3</td>
<td>57.14</td>
<td></td>
</tr>
<tr>
<td>HvKO</td>
<td>KO F1</td>
<td>GCAGGTGTGCTCGTAATG</td>
<td>64.1</td>
<td>50.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>KO R1</td>
<td>TAAACAGGACAGCGGAACT</td>
<td>63.5</td>
<td>50.00</td>
<td></td>
</tr>
<tr>
<td>HvKAO</td>
<td>KAO F2</td>
<td>AAAATCTAGACGGCTCGG</td>
<td>64.0</td>
<td>52.63</td>
<td>118</td>
</tr>
<tr>
<td></td>
<td>KAO R2</td>
<td>ACTCTACCGCGACAAACAC</td>
<td>63.8</td>
<td>52.63</td>
<td></td>
</tr>
<tr>
<td>Hv3ox2</td>
<td>3ox2 JRevf</td>
<td>TCCTCCTTCTCCTCAAGTG</td>
<td>60.9</td>
<td>50.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3ox2 JRev</td>
<td>TGTGGAACCTCCTCATC</td>
<td>61.2</td>
<td>52.63</td>
<td></td>
</tr>
<tr>
<td>Hv20ox1</td>
<td>20ox1 F3</td>
<td>CTTCATGGCCGTCTCAAAC</td>
<td>65.9</td>
<td>57.89</td>
<td>350</td>
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<tr>
<td></td>
<td>20ox1 R1</td>
<td>TGGACGGTCGATTCTCAC</td>
<td>63.8</td>
<td>52.63</td>
<td></td>
</tr>
<tr>
<td>Hv20ox2</td>
<td>20ox2 F1</td>
<td>GCCTTACACCGATTGTCTCT</td>
<td>64.1</td>
<td>57.89</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20ox2 R1</td>
<td>AGCCAGCTCCTCAAAATG</td>
<td>63.8</td>
<td>50.00</td>
<td>202</td>
</tr>
<tr>
<td>Hv20ox3</td>
<td>20ox3 F1</td>
<td>CGCTACCTTCCTCCTCAA</td>
<td>63.1</td>
<td>52.63</td>
<td>282</td>
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<tr>
<td></td>
<td>20ox3 R1</td>
<td>TCCAGGATCTGTCACCAT</td>
<td>63.4</td>
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<tr>
<td>Hv20ox4</td>
<td>20ox4 F8</td>
<td>GTGCCCTGTGGTACCACT</td>
<td>66.1</td>
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<td>104</td>
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<tr>
<td></td>
<td>20ox4 R9</td>
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<td>64.6</td>
<td>61.11</td>
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<tr>
<td>Hv2ox1</td>
<td>2ox1 F2</td>
<td>GCTCCAGAGATGGTCACA</td>
<td>64.1</td>
<td>57.89</td>
<td>186</td>
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<tr>
<td></td>
<td>2ox1 R1</td>
<td>GCCCTGCAAATGCAAGC</td>
<td>64.2</td>
<td>55.56</td>
<td></td>
</tr>
<tr>
<td>Hv2ox3</td>
<td>2ox3 F1</td>
<td>GACCAAGGAGGATTTACGA</td>
<td>64.5</td>
<td>57.89</td>
<td>186</td>
</tr>
<tr>
<td></td>
<td>2ox3 R1</td>
<td>CTGCTGGTGTCCTGTTGTA</td>
<td>64.1</td>
<td>57.89</td>
<td></td>
</tr>
<tr>
<td>Hv2ox4</td>
<td>2ox4 F1</td>
<td>TCCAGGACGCAAGCACT</td>
<td>65.0</td>
<td>57.89</td>
<td>291</td>
</tr>
<tr>
<td></td>
<td>2ox4 R1</td>
<td>GGCATGACAGGACACAG</td>
<td>65.1</td>
<td>57.89</td>
<td></td>
</tr>
<tr>
<td>Hv2ox5</td>
<td>2ox5 F2</td>
<td>ACAAGAGACGCCACCAA</td>
<td>64.6</td>
<td>52.63</td>
<td>204</td>
</tr>
<tr>
<td></td>
<td>2ox5 R2</td>
<td>AACCACAGAGCAGGACGA</td>
<td>65.7</td>
<td>57.89</td>
<td></td>
</tr>
</tbody>
</table>

2 Metabolism experiments using radio-labelled GAs

Metabolism experiments were carried out by feeding either [14C]GA₅₃, [14C]GA₁₉, or [14C]GA₂₀ (from Prof. L.N. Mander, Australian National University, Canberra, Australia) to germinated barley grains. Barley grains were imbied in control solution or ABA (2 μM and 5 μM), stratified and then grown at 20°C for 3 days. Five grains from each treatment were taken, the roots cut to about 1 cm, and then each germinated grain was placed in a microfuge tube (0.5 mL) containing either control or ABA solution plus 417 Bq [14C]GA₅₃, or 833 Bq [14C]GA₁₉. These tubes were placed in a constant 20°C room and the germinated grains were harvested after 1 d for those fed with [14C]GA₅₃ and [14C]GA₁₉, or 2 d for those fed with [14C]GA₂₀. The roots were washed in distilled water and each sample
was extracted with 80% methanol for GA analysis. There were three replicates for each treatment. The extraction procedure was described in Dewi and Chandler (2007), except that partitioning against 5% NaHCO₃ was omitted. Samples were chromatographed on reverse phase C₁₈ HPLC as methyl esters. The radioactivity in the metabolites was determined by liquid scintillation counting. To check the identification of metabolites from different fractions, a standard mix containing [¹⁴C]GA₅₃, [¹⁴C]GA₄₄, [¹⁴C]GA₁₈, [¹⁴C]GA₂₀, and [¹⁴C]GA₁ was also methylated and chromatographed on HPLC and the radioactivity in each fraction determined by liquid scintillation counting. The identity of GAs in each pooled fraction was determined by GC-SIM.

RESULTS AND DISCUSSION

Effects of ABA on the expression levels of GA biosynthetic and catabolic genes

The scutella of germinated barley grains were used as a source of RNA for Q-PCR studies because they are the major site of GA biosynthesis following germination (Dewi and Chandler, 2003). The Q-PCR results showed that expression levels of 

*HvKO1* and *HvKAO1* mRNA were generally similar between control and ABA-treated grains (Fig.1). This result is consistent with the belief that feedback and feedforward regulation does not involve the early steps of GA biosynthesis (Hedden and Phillips, 2000; Davidson *et al.*, 2005). These results also indicate that the modest accumulation of GA₅₃ in ABA-treated grains was unlikely to be due to enhanced expression of *HvKO1* or *HvKAO1* mRNAs.
Oikawa et al. (2005) reported that both OsGA20ox1 and OsGA20ox2 are important in determining plant stature in rice. In germinated barley grains, the relative expression levels of Hv20ox1 and Hv20ox2 mRNAs were higher than Hv20ox3 and Hv20ox4 mRNAs, suggesting that Hv20ox1 and Hv20ox2 proteins probably are the major isozymes that catalyze the conversion of GA3 to GA20 following germination. Mutation in the Hv20ox1 gene results in a considerable reduction in GA1 content and some visible dwarfism (P.M. Chandler, unpublished data), however, the step(s) which is/are catalyzed by Hv20ox1 as well as Hv20ox2 in vivo still warrant further determination.

In germinated grains treated with ABA (5 μM), the expression levels of Hv20ox1 mRNA were consistently lower than controls. Even though the expression of Hv20ox1 mRNA was only reduced by about 20% in ABA-treated grains (Fig.2), this slight reduction may be sufficient to affect the final amount and activity of the protein. On the other hand, the expression of Hv20ox2 increased about 20% in ABA-treated grains compared to control grains, and this might represent a mechanism of feedback regulation or balancing towards the reduction in Hv20ox1 mRNA by ABA treatment. The expression of Hv20ox3 and Hv20ox4 mRNAs also tended to increase in ABA-treated grains, however, the abundance of these mRNAs was low compared to Hv20ox1 or Hv20ox2 mRNAs, and these proteins are probably less important during early growth of barley. These results suggest that a reduction in GA1 content in ABA-treated grains might be due to decreased expression levels of Hv20ox1 mRNA. It is also possible that ABA affects the expression of other 20-oxidase genes (e.g.
Hv20ox2), but as the GA1 content declines, the level of the Hv20ox2 mRNA might increase in response to feedback regulation, causing the final amount of mRNA present to be similar, or even higher than control. Pérez-Flores et al. (2003) reported that in sorghum, the expression level of SbGA20ox mRNA in isolated embryos was repressed by ABA (50 μM). It is not known whether a greater repression of Hv20ox1 mRNA could be observed by applying higher concentrations of ABA to germinated barley grains. In the scutella of germinated grains which had been dehydrated for 2 d, the expression of Hv20ox1 mRNA was repressed (data not shown). However, the effects of dehydration treatment on the expression of GA biosynthetic genes might be different compared to the application of ABA, in which cell turgor is maintained. The expression of GA 20-oxidase mRNAs in germinated grains treated with higher ABA concentrations or in an ABA over-producer mutant(s) warrants further investigation.

![Graphs showing relative expression levels of Hv20ox1, Hv20ox2, Hv20ox3, and Hv20ox4 mRNAs](image)

Figure 2. The effect of ABA on the expression levels of Hv20ox1, Hv20ox2, Hv20ox3, and Hv20ox4 mRNAs as
determined by Q-PCR.

Barley 'Himalaya' grains were germinated in control or ABA (5 µM) medium, and harvested on 1, 3 and 5 d after growth at 20°C. Total RNA was extracted from scutella. The relative expression level was calculated from the Ct values and the PCR amplification efficiencies of samples, and normalized using a mouse gene (CYP3C38). Values are means ± SE (n = 3). The Q-PCR assay was repeated at least twice and the results were similar.

ABA treatment also caused an approximate 50% reduction in the expression of Hv3ox2 mRNA (Fig.3). Perhaps this reduction and a 20% reduction in Hv20ox1 mRNA are sufficient to contribute to the observed drop in GA1 content.

![Graph](image)

**Figure 3.** The effect of ABA on the expression level of Hv3ox2 mRNA as determined by Q-PCR.

Barley 'Himalaya' grains were germinated in control or ABA (5 µM) medium and harvested on 1, 3 and 5 d after growth at 20°C. Total RNA was extracted from scutella. The relative expression level was calculated from the Ct values and the PCR amplification efficiencies of samples, and normalized using a mouse gene (CYP3C38). Values are means ± SE (n = 3).

The Q-PCR assay was repeated at least twice and the results were similar.
The expression level of GA 2-oxidase genes was also evaluated and it was shown that ABA had no major effect on the expression levels of Hv2ox1, Hv2ox4, and Hv2ox5 mRNA. Surprisingly, the expression level of Hv2ox3 mRNA was greatly reduced by ABA (Fig.4). It is of interest to note that the apparent abundance of Hv2ox3 mRNA is higher than all other GA 2-oxidase isozymes, suggesting that it might play a major role in catabolism of GAs during early growth. It has been demonstrated that some GA 2-oxidase mRNAs are feedforward regulated (Thomas et al., 1999). In rice, the expression of OsGA2ox3 mRNA was increased by GA3 application. The recombinant OsGA2ox3 protein catalysed the conversion of GA1 to GA8, as well as GA20 to GA29, and GA29 to GA29 catabolite. It was suggested that OsGA2ox3 is responsible for maintaining the content of active GA in rice (Sakai et al., 2003). The content of GA20 and GA1 and their catabolites (GA29 and GA8, respectively) decreased in ABA-treated grains (Dewi and Chandler, 2007). It is not known which particular GA is catabolised by Hv2ox3, but it is likely that the reduced expression of Hv2ox3 mRNA in ABA-treated grains is a consequence of GA20 and GA1 being low, and it may represent a mechanism to maintain the content of active GA.

Figure 4. The effect of ABA on the expression level of Hv2ox1, Hv2ox3, Hv2ox4 and Hv2ox5 mRNAs as

---

Figure 4
determined by Q-PCR.

Barley ‘Himalaya’ grains were germinated in control or ABA (5 µM) medium, and harvested on
1, 3 and 5 d after growth at 20°C. Total RNA was extracted from scutellae. The relative expression
level was calculated from the Ct values and the PCR amplification efficiencies of samples, and
normalized using a mouse gene (CYP3C38). Values are means ± SE (n = 3). The real-time PCR
assay was repeated at least twice and the results were similar.

Effects of ABA on the metabolism of labelled GAs

The results of metabolic studies, feeding [14C]-labelled GA intermediates to germinated barley grains, suggested that ABA had an effect on the metabolism of GA\(_{19}\) to GA\(_{20}\). When [14C]GA\(_{53}\) was fed to control and ABA-treated grains, there was no difference in the amount of radioactivity detected in GA\(_{19}\), but there was a reduction in GA\(_{20}\) compared to control (Table 2). To further investigate the effect of ABA on conversion of GA\(_{19}\) to GA\(_{20}\), [14C]GA\(_{19}\) was fed to germinated grains and the radioactivity in the metabolites determined. The results showed that the mean radioactivity (of triplicate samples) in GA\(_{20}\) decreased significantly to about 56% and 43% of control in germinated grains treated with 5 M and 10 M ABA, respectively. The mean radioactivity in GA\(_{1}\) decreased with a similar pattern as GA\(_{20}\) (Table 3). In addition, when germinated grains were fed with [14C]GA\(_{20}\) for 2 d, the mean radioactivity (of duplicate samples) in GA\(_{1}\) and GA\(_{6}\) also decreased in germinated grains treated with ABA (Table 4). Appleford et al. (2006) evaluated the kinetics of one GA 20-oxidase enzyme in wheat (TaGA20ox1) and found that GA\(_{19}\) had a low affinity for the enzyme, consistent with GA\(_{19}\) accumulation observed in wheat. The reduction in activity of the isozyme that catalyses the conversion of GA\(_{19}\) to GA\(_{20}\) when ABA is present may result in a lower content of GA\(_{1}\). It is likely that Hv20ox1 is the major isozyme that catalyses the conversion of GA\(_{19}\) to GA\(_{20}\) as it is the only isozyme whose mRNA was reduced by ABA treatment.

ABA caused a reduction in the amount of radioactivity detected in GA\(_{1}\) and GA\(_{6}\), with a similar reduction in GA\(_{20}\), suggesting that the activity of GA 3-oxidase is not affected. Thus the reduced expression of Hv3ox2 mRNA in germinated grains treated with ABA is possibly due to a direct effect of ABA on gene expression or mRNA stability. It was shown in Fig. 4 that ABA reduced the expression of Hv2ox3 mRNA, and it was predicted that the protein encoded by Hv2ox3 mRNA catalyses the conversion of
GA$_{20}$ to GA$_{29}$ and possibly also GA$_{29}$ to GA$_{29}$ catabolite, based on the activity of the rice orthologue. As ABA caused a reduction in the activity of the enzyme catalyzing the conversion of GA$_{19}$ to GA$_{20}$, it may be that down-regulation of Hv2ox3 mRNA expression following ABA treatment represents a mechanism to maintain the synthesis of active GA by minimizing GA$_{20}$ catabolism. This assumption is consistent with the feedforward regulation of GA 2-oxidase mRNA. In fact, GA$_{29}$ was not detected in germinated grains treated with 5µM ABA. However, it remains unknown whether there is an increase in the catabolism of GA$_{53}$ to GA$_{37}$, because ABA caused an increase in the content of GA$_{53}$. Further work on ABA control of GA biosynthesis in barley should attempt to compare GA dioxygenase protein levels and enzymatic activities in addition to the transcript level.

Table 2. Effect of ABA on the metabolism of $^{14}$CGA$_{53}$ by germinated barley grains.

Values are means + SE of 3 biological replicates. Different letters indicate significant (T-test, P< 0.05) difference within a metabolite category

<table>
<thead>
<tr>
<th>Sample</th>
<th>Radioactivity (dpm) in each metabolite per 5,000 dpm of $^{14}$CGA$_{53}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GA$_{44}$</td>
</tr>
<tr>
<td>Control</td>
<td>713 ± 52.9 $^a$</td>
</tr>
<tr>
<td>ABA 5 mM</td>
<td>696 ± 107.7 $^a$</td>
</tr>
<tr>
<td>ABA 10 mM</td>
<td>586 ± 26.6 $^b$</td>
</tr>
</tbody>
</table>

Table 3. Effect of ABA on the metabolism of $^{14}$CGA$_{19}$ by germinated barley grains.

Values are means + SE of 3 biological replicates. Different letters indicate significant (T-test, P< 0.05) difference within a metabolite category

<table>
<thead>
<tr>
<th>Sample</th>
<th>Radioactivity (dpm) in each metabolite per 25,000 dpm of $^{14}$CGA$_{19}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GA$_{20}$</td>
</tr>
<tr>
<td>Control</td>
<td>1775 ± 50.4 $^a$</td>
</tr>
<tr>
<td>ABA 5 mM</td>
<td>979 ± 55.6 $^b$</td>
</tr>
<tr>
<td>ABA 10 mM</td>
<td>763 ± 6.24 $^b$</td>
</tr>
</tbody>
</table>
Table 4. Effect of ABA on the metabolism of $[^{14}$C]GA$_{20}$ by germinated barley grains.

Values are means ± SE of 2 biological replicates

<table>
<thead>
<tr>
<th>Sample</th>
<th>Radioactivity (dpm) in each metabolite per 25,000 dpm of $[^{14}$C]GA$_{19}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GA$_{20}$</td>
</tr>
<tr>
<td>Control</td>
<td>1775 ± 50.4 $^a$</td>
</tr>
<tr>
<td>ABA 5 mM</td>
<td>979 ± 55.6 $^b$</td>
</tr>
<tr>
<td>ABA 10 mM</td>
<td>763 ± 6.24 $^b$</td>
</tr>
</tbody>
</table>

Taken together, the results obtained from real-time PCR studies and metabolic studies using radio-labelled GAs suggest that the mechanism by which ABA reduces the content of GA$_1$ primarily involves a reduction in the expression and activity of the enzyme catalyzing the conversion of GA$_{19}$ to GA$_{20}$. These results are consistent with the finding reported in transgenic apple (*Malus pumila*), in which a dwarf phenotype associated with lower contents of GA$_1$ and GA$_{20}$, but a higher content of GA$_{19}$ was obtained by reducing the expression of *MpGA20ox1* mRNA using either sense or antisense suppression (Bulley *et al.*, 2005). The authors suggested that the conversion of GA$_{19}$ to GA$_{20}$ is the rate-limiting step in the reactions catalysed by MpGA20ox1 activity, based on the accumulation of GA$_{19}$ which is consistent with reduced GA 20-oxidase activity. Furthermore, it has been reported that the metabolism of $[^{14}$C]GA$_{12}$ in decapitated shoots of hybrid aspen (*Populus tremula* L. x *P. tremuliodes* Michx.) increased in SD conditions compared to LD conditions, causing an accumulation of $[^{14}$C]GA$_{53}$ in SD conditions, and it coincided with the down regulation of *PttGA20ox1* mRNA expression level in response to SD. It was also suggested that SD conditions caused a reduction in the activity of GA 20-oxidase as the content of GA$_{19}$ in the expanding leaf blades accumulated but GA$_1$ content decreased to 50% in SD (Erikson and Moritz, 2002). Gilmour *et al.* (1986) reported that the pH optimum for enzymatic conversion of $[^{14}$C]GA$_{19}$ was 6.5, slightly different from oxidation of $[^{14}$C]GA$_{53}$ and $[^{14}$C]GA$_{44}$ which were pH 7.0 and 8.0, respectively. Heimovaara-Dijkstra *et al.* (1994) reported that ABA (1 µM) increased the intracellular pH to almost 7.1 in barley aleurone protoplast. It could be that a higher ABA concentration also induced a higher intracellular pH, and it may cause an inhibition of enzymatic conversion of GA$_{19}$ to GA$_{20}$. Other possible mechanism(s) by which ABA inhibits activity of the enzymes catalyzing the conversion of GA$_{19}$ to GA$_{20}$ await further investigation.
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isolated from hybrid aspen (*Populus tremula* x *P. tremuloides* Michx.). *Planta*

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O-PD05

ROLES OF SOIL ARTHROPODS COMMUNITY IN LEAF LITTER DECOMPOSITION AT ARBORETUM FOREST AND BOTANICAL GARDEN OF GADJAH MADA UNIVERSITY

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ABSTRACT

Soil Arthropods community is one of the decomposer agent that has an important role in early stages of decomposition process. The roles of soil Arthropods community in litter decomposition at Arboretum Forest and Botanical Garden of Gadjah Mada University were analyzed to study of the density and diversity of soil Arthropods and roles of soil Arthropods in litter decomposition.

This research was held during September-December 2008 by litter bag method, with Completely Randomized Design (CRD) with two factors and five replications. The parameters that were examined are physical and chemical factors, leaf litter decomposition rate, and soil Arthropods community structure. The data collected were analyzed by using Analysis of Variance (ANOVA), and LSD test at 0.05 significant level.

This result showed that soil Arthropods community contributed in litter decomposition, while decomposition mesh size differences have no effect on decomposition rate at both site. The decomposition rate was slowed down by litter’s secondary metabolites like phenol and tannin. The development of soil Arthropods community with decomposition process showed the cyclic succession.

Keyword: Soil Arthropods community, leaf litter, decomposition

Introduction

Decomposition is a process which is very important to forest ecosystem’s function. Plant litter decompose through several phases that involve physical and biological processes. Physical processes in the decomposition is the leaching and the degradation of litter’s structure, while the chemical processes is a biodegradation mechanism that involves microbes enzyme. Leaves litter decomposition determined by the abundance of soil decomposers (Bradford, et. al., 2007; Shiels, 2006).

Some of the factors that affect the fluctuation and soil decomposer community are litter quality, climate, physical conditions and chemical properties of the soil (Hosseini, 2001), plants, root activity, pH, humidity, and natural disturbances caused by other animals or human (Fergusson, 2001). Soil arthropods presence are capable to change litter condition to facilitate microbes activity in the decomposition process.
Microarthropods such as collembola and acarina are the dominant groups in the leaves litter on forest soil, particularly on the humid wood litter and integrate with the soil humus (Seastedt & Crossley, 1984).

Through the variation of litter bag’s mesh size, this study was aimed to learn the relationship of the abundance and diversity of soil arthropods community with forest litter decomposition. To learn soil arthropods community, and their role in the forest litter decomposition.

Methods

1. Location and experiment design

This research was conducted in Arboretum Forest and Botanical Garden of Gadjah Mada University, Yogyakarta. Type of forest soil in this research was humus. Both of location had a tropical climate, with the moderate sun light and moist. Soil Arthropods collection conducted in the Entomology Laboratory, while the identification was done in Ecology Laboratory of Gadjah Mada University. Analysis of litter carbon and nitrogen content was conducted in the Soil Science Laboratory, while the phenol and tannin contents were conducted in the Biotechnology Laboratory, of Faculty of Agricultural Technology.

This study used litter bag method with different mesh sizes. This treatment was expected to give descriptions of soil arthropods community variations that contributed to forest litter decomposition. The field study was conducted for 3 months, from September until December 2008. The plants used in this study were Canarium sp. in Botanical Garden, and Stelechocarpus burahol (Blume) Hook. F. Thomson in Arboretum Forest. This research was conducted with factorial analysis with Complete Randomized Design (CRD), with 4 treatment, which are 1) the types of leaf litter were Canarium sp and Stelechocarpus burahol (Blume) Hook. F. & Thomson., 2) mesh sizes of litter bags, that were 1 mm and 6 mm, with 5 replications and 6 times of sampling. Parameters observed in this study were the physical environment factor, rate of litter decomposition, litter’s chemical substances, and soil arthropods community.

2. Procedure of experiment

The old leaves from both types of plants were air-dried (Ruan, et. al., 2005; Weerakody & Parkinson, 2006), for a week and then ovened at a temperature 60°C for two days (Sayer, et. al., 2006). 8 grams of leaves
inserted in each litter bag. Litter bags then being assigned and prepared under the trees for the decomposition process. The sampling time is calculated 14 days after the litter bag being placed in the field (Bernhardt & Reversat, 1982). The measurement of physical environmental factors was done in stages, at each sampling time is 14 days once. Absolute humidity measurements from each plot of land treatment was done by drying 10 grams of soil using the oven at a temperature of 100°C for 48 hours. After the drying process, the leaf litter then weighing again.

Soil arthropods separation from the litter was performed by using the funnel full green, with a 25 watt bulb (Kevan, 1962). Bottle collection included a mixture of alcohol and 1% glycerol with identity label. Amount and identification measurement of soil Arthropods were conducted to family level using the binocular microscope on 20X magnification. The differences of decomposition rate were analyzed by ANOVA. If there are significant differences between the treatment, the test continued with the LSD test at the 0.05 stage.

3. Result

a. Leaf litter chemical substances

The chemical substances that analyzed in this research were carbon (C), nitrogen (N), phenol, and tannin as shown in Table 1.

<table>
<thead>
<tr>
<th>Jenis serasah daun</th>
<th>Chemical Substances percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Carbon (C)</td>
</tr>
<tr>
<td>Canarium sp.</td>
<td>45.78</td>
</tr>
<tr>
<td>Stelechocarpus burahol</td>
<td>49.67</td>
</tr>
</tbody>
</table>

Based on Table 1, there was a difference in the percentage of chemical substance on each type of litter. The carbon percentage in Stelechocarpus burahol leaves litter were higher than in Canarium sp, it was 49.67%. Nitrogen content in the Canarium sp leaves litter were higher than in the Stelechocarpus burahol leaves litter, it was about 1.64%. Tannin and phenol substances in Canarium sp leaves litter were higher than in Stelechocarpus burahol leaves litter, the values were 4.57% and 4.66%. These differences reflect the quality of leaves litter.
b. **Environmental factors**

Physical environmental factors on the Botanical Garden and Arboretum Forest in this research is shown in Table 2.

<table>
<thead>
<tr>
<th>Environmental Factors</th>
<th>Botanical Garden (a)</th>
<th>Arboretum Forest (b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil temperature (°C)</td>
<td>25.04°a</td>
<td>24.41°a</td>
</tr>
<tr>
<td>Air temperature (°C)</td>
<td>27.54°a</td>
<td>27.14°a</td>
</tr>
<tr>
<td>Relative soil humidity (%)</td>
<td>47.91°a</td>
<td>46.24°a</td>
</tr>
<tr>
<td>Absolute soil humidity (%)</td>
<td>19.12°a</td>
<td>16.19°a</td>
</tr>
<tr>
<td>Air humidity (%)</td>
<td>82.70°a</td>
<td>83.46°a</td>
</tr>
<tr>
<td>Soil pH</td>
<td>6.64°a</td>
<td>6.10°a</td>
</tr>
</tbody>
</table>

Based on Table 2, the physical environment factors significantly different (P ≥ 0.05) at each location.

c. **Rate of litter decomposition**

Decomposition rates in Botanical Garden and Arboretum Forest can be seen in Table 3.

<table>
<thead>
<tr>
<th>Kind of leaf litter</th>
<th>Mesh size</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>8</th>
<th>10</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Canarium sp.</td>
<td>1 mm</td>
<td>3.72°a</td>
<td>11.67°a</td>
<td>13.18°a</td>
<td>18.22°a</td>
<td>22.22°a</td>
<td>27.00°a</td>
</tr>
<tr>
<td></td>
<td>6 mm</td>
<td>3.22°a</td>
<td>12.91°a</td>
<td>13.36°a</td>
<td>17.12°a</td>
<td>17.38°a</td>
<td>23.75°a</td>
</tr>
<tr>
<td>Stelechocarpus burahol</td>
<td>1 mm</td>
<td>1.49°a</td>
<td>12.56°a</td>
<td>13.17°a</td>
<td>19.01°a</td>
<td>32.17°c</td>
<td>43.38°c</td>
</tr>
<tr>
<td></td>
<td>6 mm</td>
<td>1.64°a</td>
<td>10.85°a</td>
<td>12.12°a</td>
<td>22.09°a</td>
<td>33.25°c</td>
<td>42.44°</td>
</tr>
</tbody>
</table>

The ANOVA test (P ≥ 0.05) shows that Canarium sp leaves litter decomposition rate generally not different, while the Stelechocarpus burahol leaves litter decomposition rate were different in some treatments.

d. **Soil Arthropods Community**

There were some differences between the number and types of soil arthropods community composition in both of locations. From the result, amount of soil arthropods groups in Canarium sp leaves litter were more than soil arthropods in Stelechocarpus burahol leaves litter, while the abundance of soil arthropods in Stelechocarpus burahol leaves litter were more than in Canarium sp.

Kinds of Canarium sp soil arthropods in litter bag 1 mm were 33 groups, and the abundance was 1709.8 individuals. In the 6 mm mesh size were 34 groups, and the abundance was 2258.4 individuals. Soil arthropods in Stelechocarpus burahol litter bag mesh size 1 mm were 30 groups, with abundance was 1593.6 individuals, while in the mesh size 6 mm were 29 groups with abundance was 2998.1 individuals.

e. **Ordination of Soil Arthropods Community**

The ordination method used to analyze the development of animal community geometrically. Soil arthropods ordination on each treatments are presented in Figures below.
Figure 1. The ordination of soil arthropods community on *Canarium sp.* leaves litter in litter bag’s mesh size 1 mm.

Figure 2. The ordination of soil arthropods community on *Canarium sp.* leaves litter in litter bag’s mesh size 6 mm.

Figure 3. The ordination of soil arthropods community on *Stelechocarpus burahol* leaves litter in litter bag’s mesh size 1 mm.
Based on the ordination analysis, the soil litter arthropods community in both of locations change time after time. Circle sizes on ordination reflect the Prominence Value (PV) of soil arthropods community on every week. The distance between circles shows the difference of the soil arthropods community.

4. Discussion

Forest litter decomposition had no differences in both locations. This condition may caused by physical environmental factors that were similar in both locations. Those locations have similarity in physical environment factors, and this is a normal range in tropical forest condition.

Obviously the litter quality affects the activity of soil arthropods community in the decomposition process. The results indicated that *Stelechocarpus burahol* leaves had higher C/N than *Canarium sp.*, while the tannin and phenol contents in *Canarium sp* were higher than in *Stelechocarpus burahol*. The substances of lignin, tannin, phenols and other carbohydrate such as cellulose significantly correlated with decomposition rate. Tannin and phenol decreased litter palatability to be consumed by the soil arthropods.

Tannin and phenol substances are secondary metabolites and antimicrobes. The high content of phenol and tannin caused both leaves litter less palatable for soil arthropods as decomposer agent on the beginning of decomposition process. Therefore, the decomposition rate in both of leaves litter more influenced by tannin and phenol substances.
a. Contribution of Soil Arthropods Community

Based on the results, the role of soil arthropods clearly visible at the beginning of decomposition process, this confirm the previous study by Alhamd, et. al., (2004).

Collembola and Acarina were kinds of soil arthropods contributed more in litter decomposition in both locations. Soil arthropods contribution to the decomposition are by breaking the litter into small pieces, transports litter from one place to another, defecation, inoculates fungi propagules and facilitated microbes activity to colonize.

Soil arthropods bites expand the surface of litter wound. In addition of feeding activities, soil arthropods can also remove faeces on the forest floor. The bacteria could live in digestive organ of soil arthropods. Soil arthropods faeces is useful in addition to the growth medium of fungi and bacteria, also can be used again as nutrient for them.

Soil arthropods create microhabitat in litter layer, so that kind of group can invite other decomposers (Hishi, et. al., 2007). Soil arthropods can also transport organic material to the outside or inside of the soil (Dindal, 1990). The process involves materials transport can increase the pore size of the soil (Kevan, 1962).

Treatment with the differences of mesh size (Guzman & Sanchez, 2004) may select some groups of soil arthropods to enter, so can make an impact to the decomposition rate. In the mesh size of 1 mm could be entered by smaller soil arthropods (≤ 1 mm), while the mesh size of 6 mm could be entered by bigger soil arthropods. Higher abundance and diversity in the mesh size of 6 mm could cause the decomposition rate more rapidly, because arthropods’ body size affects the size of bites on leaves litter (Nugroho, 2000; Dindal, 1990; Swift, 2004). With bigger litter wound area, it made the litter easier to be colonized by microbes.

Similarity in the composition of soil arthropods in both of mesh sizes indicate that the soil arthropods at the location were small sizes. They were relatively smaller compared with the previous research conducted by Guzman & Sanchez, (2004) in Mexico tropical forests, so that all groups of soil arthropods could enter to both of mesh sizes since the beginning of decomposition process. Nevertheless, the results of this study still shows that the soil arthropods has an obvious role in litter decomposition in both locations.
b. **Soil Arthropods Community**

The abundance and composition of soil arthropods community structure were different in both locations. The number of families in Botanical Garden were higher than in Arboretum Forest, while the number of soil arthropods individu in Arboretum Forest were higher than in Botanical Garden.

The differences of the abundance can also be seen temporally, and always different in each sampling. The soil arthropods communities develop to more complex structure by time. Early communities were dominated by Psocoptera known that they are prefer low humidity and fresh leaves litter.

The number of families and soil arthropods abundance's in both locations increased until week 8. The Increased of soil arthropods influenced by the ability to utilized the soil substrate, the more of them could survive and reproduce. This supports for the new generation of soil arthropods in these habitats.

After week 8 the soil arthropods abundance declined. This was resulted by the reduction of leaves litter resources. Along with the decomposition processes, there are also occur a gradual succession of soil arthropods communities in both locations. It was directional and cumulative over the time (Barbour, *et. al.*, 1987).

Some things that could change over the succession of soil arthropods community are the decreasing number of herbivore (phytaphagous), and increasing diversity of predators as the impact of succession process. The succession could also increasing the number of diversity, number, and parasites number on the soil arthropods community (Odum, 1971).

The composition of soil arthropods community in both location were dominated by phytophagous, mycophagous, saprophagous, microphytophagous, and detritivore. The increase of the number of soil arthropods will improve the diversity of predators. The higher predator diversity was influent by the prey availability. The physical factors, litter quality, and microbes are enable to make a new microhabitat and niche in soil arthropods community. The dynamics of soil community is complex and allows the coexistence or loosing groups of species in the community according to their ability to compete with the other competitors (Bever, 2003).

C. **Ordination of soil Arthropods Community**
Based on the ordination, it could be seen that there are the development of soil arthropod community structures, starts from the simple to the complex community structure. This development could be seen from the higher PV along the study, but the soil arthropods community development was different in both locations. This is caused by differences in the mesh size. In the bigger mesh size, the soil arthropods community developed faster than the smaller one.

Soil arthropods community development in both locations showed the succession in a particular direction. Differences in the mesh size affected the soil arthropods community succession. In the bigger mesh size, the PV was higher and the succession is linear, while in the smaller mesh size shows the cyclic succession. The succession of soil arthropods community different in all locations, but either linear succession and cyclic succession could not be described in detail.

From the discussions above can be concluded that the soil arthropods community has a role in leaves litter decomposition in all locations. Differences of mesh sizes did not affect the decomposition rate because of the body size of soil arthropods relatively small in all locations, allowing each soil arthropods groups to enter the litter bag.

Litter quality which affected the rate of the decomposition were phenol and tannin compound, because these chemicals are toxic and unpalatable to soil arthropods community. Soil arthropods community changes along with the decomposition processes. Soil arthropods community changes in all locations indicated by a cyclic succession.

5. References


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O-PD06  

Wnt SIGNALINGS INVOLVEMENT IN THE DEVELOPMENT OF PROVENTRICULAR GLAND OF CHICKEN EMBRYO

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ABSTRACT

We had reported the expression and possible function of \textit{Wnt5a}, a non-canonical \textit{Wnt}, during the development of chicken proventricular gland. In that studies we found that \textit{Wnt5a} has begun to be expressed in very early stage of development (E2) in the mesenchyme of anterior intestinal portal. The expression was then broadening to the presumptive of proventriculus (PV) and later became limited in mesenchyme area just beneath the developing glands. It has widely known the involvement of reciprocal communication between epithelium and mesenchyme in the formation of digestive organs. The examination of the expression of some marker genes for stomach gland development after over-expression or inhibition of \textit{Wnt5a} unveiled the possible involvement of this gene in the differentiation of proventricular gland. It is suggested that \textit{Wnt5a} is one of numerous mesenchymal factors regulate the development of proventricular gland through non-canonical pathway which is orchestrated with canonical \textit{Wnt} signaling pathway. Here we discuss this possibility.

\textbf{Key words:} \textit{Wnt5a}, \textit{Wnt} signalings, proventricular gland, chicken embryo.

INTRODUCTION

\textit{Wnt} proteins are a family of secreted glycoproteins that play essential roles in many aspects of animal development\textsuperscript{1,2).} They perform such functions through the activation of cellular processes via different signaling pathways\textsuperscript{3,4).} As an example of this, members of the \textit{Wnt1} class of proteins, including \textit{Wnt1}, \textit{Wnt3a}, \textit{Wnt8} and \textit{Wnt8b}, transmit their signals through the canonical \textit{Wnt/β-catenin} pathway\textsuperscript{5)}, whereas those of the \textit{Wnt5} class, including \textit{Wnt4}, \textit{Wnt5a}, \textit{Wnt7} and \textit{Wnt11}\textsuperscript{1,4,6,7)}, transduce signals through a non-canonical \textit{Wnt/Ca\textsuperscript{2+}} pathway\textsuperscript{8,9)} and a planar cell polarity (PCP) pathway\textsuperscript{10).} \textbf{Recently it was demonstrated that \textit{Wnt5a} also}
negatively regulates Wnt/β-catenin signaling\textsuperscript{11}) through the stimulation of Wnt/Ca\textsuperscript{2+} signaling\textsuperscript{9,12}) and GSK-3-independent β-catenin degradation\textsuperscript{13}).

Some members of the Wnt gene family are expressed in a distinct pattern along the developing digestive tract\textsuperscript{14,15}). Among these, Wnt5a, a member of the non-canonical Wnt signaling factors is reported expressed in the proventriculus (PV) at E4.5\textsuperscript{16}). Later it has been known that this gene is expressed in the mesenchyme of the developing digestive tract of the chicken embryo\textsuperscript{17,18}).

Digestion is a complex system which provides continued processing of food, physically and chemically, from raw to a “ready to be used” and finally waste forms. In vertebrate, this system is composed of a row of hollow organs running from the anterior to the posterior end of body to form a tract. Reserving the only machine responsible for whole processing of food, digestive system consists of a series of compartments with specific function, enzymatic break-down, mechanic break-down, absorption and elimination, respectively. Each compartment has a specific structure to support its unique function. In the chicken, the most elaborate compartment to digest the food is stomach, with its glandular in the anterior part (proventriculus, PV) and muscular in the posterior part (gizzard). Studies on digestive tract development provide valuable knowledge on how that shape is formed and the special function of each compartment is gained.

Since two embryonic tissues, mesenchyme and endoderm are involved, the distinct role of both tissues and the reciprocal communication between them are very important in order to construct the defined structures. The epithelial-mesenchymal interactions that occur during the development of the stomach are pivotal for the differentiation of both of these tissue types\textsuperscript{19,20}). Various genes have been known to play important roles in specific part and specific time. These genes include Sonic hedgehog (Shh)\textsuperscript{21), Notch, families of bone morphogenic proteins (BMPs), fibroblast growth factors (FGFs), and Wnts. Each gene or gene family takes part in the regulation of digestive tract development through an intricate molecular mechanism.

We had reported that Wnt5a, a member of Wnt family proteins, was expressed very early in the mesenchyme of anterior interstinal portal (AIP) which later develop becomes presumptive proventriculus (PV) area. In the later stages of development the expression of Wnt5a was decreased, except for the mesenchyme just beneath the luminal epithelium and around the glandular epithelium where very weak signals continued to be observed\textsuperscript{22}). It is suggested that this gene might
involved in the regionalization\textsuperscript{23} and development of chicken proventricular gland since \textit{Wnt5a} also participates in epithelial-mesenchymal signaling and there is some evidence that it may specify region identity in the anterior foregut in mouse\textsuperscript{24}.

\textbf{MATERIAL AND METHODS}

\textbf{Probes}

Digoxigenin (DIG)-labelled anti-sense RNA probes for c\textit{SP}\textsuperscript{20}, \textit{ECPg}\textsuperscript{25}, and c\textit{Smad8} (ChEST63012) were used to examine the expression of developmental markers of the chicken embryonic stomach. That for \textit{Wnt5a} (a gift from Dr. Nohno\textsuperscript{26}) was used to examine the expression of both endogenous and exogenous \textit{Wnt5a} and that for \textit{DS-1 (RSV retrovirus envelop protein) to check the efficiency of infection of the RCAS (replication-competent avian sarcoma virus)-Wnt5a and RCAS-\textit{EGFP} virus. The probe was prepared according to the manufacturer’s instructions (DIG RNA Labeling Kit; Boehringer Mannheim GmbH, Germany). Either T3 or T7 RNA polymerase (Stratagene, La Jolla, CA, USA) was used to transcribe RNA probes from the linearized templates\textsuperscript{27}.

\textit{Section in situ hybridization}

In situ hybridization of sectioned samples was carried out according to the method previously described by Ishii \textit{et al.}\textsuperscript{28}. Briefly, the samples were fixed in 4\% PFA overnight at 4\textdegree C, embedded in the Optimal Cutting Temperature (OCT) compound (Sakura Finetechnical, Tokyo) and then sectioned at 12 \textmu m in a cryostat. Sections were treated with proteinase K (1 \textmu g/mL) at 37\textdegree C for 7 minutes, followed by re fixation in 4\% PFA and hybridization to digoxigenin-labeled RNA probes at 68\textdegree C overnight. Each probe was hybridized to the adjacent sections. Color development for the \textit{Wnt5a} RNA probe\textsuperscript{26} was carried out in the dark for about 2 days at room temperature, whereas for the other RNA probes this process carried out for several hours at room temperature. At least 5 embryos successfully infected were examined in each experiment.
**Viral construction**

Wnt5α-expressing RCAS virus (RCAS-Wnt5α) was constructed by generating the entire coding region of Wnt5α from pBluescript (SK-) with a Spe1 site at the 5’-end and Not1 site at the 3’-end and inserting it into RCAS L-44 vector. To eliminate the start codon upstream of the Spe1 site in the vector, I cut the vector sequence using Cla1 and Spe1 restriction enzymes, followed by treatment with Klenow fragment. Production of viral particles was performed using DF-1 competent cells grown at 37°C in 5% CO₂. The viral particles were harvested by collecting the culture medium and concentrated by ultracentrifugation. The viral preparations were then stored in 5μl aliquots at -80°C until use. EGFP-expressing RCAS virus was constructed by Shin, Dsh-ΔC-expressing RCAS virus was constructed by Yamada from cDNA of Dsh-ΔC given by Prof. Masazumi Tada. RCAS-Sfrp2 cDNA was gift from Prof. Francis-West.

**Overexpression of Wnt5α and inhibition of Wnt signalings**

Embryos from the C/O strain, which are susceptible to all subgroups of the avian retrovirus, were used to introduce exogenous genes. The embryos were incubated at 38°C and staged either according to34 or by the number of incubation days. To expose the chicken embryos, the shell of 2.5 to 3 day old eggs was removed and the extraembryonic membranes were cut open. Concentrated RCAS virus solution containing 0.01% fast-green was injected directly into the presumptive stomach area with a fine glass needle from the latero-dorsal side of the embryo. The opened shell was then covered with cellophane and the embryos were further incubated at 38°C for the determined period. Wnt5α-expressing RCAS virus and Dsh-ΔC or Sfrp2-expressing RCAS virus were used to examine the effect of overexpression of Wnt5α and inhibition of non-canonical or canonical Wnt signaling activities, respectively.

**RESULT AND DISCUSSION**

Wnt signaling pathways are classified into canonical and non-canonical groups. Some canonical and non-canonical Wnt genes are expressed during the development of the digestive tract. It is known that canonical Wnts inhibit the development of stomach-specific epithelium in the mouse. Since it is now well
known that non-canonical Wnts possess the potency to antagonize their canonical counterparts \(^{11,37}\), it is possible that, also in the development of PV, there is an interaction between canonical and non-canonical Wnt signaling. Non-canonical Wnts may thus antagonize the effects of canonical Wnts and serve to regulate early development in vertebrates.

**Overexpression of Wnt signaling**

Overexpression of *Wnt5a* resulted in the upregulation of the expression of some stomach marker genes including *cSP* (Fig. 1A’), *cSmad8* (Fig. 1B’), and *ECPg* (Fig. 1E’) but does not induce precocious gland formation indicated by lack of epithelial invagination into the mesenchyme (Fig. 1A’, B’). It also induces the ectopic expression of *cSP* in both the proximal and distal regions of the glandular epithelium (Fig. 1D’). Ectopic expression of *cSP* seems to be directly related to the ectopic expression of *Wnt5a* (Fig. 1C’, D’). This indicates that the overexpression of *Wnt5a* does not induce the differentiation of luminal cells to a glandular fate, yet the possibility to induce luminal epithelial cells shape changing. The similar results were obtained by the inhibition of canonical Wnt signaling using Sfrp2 (Fig. 2). This stresses the possibility that Wnt5a involves in the development of glandular stomach through non-canonical pathway.

From this study, we found that in addition to BMP2, *Wnt5a* also positively regulates the expression of *ECPg*. Given that BMP and Wnt often cooperate during organogenesis\(^{38,39}\), now we postulate that Wnt5a also play very important roles in the differentiation of the PV in the chicken embryo\(^{40}\).
Fig. 1: Expression of cSP (A, A’, D, D’), cSmad (B, B’), Wnt5a (C, C’), and ECPg (E, E’) in PV of control embryos (A-E) and embryos infected with RCAS-Wnt5a (A’-E’) at E2.5 to E3.0. Sections were processed and examined at E5.5 (A, A’, B, B’) and E9.5 (C, C’, D, D’, E, E’). Black brackets depict upregulation of cSP, black arrows in B’ indicate the upregulation of cSmad, black and red arrows in C’ indicate the ectopic expression of Wnt5a in luminal and glandular epithelia, respectively, black and red arrows in D’ indicate upregulation of cSP in luminal epithelium and ectopic expression cSP in glandular epithelium, respectively. Red bars 200 μm.

Inhibition of Wnt signalings

The cytoplasmic Wnt signalings use commonly Dsh molecules but through its different functional domains41). To examine the possible contribution of these canonical Wnt genes in the development of PV, we inhibited canonical signaling pathway using Sfrp232,42). Overexpression of Sfrp2 resulted in the upregulation of cSP and ECPg expression (Fig. 2E and F, respectively) and the ectopic expression of cSP in the glandular epithelium (Fig. 2E, thin arrow). To some extent, these phenotypes are similar to those elicited by Wnt5a overexpression (Fig. 1D’, E’).
In order to understand the possible contribution of non-canonical Wnt5a more accurately, we inhibited its signaling pathway using Dsh-∆C, the dominant negative form of Dsh lacking DEP domain which is used by non-canonical Wnts. Examination on E9.5 of infected PV revealed that inhibition of non-canonical Wnt activities results in the upregulation of cSP and ECPg expression (Fig. 2E, F) and induction of ectopic expression of cSP in the distal part of the glandular epithelium (Fig. 2H, thin arrow) and ECPg in the luminal epithelium (Fig. 2I, black arrows). The inhibition of non-canonical pathway also affect on morphology of both luminal and glandular epithelium. Epithelial layers demonstrated uneven thickness and ruptures (Fig 2H, H' and I' red arrows), that are hardly observed neither in Wnt5a nor Sfrp2 overexpressed PV (Fig. 2B, C, E, F). We could not clearly state whether this condition is occurred as a result of the lamina basalis disarrangement because of the blockade of F-actin synthesis and arrangement or the lack of cell adhesion.

<table>
<thead>
<tr>
<th>env</th>
<th>cSP</th>
<th>ECPg</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>RCAS-EGFP</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>RCAS-Sfrp2</td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>RCAS-Dsh-∆C</td>
<td></td>
</tr>
<tr>
<td>H</td>
<td>RCAS-Dsh-∆C</td>
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</tr>
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<td>H'</td>
<td>RCAS-Dsh-∆C</td>
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</tr>
<tr>
<td>I</td>
<td>RCAS-Dsh-∆C</td>
<td></td>
</tr>
<tr>
<td>I'</td>
<td>RCAS-Dsh-∆C</td>
<td></td>
</tr>
</tbody>
</table>
Fig. 2: Expression of cSP and ECPg and glandular epithelial morphology in the RCAS-Sfrp2 (D-F) and RCAS-Dsh-ΔC (G-I') infected PV examined at E9.5. (A-C) control embryos, (A, D, G) demonstrate the expression of viral envelop, (B, E, H, H') demonstrate the expression of cSP, (C, F, I, I') demonstrate the expression of ECPg. (H'-I') demonstrate higher magnification of demarcated areas in (H) and (I), respectively. Black arrows in (I) point ectopic expression of ECPg in the luminal epithelium, thin arrows in (E and H) point the ectopic expression of cSP in the distal part of glandular epithelium, red arrows in (H, H' and I') point disrupted epithelial layers. Red bars in (A-I) = 200 μm, black bars in (H' and I') = 100 μm.

Giving together the results of inhibition of both Wnt canonical and non-canonical pathways had endorsed the contribution of Wnt5a in the normal tissue arrangement and cell differentiation during the development of chicken proventricular gland through non-canonical pathway. It needs further examination to elucidate whether the lack of Wnt5a might induce a tumor structure considering to that potentially disruption of both luminal and glandular epithelia.

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O-PD07

ENHANCING OF GROWTH, ESSENTIAL OIL YIELD AND COMPONENT OF YARROW PLANTS (Achillea millefolium) GROWN UNDER SAFE AGRICULTURE CONDITIONS USING ZEOLITE AND COMPOST

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Abstract

The unique cation exchange, adsorption, hydration-dehydration and catalytic properties of natural zeolites, have promoted their use in clean agriculture as soil amendments and slow-release fertilizers. This research was conducted in open field to investigate the effects of natural zeolite, organic fertilizer (compost) and combination of them on herb growth, oil yield and components of Yarrow plants (Achillea millefolium). The results indicated that zeolite mixed with organic fertilizer led to significant increase in fresh weight, dry weight, number of flowers, total chlorophylls, carbohydrates content, oil yield as well as major ingredients of essential oil, and mineral nutrients, in comparison with the recommended dose of chemical fertilizers NPK (control) under the same conditions. These results undoubtedly confirm that zeolite and compost mixture could replace the application of chemical fertilizers and consequently improve the quality and quantity of Achillea yield. This application may have direct impacts on safety and efficacy of herbal active constituents which entail for medicinal and aromatic products, and also minimizing economic costs and pollution of agricultural environment.

Key Words: Zeolite, Organic fertilizer, Chemical fertilizers, Yarrow plant, Safe agriculture

INTRODUCTION

Effective agriculture, sanitary, safety treatments and collection practices for medicinal and aromatic plants is only the first step in quality assurance, on which the safety and efficacy of herbal medicinal and aromatic products directly depend upon, and also play an important role in sustainable and medical use (WHO guidelines). Lately, the safe agriculture is one of the main attitudes in the world (El-Kouny, 2002). Also, there has been an increasing awareness of the undesirable impact of mineral fertilizers on the environment, as well as the potentially dangerous effects of chemical residues in plant tissues on the health of human and animal consumers.

Natural Zeolite (clinoptilolite) is an amazing crystalline mineral capable of adsorbing and absorbing many different types of gases, moisture, petrochemicals, heavy metals, low-level radioactive elements and a multitude of various solutions and
high cation exchange capacities, also produces long term soil improvements as well as slow release fertilizer of nitrogen. Zeolites can also act as water moderators, in which they will adsorb up to 55% of their weight in water and slowly release it under plant demand. (Jean and Dupont, 1983)

*Achillea millefolium* known as Yarrow, Thousand-leaf and Angel flower of Asteraceae family comprises 115 species, Yarrow is a perennial herb that produces one to several stems (8 to 14 inches tall) from underground horizontal rootstock (rhizome) (Bartram, 1995) For medicinal purposes it has been used as administered both internally and externally. It is valuable for treating wounds, stopping the flow of blood, treating colds, fevers, kidney diseases, menstrual pain (Duke,1985, Bown,1995, Chevallier,1996 and Moerman,1998) .The whole plant is used, both fresh and dried (Foster,1990).

This research scrutinizes the effects of organic fertilizer and natural zeolite on Yarrow plants in comparison with chemical fertilizers (N, P and K) as control.

**MATERIAL AND METHODS**

This research was carried out at the experimental farm of Wadi El-Notron, at private farm (new reclaimed land) as open field, during two consecutive seasons (2006-2007 and 2007- 2008). Mechanical and chemical analyses of the soil were performed according to (Richards, 1954 and Jackson, 1973) as shown in (Table 1) also; chemical characteristics of compost (Table 2), which obtained from Soil, Water and Environment Research Institute, Agriculture Research Center (A.R.C) were accomplished as described by Page *et al.*, (1982) before planting.

**Table 1.** Some physical and chemical properties of experimental soil.

<table>
<thead>
<tr>
<th>Physical properties</th>
<th>Cations (m eq / l)</th>
<th>Anion (m eq / l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sand (%)</td>
<td>90.5</td>
<td>Ca++ 4.16</td>
</tr>
<tr>
<td>Silt (%)</td>
<td>6.50</td>
<td>Mg++ 1.52</td>
</tr>
<tr>
<td>Clay (%)</td>
<td>3.0</td>
<td>Na+ 3.24</td>
</tr>
<tr>
<td>Texture grade</td>
<td>Sandy</td>
<td>K+ 0.21</td>
</tr>
<tr>
<td>pH (soil paste)</td>
<td>7.8</td>
<td></td>
</tr>
<tr>
<td>EC (dS/m at 25 °C)</td>
<td>1.37</td>
<td></td>
</tr>
</tbody>
</table>
Table 2. Chemical analysis of compost

<table>
<thead>
<tr>
<th>F. Property</th>
<th>G. Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH (1:5)</td>
<td>7.5</td>
</tr>
<tr>
<td>EC (1:5 extract) ds/m</td>
<td>3.1</td>
</tr>
<tr>
<td>Organic-C %</td>
<td>33.11</td>
</tr>
<tr>
<td>Organic matter %</td>
<td>70</td>
</tr>
<tr>
<td>Total-N %</td>
<td>1.82</td>
</tr>
<tr>
<td>Total-K %</td>
<td>1.25</td>
</tr>
<tr>
<td>C/N ratio</td>
<td>14:1</td>
</tr>
<tr>
<td>Total-P %</td>
<td>1.29</td>
</tr>
<tr>
<td>Fe-ppm</td>
<td>1019</td>
</tr>
<tr>
<td>Mn-ppm</td>
<td>111</td>
</tr>
<tr>
<td>Cu-ppm</td>
<td>180</td>
</tr>
<tr>
<td>Zn-ppm</td>
<td>280</td>
</tr>
<tr>
<td>Total content of Bacteria</td>
<td>$2.5 \times 10^7$</td>
</tr>
<tr>
<td>Weed seeds</td>
<td>0</td>
</tr>
<tr>
<td>Phosphate dissolving Bacteria</td>
<td>$2.5 \times 10^6$</td>
</tr>
<tr>
<td>Humidity (%)</td>
<td>25</td>
</tr>
</tbody>
</table>

Natural zeolite as granules used in this research was obtained from Yogyakarta, Indonesia. The plantlets of about 13 cm with intact rhizomes were obtained from experimental farm of Faculty of Pharmacy, Cairo University. Planted on the 10th of October (2006) in the experimental field, with a distance of 60 cm between rows, and spacing 50 cm between plants. Compost at (5 ton/fed.) as well as zeolite (210 k/fed) and combination of them (1:1) were added 20 days before planting. Chemical fertilizers as recommended dose were added at the rate of 150 k/fed as ammonium nitrate (33%) divided into two doses, the first was 3 weeks after planting and the second was six weeks later, both calcium superphosphate (15.5%) at the rate of 200 k/fed and potassium sulphate (48%) at the rate of 50 k/fed were added before planting. Agricultural practices were followed as recommended throughout the growing season.

At the first season on the 20th of February, and the 20th of March (2007) during flowering stage, plants were cut 10 cm above the soil surface as the first and the second cut respectively. Harvest repeated on the 20th of February, 20th March (2008) with the same way. The data of growth characters, plant height, fresh and dry weight, number of flowers, fresh weight, and chemical constituents represented in total chlorophylls, carbohydrates content, mineral nutrients (N, P, K), and oil yield were recorded as the first and the second cut for both seasons. The data in both
seasons were statistically analyzed and the means were compared using LSD at 5% significance level (Gomez and Gomez, 1984).

Total nitrogen was determined by Kjeldah method described by Bremner and Mulvaney (1982). Total phosphorus was estimated colorimetrically as described by Jackson (1973). The concentration of K was determined by the Atomic Absorption Spectrophotometer (GBC, 932 AA). Total chlorophylls content was determined using the method described by Normai (1982), while the content of carbohydrates in dried leaves samples were determined using the method described by Dubois et al. (1956). Chemical analysis for essential oil was conducted using the Ati-Unicam gas liquid chromatography (GLC), 610 series, to determine their main constituents as described by Gunther and Joseph (1978).

RESULTS AND DISCUSSION

I. Growth characters

The obtained results of plant growth characters revealed that compost with zeolite mixture treatment significantly increased all growth characters in both seasons of Achillea plants compared to the control treatment (plants receiving the recommended NPK dose) as shown in (Table 3). Supportive evidence for these results was reported by Pirela, et al. (1984), El-Gahban et al. (2002) and Aziz and Iman (2004).


<table>
<thead>
<tr>
<th>Treatment</th>
<th>Season</th>
<th>Plant height (cm)</th>
<th>Herb fresh Weight (g/plant)</th>
<th>Herb Dry weight (g/plant)</th>
<th>Number of flower per plant</th>
<th>Fresh weight (ton/fin)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N.P.K (Control)</td>
<td>FS</td>
<td>77.3 a</td>
<td>3072.6 b</td>
<td>950.3 d</td>
<td>12.00 cd</td>
<td>35.4 d</td>
</tr>
<tr>
<td></td>
<td>SS</td>
<td>82.2 c</td>
<td>3156.7 e</td>
<td>972.2 c</td>
<td>14.00 bc</td>
<td>37.6 c</td>
</tr>
<tr>
<td>Compost</td>
<td>FS</td>
<td>54.2 g</td>
<td>1393.4 h</td>
<td>517.3 k</td>
<td>6.00 a</td>
<td>18.1 g</td>
</tr>
<tr>
<td></td>
<td>SS</td>
<td>59.7 f</td>
<td>1506.0 i</td>
<td>546.6 j</td>
<td>10.00 b</td>
<td>20.5 f</td>
</tr>
<tr>
<td>Zeolite</td>
<td>FS</td>
<td>72.4 a</td>
<td>2110.2 b</td>
<td>728.5 d</td>
<td>11.00 d</td>
<td>28.7 a</td>
</tr>
<tr>
<td></td>
<td>SS</td>
<td>78.6 e</td>
<td>2267.4 d</td>
<td>746.6 e</td>
<td>12.00 cd</td>
<td>29.6 e</td>
</tr>
<tr>
<td>Compost+ Zeolite</td>
<td>FS</td>
<td>93.5 b</td>
<td>3320.6 d</td>
<td>1039.7 c</td>
<td>15.00 b</td>
<td>40.5 d</td>
</tr>
<tr>
<td></td>
<td>SS</td>
<td>102.3 c</td>
<td>3454.8 a</td>
<td>1087.8 a</td>
<td>22.00 a</td>
<td>45.3 a</td>
</tr>
</tbody>
</table>

Means with the same letter in a column are not significantly different at LSD 5%

F.S: first season  S.S: second season
II. Chemical analysis

As mentioned of growth characters data, the results of chemical analysis (Table 4) have the same conclusion where, all parameters significantly increased as a result of compost with zeolite mixture treatment application compared to control plants. These results are convenience with those reported by Kallo (1986), El-Gahdban et al. (2002) and Aziz and Iman (2004).

Table 4. Comparative effects of compost, zeolite and combination of them on nutrient content (N, P, and K), oil yield, total chlorophyll (mg/g fresh weight) and total carbohydrates (% of dry matter weight) of Achillea plants during 2006-2007and 2007-2008 seasons.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Season</th>
<th>Total chlorophylls</th>
<th>Carbohydrate content</th>
<th>N %</th>
<th>P %</th>
<th>K %</th>
<th>Oil yield (ml/plant)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N.P.K (Control)</td>
<td>FS</td>
<td>2.60</td>
<td>21.33</td>
<td>1.25</td>
<td>0.28</td>
<td>1.58</td>
<td>0.22</td>
</tr>
<tr>
<td></td>
<td>SS</td>
<td>2.74</td>
<td>24.34</td>
<td>1.30</td>
<td>0.37</td>
<td>1.68</td>
<td>0.28</td>
</tr>
<tr>
<td>Compost</td>
<td>FS</td>
<td>2.11</td>
<td>16.42</td>
<td>1.08</td>
<td>0.18</td>
<td>1.21</td>
<td>0.13</td>
</tr>
<tr>
<td></td>
<td>SS</td>
<td>2.25</td>
<td>18.39</td>
<td>1.12</td>
<td>0.22</td>
<td>1.37</td>
<td>0.18</td>
</tr>
<tr>
<td>Zeolite</td>
<td>FS</td>
<td>2.30</td>
<td>19.51</td>
<td>1.21</td>
<td>0.26</td>
<td>1.66</td>
<td>0.18</td>
</tr>
<tr>
<td></td>
<td>SS</td>
<td>2.46</td>
<td>20.08</td>
<td>1.31</td>
<td>0.31</td>
<td>1.69</td>
<td>0.20</td>
</tr>
<tr>
<td>Compost+ Zeolite</td>
<td>FS</td>
<td>3.04</td>
<td>23.67</td>
<td>1.31</td>
<td>0.32</td>
<td>1.76</td>
<td>0.27</td>
</tr>
<tr>
<td></td>
<td>SS</td>
<td>3.24</td>
<td>26.19</td>
<td>1.45</td>
<td>0.41</td>
<td>1.84</td>
<td>0.36</td>
</tr>
</tbody>
</table>

Means with the same letter in a column are not significantly different at LSD 5%

F.S: first season S.S: second season

III. Essential oil composition

The results in Table 5 showed that, the essential oil of Achillea plants is characterized by high amount of chamazulene (16.79 - 26.6 %) from sesquiterpenes as the principal components of the oil. The highest amount of chamazulene was increased (26.6 %) in compost + zeolite treatment as compared with the control treatment (NPK). Also monoterpane hydrocarbons are an important group of terpenes, and the major components were β-Pinene (5.67 – 18.47%) which clearly
increased in compost + zeolite treatment in comparison with (NPK) treatment, and Sabinene (5.88 -11.59 %), that give the same trend. Oxygen containing terpenes 1,8-Cineol (2.09 - 4.17 %) was gave a relative increment with compost + zeolite treatment compared with (NPK) control. These results are in agreement with those obtained by several authors, Hofmann and Fritz (1993), Grth and Czygan (1999) as well as Aziz and Eman (2004).

Table 5. Effect of compost, zeolite and combination of them on the essential oil components of Achillea plants during 2007-2008.

<table>
<thead>
<tr>
<th>Treatment Component</th>
<th>NPK</th>
<th>Compost</th>
<th>Zeolite</th>
<th>Compost + Zeolite</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-Pinene</td>
<td>6.37</td>
<td>2.48</td>
<td>4.71</td>
<td>9.46</td>
</tr>
<tr>
<td>Chamazulene</td>
<td>21.38</td>
<td>16.79</td>
<td>20.11</td>
<td>26.61</td>
</tr>
<tr>
<td>Camphor</td>
<td>0.24</td>
<td>0.11</td>
<td>0.18</td>
<td>0.81</td>
</tr>
<tr>
<td>β-Pinene</td>
<td>5.67</td>
<td>15.60</td>
<td>11.64</td>
<td>18.47</td>
</tr>
<tr>
<td>Limonene</td>
<td>0.61</td>
<td>0.48</td>
<td>0.25</td>
<td>0.95</td>
</tr>
<tr>
<td>P-cymene</td>
<td>0.83</td>
<td>0.59</td>
<td>0.18</td>
<td>2.14</td>
</tr>
<tr>
<td>1,8-Cineol</td>
<td>2.23</td>
<td>3.47</td>
<td>2.09</td>
<td>4.17</td>
</tr>
<tr>
<td>β-Caryophyllene</td>
<td>9.86</td>
<td>13.07</td>
<td>11.88</td>
<td>15.08</td>
</tr>
<tr>
<td>Bornyl acetate</td>
<td>0.40</td>
<td>0.64</td>
<td>0.28</td>
<td>0.78</td>
</tr>
<tr>
<td>Cadinene</td>
<td>1.87</td>
<td>0.51</td>
<td>0.99</td>
<td>0.75</td>
</tr>
<tr>
<td>α-humulene</td>
<td>1.98</td>
<td>2.66</td>
<td>2.08</td>
<td>3.44</td>
</tr>
<tr>
<td>γ-terpinene</td>
<td>3.26</td>
<td>0.79</td>
<td>1.55</td>
<td>1.47</td>
</tr>
<tr>
<td>α-Farnesene</td>
<td>2.21</td>
<td>1.86</td>
<td>2.46</td>
<td>1.74</td>
</tr>
<tr>
<td>Sabinene</td>
<td>10.51</td>
<td>8.42</td>
<td>5.88</td>
<td>11.59</td>
</tr>
<tr>
<td>Total</td>
<td>67.42</td>
<td>67.47</td>
<td>64.28</td>
<td>97.46</td>
</tr>
</tbody>
</table>

**Conclusion**

The obtained results in this study are strongly proved that the mixture of compost and zeolite was enhanced the growth characters, oil yield and chemical composition of Achillea plants. Therefore it could be concluded that, the chemical fertilizers of NPK could be replaced by the compost with zeolite mixture for improving the quality of the produced herb, oil yield and components under safe agriculture conditions, in addition to decreasing the production costs and environmental pollution.
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O-PD08

FUNGAL DIVERSITY INSIDE THE SEMULUH CAVE, SEMANU, GUNUNG KIDUL, YOGYAKARTA

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Cave ecosystem has a specific condition such as high humidity, constant temperature, and low light intensity. Organisms including fungi which live inside a cave are peculiar and specialized for the cave condition. However, fungi are remarkable for their antiquity, diversity, ubiquitous, distribution and longevity, led to investigations of the fungal diversity. It is recognized that fungi ordinarily live in almost environment such as soil, among seeds, marshy plants, sewage, polluted water, peat soil, and also in caves. This research is purposed to isolate and to identify fungi at different zone in Semuluh Cave, Ngepohsari Village, Subdistrict of Semanu, Gunung Kidul, Yogyakarta. The sampling was carried out in dry season on July 16, 2008. Environment factors such as temperature, relative humidity, and pH were recorded in 3 stations: twilight zone, middle zone, and dark zone. Soil samples in each station were collected and isolated on RoseBengal Agar Medium. After 7 days incubation at 2325°C, isolated fungi were identified their colony and hypha morphology under microscope. As results, a total of 16 species of fungi was identified from the cave which 5 fungal species in the twilight zone, 5 fungal species in middle zone, and 6 fungal species in dark zone. Aspergillus was the most dominant genus found in this investigation and each zone has a different kind of fungal species. In addition, pH and humidity at all zones were almost similar, but their temperature showed significance different.

Keywords: Cave, Fungi, Hypha, Colony, Diversity
O-PD09

POSSIBLE MECHANISMS OF DROUGHT TOLERANCE IN PEANUT BASED ON ANALYSIS OF SOMACLONAL LINES DERIVED FROM IN VITRO SELECTION

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EXTENDED ABSTRACT

Somaclonal variation and in vitro selection were used to improve drought stress tolerance of a sensitive peanut cultivar (*Arachis hypogaea* cv. Kelinci). In vitro selection of peanut embryogenic calli has resulted in occurrences of somaclonal variation among regenerated plantlets. Addition of polyethylene glycol 6000 (PEG 6000) in the embryogenic callus inducing medium resulted drought stress tolerance peanut lines. The objective of this study was estimate the possible mechanisms of drought tolerance in peanut based on analysis of somaclonal lines derived from in vitro selection using 15% PEG 6000.

PEG 6000 insensitive somatic embryos of peanuts was germinated, regenerated into plantlets and then were transferred into 40 x 40 cm plastic pot containing a mixture of top-soil, sand and manure (2:1:1, v/v), and grown to maturity in the glass-house (R0 lines). Families of R1 and R2 progenies derived from R0 somaclonal lines were grown for evaluation. A family of peanut plants derived from seeds were also planted and used as control. Two evaluated families, namely R2 somaclonal lines and control line, were split into two groups. One group were subjected to stress condition during vegetative and generative periods (12–80 days after planting) by watering them only after their 75% leaves have wilted, while another was grown optimally by watering them every two days. Response of the evaluated families against drought stress, their density of stomata, total leaf proline content, root : shoot ratio, and primary root length were determined.

The drought susceptibility index (S) was used as a measure of response against drought stress in terms of minimisation of the the reduction in yield caused by unfavourable compared favourable environment. It was calculated according to Fischer dan Maurer (1978) for each line as follow:

\[ S = \frac{(1 - Yd/Y)}{(1 - Xd/X)} \]

Yd is the fertile pod number under drought, Y is the fertile pod number under optimum condition, Xd is the average of the fertile pod number of all evaluated plant lines under drought, while X the average of the fertile pod number of all evaluated plant lines under optimum condition. The drought tolerance was determined by criterium: S ≤ 0,5 is tolerant; 0,5 < S ≤ 1,00 is moderate tolerant and S >1,00 is sensitive.
Total leaf proline content of both drought stress and non stress plants of each line were measured on 5 randomly chosen plants at 75 days after planting. The fully expanded leaves were taken out randomly, rinsed several times with distilled water, dried on filter paper, immediately frozen in liquid nitrogen, and then freeze dried. The samples were weighed before analyses, then homogenized with sulpho-salicilic acid as solvent. Then they were filtered and to the extract were added 2,5% ninhydrine solution and glacial acetic acid. In test tubes, the reaction mixture were kept in water bath at 100°C for 1 hour to develop the colours. Soon after removal from the water bath, the test tubes were cooled in ice bath and toluene was added to separate chromophore. The absorbance was read in spectrophotometer at 520 nm, as indicated by Bates et al. method(1973). Proline content was calculated by comparing the samples absorbancies with the standard proline curve in a specific concentration range.

The density of stomata was observed by finger print method on the first two fully expanded leaves from 10 randomly chosen plants at 75 days after planting. Leaf impressions were made by applying a layer of transparent fingernail polish to abaxial leaf surfaces. After drying for 10 minutes, the imprint were removed from the leaves with clear adhesive tape, attached to microscope slides and the amount of stomata were counted under light microscope (200x).

Root : shoot ratio and primary root length were measured on 10 randomly chosen plants at harvest time. Root : shoot ratio was counted based on dry weight after drying fresh material in an oven at 80 °C for 48hours. Primary root length was measured from the crown to the tip of the longest root. The dependent variable drought susceptibility index (S) was regressed on the some independent (predictor) variable, such as density of stomata, total leaf proline content, root : shoot ratio, and primary root length.

Results of the experiments indicated there was a significant linear association between density of stomata and drought susceptibility index (S). A predicted S (y) can be estimated from density of stomata (x) by regression equation as follow: y = -4.48 + 0.056 x, with an R² = 0.2286. The association was positive, so that it can be declared the higher density of stomata, the higher drought susceptibility index; or the lower drought tolerance. Coefficient of determination 0.2286 indicated that 22.86% of S variability can be predicted by density of stomata.

Furthermore, there was a linear association between total leaf proline content and drought susceptibility index (S). A predicted S (y) can be estimated from total leaf proline content (x) by regression equation as follow: y = 7.45 – 0.002 x, with an R² = 0.4151. The association was negative, so that it can be declared the higher density of stomata, the lower drought susceptibility index; or the higher drought tolerance. Determination coefficient 0.4151 indicated that 41,51% of S variability can be predicted by total leaf proline content. In contrast, there were no significant linear association between root : shoot ratio and primary root length with drought susceptibility index (S).
Such results indicated that the possible drought tolerance mechanisms existed among somaclonal lines of peanut derived from in vitro selection were caused by low density of stomata and abundant leaf proline in the cell.

**Key words**: peanut somatic embryos, drought tolerance, total leaf proline, density of stomata
O-PD10

THE DEVELOPMENT OF THE EMBRYO OF GATUL FISH (Poecilia SP.) FROM A SPRING IN STATE UNIVERSITY OF MALANG AREA

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ABSTRACT

Gatul fish is widely known as guppies. According to its morphology Gatul fish is belongs to Genus Poecilia of Familia Poeciliidae. This fish bears the embryos and deliver it as larva. Gatul fish is broadly used as bioindicator for pollution and to eliminate malaria problem in many South American countries. Yet, in Indonesia the biological study upon Gatul fish is very limited. This research was aimed to study the development of Gatul fish embryo. Embryos from the ovary of gravid females from a spring in State University of Malang area were served as subject of this research. Different stages of embryos were examined to study its development. This current study showed that Gatul fish we had studied is a Livebearer. Females develop only one ovary. In that ovary ovum and embryos develop together in different stages. The division type of embryo is meroblastic, embryonic body develops at the animal pole. At the later development the body of embryo embraces the yolk sack. Yolk is absorbed into the body of newborn soon after delivery and yolk sack becomes abdominal wall. The number developing embryos in one ovary range 13 to 22. The size of embryos range from 2,8 to 3 mm in diameters or ± 6 mm in length from snout to tail.

**Keywords:** Gatul fish, embryo development, Poecilia sp.

INTRODUCTION

Gatul fish is a member of Family Poeciliidae. These fish live in river, pond, swamp, and lake in almost all regions in Indonesia. Gatul fish can also survive a polluted environment. That’s why it can be used as bioindicator. Beside, South American societies use this fish to eliminate the problem of malaria because this fish eat mosquito larva, but less utilized by Indonesian society. For this reason the South American countries exported Guppies tropical and sub tropical countries such as: Spain, southern France, Italy, the Balkan Peninsula, southern Romania, Ukraine, Caucasus, and Asia \(^1\).
As a member of Poecilidae Gatul fish is Livebearer. Fertilization is internal, male has external copulatory organ named gonopodium. It has a fast reproductive rate with a marked number of ovum found in the ovary which ranged from 15 to 20. The embryos develop in the ovary and delivered through cloaca \(^2\).

In our preliminary study we found that the embryos of Gatul fish developed in ovary in different stages. The number embryos ranged from 15 to 22. Based on that finding we examined the development of Gatul fish embryo from adult females living in State University of Malang area.

**MATERIALS AND METHODS**

**Materials (Animal)**

Embryos from gravid female of Gatul fish (*Poecilia* sp) living in Spring in State University of Malang area.

**Methods**

Gravid females of Gatul fish were captured using fishing nets and maintained in small aquarium for further examination. Captured fish then decapitated. To open the abdomen dissected the ventral site starting from cloaca to the anterior using fine scissors. Opened abdomen was examined using dissecting microscope. Ovary then isolated and placed in a petri dish containing spring water. The thin connective tissue of the ovary carefully teared to expose the embryos using fine pinsets. The embryos then examined under dissecting microscope to determine its stage. During the examination the embryos are soaked in spring water in Petri dish. The determination of embryonic development was refered to Medaka fish (*Oryzias latipes*) development \(^3\).
RESULTS AND DISCUSSION

Fig. 1: Reproductive system of female Gatul fish. (A) The ovary in the abdominal cavity; circle of green dots indicate the ovary. (B & D) Position of reproduction system related to digestive system. (B-D) green bracket indicate the ovary, black arrows digestive system, blue arrows indicate the liver, orange bracket indicate oviduct, green arrows indicate cloaca, white arrows indicate ovum has vitellogenesis, red arrows showed ovum that has not yet done vitellogenesis, orange arrows indicate mesovarium, purple arrows indicate the embryo, the yellow arrows indicate ovary wrappers. (C) isolated reproductive system. Yellow line = 5 mm.

Female reproductive organs of fish Gatul composed of ovary and oviduct. There is only one ovary developed (Fig. 1A-D). The ovary is surrounded by the digestive system (Fig. 1A). This organ is bound by connective tissue (mesovarium) to other organs (Fig. 1C). The ovary might contain only developing ovum or developing ovum and embryos (Fig. 1D; 2F-I; 3B & D). Ovary and oviduct leads to the cloaca (Fig 1B & C). Each ovary is wrapped with transparent thin connective tissue. In the ovary of mature female we found embryos at different stages of development as had been found in mature Poeciliopsis retropinna and Heterorandria farmosa as well ⁴).
The number of embryos produces by Gatul fish a bit different compared to the other member of Poeciliidae in general. Each embryo in the ovary is covered by a transparent membrane which originated from follicles sack. Ovum and embryos size varies between 2.7 mm - 3 mm. Individual females deliver its larvae developed in the ovary as of other Livebearer such as Guppy fish (Poecilia reticulata) (2). Females have the ability to store sperm, so the sperm can fertilize the developed ovum. Early adults produce 10 to 15 embryos, while the older produces 18 to 22 embryos. Member is a bit different from the number of embryos produced by other 2).

Fig. 2: The ovary of gravid female. (A) Embryo and ovum in the ovary; green arrow indicates the embryo, the red arrow indicates the egg, (B) - (I) embryos in the ovary; blue arrows indicate heart, purple arrows indicate eyes, black arrows indicate vertebra, green arrows indicate an ovum that has not yet done vitellogenesis, red arrows indicate the ovum that has done vitellogenesis, pink arrows show blood vessels, green brackets indicates the early stage of embryos.

The stages of embryonic development in different ovaries are presented in Fig. 3A-D. Fig. 3A&C shows ovaries which contain embryos in close developmental stages, while Fig. 3B&D show ovaries which contain developing both ovum and embryos. Fig. 3A,2-4 show the embryos that already have heart and eye but the
body and eyes pigment have not clearly seen yet. Beside, there are more advance development which already developed eye pigment and dorsal aorta (Fig. 3A5).

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Fig. 3: Developmental stage of the embryo from different ovaries, there is an ovary contains embryos in different of development embryos in one ovary; green arrows indicate the heart, blue arrows indicate the eyes, (A2) The body of the embryo is not yet visible. (B) - (D) embryos other ovaries; green arrows indicate an ovum that has not yet done vitellogenesis, red arrows vitellogenesis ovum, white arrows indicate vertebrae, orange the arrow indicates yolk, blue arrows indicate eyes, yellow arrows indicate brain, black arrows indicate blood vessels.
Fig 3B1-2 shows embryos that the vertebrae, pigment bodies, brain, and eyes have perfectly formed. In the one ovary there is also an ovum that has not been vitellogenesis (Fig 3B-C). Embryo has a size of a large yolk sack before birth. Yolk is absorbed into the body of the embryo (Fig. 3D) and the embryo soon became active kids swimming fish (Fig. 3D1).

In fig B-D there are embryos in advance development. Each embryos display a mature morphology, i.e. head and body had been developed, eyes had become functional one with distinct pupil (black) and choroids layer (metallic). The heart has begun to beat; body pigment had been developed from head area up to tail. Soon after removal of the embryonic sack later (folicle sack) the embryos are actively swimming and its yolk immediately absorbed (Fig. 3D2). Embryonic development of Gatul fish from zygote to hatch is passing 3 stages, i.e cleavage of the embryo, development of the embryo, and free embryo respectively as refer to Peter 5). We have not yet found a complete set development stages comprising detail of each stage.
Fig. 4: The development stage of Gatul fish embryo. (A-C) early stage embryo; red arrow shows the animal pole (blastodisc). (D-Q) Developing embryos; green arrows indicate the embryo's body, green brackets indicate the body segment, white arrows indicate the embryo's body, red arrows indicate the eyes, black arrows indicate blood vessels, green arrows indicate the heart rate, purple arrows indicate heart going, Arrow indicates yolk brown, blue brackets indicate the brain, arrows showed pigment blue body, orange arrows show the vertebrae, pink arrows indicate the tail fin. (R-S) Newly hatched embryos. Green bars = 1 mm.

In this research we found a zygote (Fig. 4A), early cleavage embryos (Fig. 4B) which shows that the cleavage is meroblastic 6 occurs on animal pole as of Medaka fish (Oryzias latipes) 3 Embryo developed from those actively dividing cell at the animal pole, while yolk and yolk sack developed from vegetal pole. In the next stage we found the embryos that already have distinct head shape and developing eyes. At this moment this embryo has not yet formed blood vessels (Fig. 4D - E). Later, blood vessels were found in the dorsal part of the embryo's body and on the
ventral side relative to the developing head (Fig. 4F). Body of the embryo's is still transparent and flattened (Fig. 4F). The next stage of development body tube was formed and became elongated; the eyes begin to form as the optic vesicle and yet there are pigment (Fig. 4G-I), dorsal blood vessels are developing (Fig. 4H); ventral blood vessel would have formed the heart and started beating (Fig. 4I-K). The optic vesicle then developed more pigment and became optic cup (Fig. 4K) and then develop into perfect eyes with metallic pigment on choroid layer, pupils is black and can be distinguished from other parts (Fig. 4L). Dorsal blood vessel and those emerged from heart the developing branched to form peripheral vessel which ventrally grow to cover yolk sack (Fig. 4M-O). Furthermore body pigment formed at the head area extends into the tail (Fig. 4N-S), eye and vertebrae almost perfect, yolk sack is shrink (Fig. 4O-S). Embryos which has reached a perfect structure hatch from follicles (embryonal sack) (Fig. 4R&S), then go out (delivered) through oviduct and cloaca. The yolk is absorbed into the body of newborn soon after delivery. This newborn is actively swimm. So far we could not yet have explanation on how those embryos are moving during the delivery. Beside, a complete set of developmental stage of Gatul fish embryo comprising the detail of each stage has not yet accomplished. However, we can show that the Gatul fish embryos development is attractive.

REFERENCES


O-PD11

THE EFFECT OF STORAGE PERIOD AND GIBBERELLIN ON DORMANCY BREAKAGE AND AMYLASE ACTIVITY OF RICE SEEDS (Oryza sativa L. var C64)

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Dormancy is normally occurred in newly harvested rice seeds. An experiment was conducted to evaluate the effect of storage period and gibberellin on dormancy breakage and amylase activity of rice seeds (Oryza sativa L. var. C64). Newly harvested rice seeds were stored for either 0 week, 2 weeks or 4 weeks at room temperature, then germination test was conducted using four concentration of Gibberellin (GA), namely 0 ppm (control), 100 ppm, 150 ppm and 200 ppm. Twenty seeds were used for germination test with three replicates for each treatment. Amylase activity was determined on germinated seeds without GA treatment.

The results showed that newly harvested rice seeds were dormant as the average of germination percentage was less than 1%. Seeds that were stored for 2 weeks showed an average germination percentage of 22%, whereas those seeds that were stored for 4 weeks showed an average germination percentage of 78%. Application of GA up to 200 ppm showed no effect on the germination percentage of seeds that were stored for different periods, suggesting that dormancy breakage is not determined by GA concentration, but could be due to the sensitivity toward GA. The amylase activity on germinated seeds increased accordingly to the increase in duration of storage, indicating that the loss of dormancy during storage is associated with the responsiveness of aleurone cells to GA which leads to an increase in amylase activity.

Key words: rice seed (Oryza sativa L. var C64), dormancy, gibberellin, amylase activity.
O-PD12

CRUDE EXTRACT OF Catharanthus roseus POTENTIALLY HALT SPERMATOCYTE DIVISION IN GRASSHOPPER SPERMATOBIOGENESIS

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ABSTRACT

Catharanthus roseus produces numerous alkaloids well known as vinca alkaloids. Among those, vinblastine and vincristine, are used extensively in cancer chemotherapy. They disturb mitosis by bind to and inhibit the function of mitotic spindle. The administration of vinblastine and vincristine in cancer treatment shows side effect including severe peripheral neuropathies. People believe that C. roseus as herbs has lower side effect than modern medicine. Crude ethanol extract of this herb contains vinblastine, vincristine, vindoline, and catharantine. It might give different effect compared to pure vinblastine or vincristine. The general purpose of this research is to know the effect of C. roseus crude extract in cell division. We used grasshopper spermatocyte as a model since this cell actively dividing. We examined the duration of cell division from metaphase to early telophase and cell length during metaphase and early telophase, and non kinetochore microtubule polimerization rates. We applied the 0.1%, 0.3%, 0.5%, 0.7%, or 0.9% of C. roseus crude extract in Carlson solution to each group. For the control we used Carlson solution. Data were analyzed using anova and continued with Least Significant Difference (LSD) test. The results so far shows that crude extract of C. roseus has significantly extend cell length during metaphase and early telophase were significantly shortened and the most significant effect was 0.9% (p>0.05). Furthermore, the rate of chromosome movements were significantly getting lower. From this research we conclude that crude extract of C. roseus potencially halt cell division.

Key words: Catharanthus roseus, crude extract, spermatocyte, cell division

INTRODUCTION

Catharanthus roseus is one of medicinal plant in Indonesia. Its herb produces numerous alkaloids well known as vinca alkaloids. Among those, vinblastine and vincristine, are used extensively in cancer chemotherapy 1). Catharanthus alkaloids comprise a group of about 130 terpenoid indole alkaloids (TIAs) 2). No other single
plant species is reported to produce such a wide array of complex alkaloids 3). The roots of the plant accumulate ajmalicine and serpine, which are important components of medicines for controlling high blood pressure and other types of cardio-vascular maladies. However, the plant is particularly known for its economically important leaf-specific bisindole alkaloids, vinblastine and vincristine, which are potent antineoplastic agents 4,5) and indispensable constituents of most cancer chemotherapies. Indeed it was the destruction of the spindle, leading to a loss of chromosome segregation with consequent inhibition of cell division and cell death that gave rise to the term 'antimitotic agent' 6). Besides alkaloids, other secondary metabolites have been isolated from C. roseus, including monoterpenoid glucosides (loganin, secolloganin, sweroside, deoxyloganin, dehydrologanin), steroids (catasteron, brassinolides), phenolics, flavonoids and anthocyanins 2).

Vincristine and vinblatine, are well-established cancer chemotherapeutic agents generally believed to interfere with the microtubule of the spindle apparatus. These compound are used widely in the treatment of both childhood and adult cancers. Their cellular target is the β-tubulin subunit of α/β-tubulin heterodimers, and they act to inhibit cell division by disrupting microtubule dynamics 7). However, they disrupt the microtubule not only spindles of dividing cells but also microtubules of cell types including the neuron.

Microtubular network is important for the execution of many cell functions. They play an important role in cell division 6). Tubulin is the basic protein of the microtubules and molecules of tubulin are arranged in dimers containing both its forms: α-tubulin and β-tubulin. Microtubules are continuously changeable structures 8) and polymerisation and depolymerisation of MTs is regulated by extracellular and intracellular factors 9). Microtubule comes from the core area called MTOC (Microtubule Organizing Center). In mitosis, kinetochorial microtubule adjust chromosome on the spindle, organize, and move the chromosomes to the equatorial during metaphase. While non-kinetochorial microtubule have a role in maintaining attraction force and push the poles 7). The cytoskeleton can be damaged through the effect of many external factors or chemical agents 10-11). As such, the tubulin/microtubule system remains an important target for anticancer therapy 7,8).

Previous studies showed that 0.01% of vinkristin halt the chromosome movement, while 0.005% delayed chromosome movement 11), disturbed spermiogenesis 12), and delayed cell elongation during anaphase 13). Vinca alkaloids also induce apoptosis in tumor cells 14). Inhibition of proliferation by vinblastine and
vincristine appeared to be due to spindle microtubule perturbation, rather than depolymerization of the microtubules. Vinca alkaloids act specifically on spindle microtubules to alter their dynamic behavior by subtly modulating the dynamics of tubulin addition and loss at microtubule ends and thereby, their ability to function properly in chromosome movement 15).

People believe that C. roseus as herbs has lower side effect than modern medicine. Crude ethanol extract of this herbs might contains glucosides 21, vinblastine, vincristine, vindoline, and catharantine 16). It suggested give different effect compared to pure vinblastine or vincristine. In this research we examined the effect of C. roseus crude extract in cell division using grasshopper spermatoocyte as a model since this cell actively dividing. In addition grasshopper spermatoocyte cells have a relatively large in size, chromosome number is relatively small and clear cytoplasm so it is easy to observe 17).

MATERIALS AND METHODS

Catharanthus roseus Extract

C. roseus were provided by Balai Materia Medica. Leaves were air dried at room temperature. Dried leaves were grinded to become powder then extracted using soxhlet extractor in 96% ethanol. Extract were collected and evaporated using Bunchi vacuum rotavator. Yellow concentrated extract of C. roseus kept in 4\(^\circ\)C until used.

Primary Spermatocyte Preparation

Mature male grasshoppers (Cantatops angustifrains) were caught from the area of State University of Malang using insect net. Spermatocyte isolation was done by cutting the grasshopper abdomen diagonally, at 4\(^{th}\) segments from distal end using fine scissors. Isolated testicles then soaked in 0.9% Carlson's solution while discarding the fat body from the testicles. Then testicles were cut open to disperse spermatocyte from testicular lobus in to 0.1, 0.3, 0.5, 0.7, or 0.9% of C. roseus crude extract in carlson solution. For the control, isolation of primary spermatocyte was soaked in 0.9% Carlson’s solution. After 10 minutes of pretreatment, cells suspension is mounted on object glass and covered for examination.
Data

To examine the duration of division we count the time needed by primary spermatocyte to complete anaphase, started from late metaphase to early telophase. To measure the rate of chromosome movement, calculated by the formula, \( v = \frac{s}{t} \). \( S \) is the distance between the equator and pole and \( t \) is time to complete anaphase.

RESULT AND DISCUSSION

Dividing Grasshopper Primary Spermatocytes

Primary spermatocytes was the largest cells among the other cells in grasshopper testicular. It is division normally going through prophase, metaphase, anaphase, and telophase and respectively, accomplished by cytokinesis.

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<th>Metaphase</th>
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Fig. 1: Cell division series of grasshopper primary spermatocyte. (A-F) Cell division series in control treatment, (G-L) Cell division series in 0.1% *C. roseus* crude extract, (M-R) The failure of cytokinesis in 0.3% *C. roseus* crude extract.
The observation of primary spermatocyte division showed that during prophase cell shape was round, there disruption of nuclear envelope, which breaks into membrane vesicle, the chromosome randomly distributed and began to condense. At metaphase, cell shape remained unchanged and the chromosome aligned at the equator. In anaphase, cells elongated by polimerization of non kinetochoiral microtubule, while chromosomes began to move to each pole as a result of kinetochoiral microtubule depolimerization. Telophase is characterized by chromosome that had reached the pole and the begining of membrane ingestion in equator, then the cells immediately undergoes cytokinesis which devides cell into two daughter cells.

It seemed that primary spermatocyte which had been soaked in 0.1% crude extract could undergo a complete division as a normal one, while those which had been soaked in 0.3% could not complete the division, caused by the failure of cytokinesis.

**Duration of Anaphase**

![Duration During Anaphase](image)

Fig. 2: Duration during anaphase (minutes)

Anaphase is stage where the dynamic of microtubule is very high. On this moment the kinetochoiral microtubules pulling the chromosomes to the opposite poles. Non-kinetochorlial microtubules push the poles farther apart. From our study we found that there were differences in the average of cell division duration at differences of concentration. The higher concentration of *C. roseus* crude extract led to the prolonged time required to complete the anaphase (Fig.2). The number of
cells that can not reach early telophase increased at higher concentration (0.5%, 0.7%) and almost all spermatocyte can not reach telophase. These result suggest that, *C. roseus* crude extract disturb microtubule dynamic, thus leads to prolong or blockade of cell division in certain stage. Previous studies showed that raising the concentration of vinca alkaloid led to gradual accumulation of the cell arrested at metaphase 19), 0.005% vincristine treatment delayed anaphase duration 20), while crude extract started to delay anaphase duration at 0.5%. It means needed ten times concentration of crude extract to give same effect as pure vincristine. From those result we can conclude that crude extract less effective to halt cell division compared to pure vincristine.

The Rate Of Chromosome Movement

At anaphase, cell undergoes two separate processes which is related to the elongation of cell, i.e. the movement of chromosomes to the poles and the elongation of polar spindle further separates the poles 21). Those two process is a result of dynamic process. To know the effect of *C. roseus* crude extract on microtubule dynamic during cell division process, we measured the cell length during anaphase. One way anova analysis showed that cell length was significantly influenced by *C. roseus* crude extract. Based on LSD analysis test, 0.9% extract gave the significant influence (P<0.05). Increasing the crude extract concentration led to decrease of cell length changing. At chromosome hardly move, thus the cell size remained unchanged (0.9%).

![Cell Length Changing](image)

**Cell Length Changing**

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<tr>
<th>C. roseus Crude Extract Treatment</th>
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The duration of anaphase and cell length changing were used to determined the chromosome movement rates. The result showed that there are differences in rate of chromosome movement in different concentration. The results of one-way anova showed that there was significant effects of *C. roseus* crude extracts on rate of chromosome movement (P <0.05).

The result of these study suggest that crude extract may disturb microtubule dynamics. Previous studies showed that 0.005% of pure vincristin could delay the rate of chromosome movement. Furthermore, increasing pure vincristine in to cell suspension two times halted the chromosome movement (0.01%) \(^{11}\). While *C. roseus* crude extract delayed the rate of chromosome movement started from 0.3%-0.9%, and entirely halt at 0.9%. It means to give the same effect in rate of chromosomes movement as pure vincristine we need higher concentrations of *C. roseus* crude extract, the disturbances of kinetochore microtubule depolimerization and non-kinetochore microtubule polymerization processes by vinca alkaloids \(^{22}\) perturb on chromosome movement \(^{11}\).

REFERENCE


O-PD13

CLASSICAL QUANTITATIVE STRUCTURE-ACTIVITY RELATIONSHIP STUDIES OF FLAVYLUM SALTS AS XANTHINE OXIDASE INHIBITORS

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A classical Quantitative Structure-Activity Relationship (QSAR) model of xanthine oxidase inhibitory flavylum salts, which enables prediction of the inhibitory potency of anthocyanidins as a function of their molecular properties, has been developed. To compare the inhibitory potency of the flavylum salts considered and to establish the relationships between structure and activity, the dissociation constant of the enzyme-inhibitor complex, $K_{EI}$, was used as experimental biological activity. The variety in molecular descriptors is considered to clarify the effect of electronic, hydrophobic, and steric properties on $K_{EI}$. As usual, classical QSARs were calculated using stepwise multiple linear regression to fit the biological activity to molecular properties. This served to elucidate the relative significance of each independent variable in explaining the xanthine oxidase inhibitory activity as determined by regression analysis. Different models for predicting the inhibition of xanthine oxidase by a particular tautomeric form of anthocyanidins can be achieved by considering various combinations of molecular descriptors. On the basis of the multiple regression analysis performed, tautomeric forms $A_{74}^-$ and $A_{56}^-$ seem to be most active. Multiple regression analysis also indicated that combinations of the hydrophobicity descriptor $\tau$ and indicator variable $I$ were responsible for variation in the enzyme inhibitory activity. The results obtained in the present work help to understand which of the several tautomeric anhydrobase species present in nearly neutral solution are mainly responsible for the inhibition of xanthine oxidase. Moreover, the proposed QSAR models should be helpful in guiding synthetic chemists to improve syntheses of new candidate compounds possessing an enhanced inhibitory activity on xanthine oxidase and as a result, a new therapeutic agent could be designed.

**Keywords:** Classical QSAR, flavylum, xanthine oxidase, anthocyanidins, inhibitory activity.

not presented
O-PD14

ANAC032, A MEMBER OF ARABIDOPSIS NAC PROTEINS, RESPONSE TO HIGH SALT AND OSMOTIC STRESSES

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Transcription factors play important roles in regulation of gene expression, and they are usually members of multigene families. One of the largest families of plant-specific transcription factors are NAC proteins. NAC (NAM, ATAF1, ATAF2, CUC2)-domain proteins are a class of transcription factors known to control multiple processes including plant development, defense, and hormone and stress responses. In this study, we have identified a gene of NAC family which was highly induced by abiotic stresses using microarray analysis and designated it as ANAC032. ANAC032 is localizing in the nucleus. Expression of ANAC32 is strongly induced by various stresses, including drought, salt, abscisic acid (ABA) and Mannitol treatments. Transgenics overexpressing ANAC032 significantly showed improved tolerance to drought and high-salt stress and sensitive to ABA. On the other hand, the null mutants of this gene showed significant decreased sensitivity to high concentration of ABA and decreased tolerance to high-salt and drought stress. The expression of some osmotic-responsive genes, such as RD29A and ERD1 were increased in ANAC032-overexpressing plants than the wild-type. Collectively, these results indicate that ANAC032 functions as a transcriptional regulator that can regulate the expression of stress-related genes in response to abiotic stresses through an ABA-dependent regulation pathway. We propose that ANAC032 has a major role in the NAC-family for conferring ABA-dependent drought and salinity tolerance and is highly potentially useful in the genetic improvement of its stress tolerance.

Keywords: Transcription factor . NAC protein . ANAC032 . salt and osmotic tolerance. ABA sensitivity
O-PD15

GROWTH RESPONSE OF NATURAL PHYTOPLANKTON TO ENRICHMENT OF UREA AND OTHER FORMS OF DISSOLVED NITROGEN

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The Broadwater of Myall Lakes, NWS Australia is brackish water which has a high variation in water quality in particular salinity and nutrient concentration. In these experiments, we determined the growth and species assemblages of natural phytoplankton community exposed to nutrient enrichment. Laboratory incubation condition was used to measure 10 days biomass and group-specific response of phytoplankton community. Four experimental occasions were conducted in autumn (Apr and May’05) and early summer (Nov and Dec’05) with two experiments each season. Biomass of phytoplankton was determined based on chlorophyll fluorescence, extracted chlorophyll a and cell abundance of phytoplankton, and community structure/species assemblages was based on manual identification until genus level using upright light microscope. During four experiment events, chlorophyll growth response was significantly higher in N+P treatment than control and N treatment only, with growth rate was slow which occurred 24-48 hours following nutrient addition. There was inconsistent trend of biomass in terms of cell abundance in respond to nutrient enrichment between experiment occasions, except for May Experiment. Generally, our study found that the greatest difference of phytoplankton growth/biomass at the Broadwater of Myall Lakes was at site level instead of nutrient treatments. Our study also revealed that urea and other forms of dissolved N stimulated growth of group specific of phytoplankton, with P addition contributed considerably to changing in community structure of phytoplankton.

The result of this study suggest that urea was not a factor for Cyanobacteria bloom as compared to the other dissolved N forms, consequently urea does not give a further enhance for Cyanobacteria bloom formation in The Broadwater of Myall Lakes system.

This study revealed that enrichment of different forms of dissolved nitrogen stimulated the growth of phytoplankton taxa in ways that resulted in significant differences in species assemblages among treatments for most sites. Extra addition of P can initiate bloom conditions for cyanobacteria. In order to reduce this possibility, P flow from catchment areas has to be prevented.

Keywords: phytoplankton, community structure, nutrient and urea

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1. Introduction

One of the most serious management issues for coastal waters within the past few decades has been antrophogenic loading of nutrients and sediment, with subsequent eutrophication-enhanced phytoplankton growth (D'Elia et al., 1986; Janse, 1997; Ornolfsdottir et al., 2004). Excessive biomass associated with algal blooms is problematic for many native plant and animal species inhabiting polluted waters and has impacts on both the recreational and commercial use of waterways. Nuisance blooms may be characterized by objectionable taste and odour (Dzialowski et al., 2005) and the production of toxins (Codd, 2000; Kanoshima et al., 2003). Understanding factors controlling undesirable algal growth in coastal areas is an important step towards developing effective management strategies for sustaining the "health" of sensitive coastal waterbodies (Elser et al., 1990; Dodds et al., 1993).

It is well known that Nitrogen (N) and Phosphorous (P) are essential macro elements for phytoplankton growth, with nutrient requirements for phytoplankton growth based on the Redfield Ratio of 106C: 16N: 1P (Redfield, 1958). In general, phytoplankton tend to be limited by N in marine waters and by P in freshwater systems (Elser, 1990), however the limiting nutrient for phytoplankton in estuarine/brackish waters may vary seasonally (D'Elia et al., 1986) and depend on nutrient input ratio (Graneli, 1987; Zou et al., 2001).

Numerous studies, mostly marine, have shown that urea is an important source of N for phytoplankton (Rees and Syrett, 1979; Kristiansen, 1983; Lund, 1987; Mitamura et al., 2000), often ranking equal or higher in importance than nitrate (Kudela and Dugdale, 2000). Generally, urea contributes only a small fraction of the total dissolved organic N pool in coastal and estuarine waters (McCarthy, 1972; McCarthy, 1977; Gilbert et al., 1998). Urea (CO(NH)2) serves not only as a N source but also as a readily available source of carbon for photosynthesis (Berman and Chava, 1999). Gilbert et al. (2005) suggest that urea could potentially trigger the development of harmful algae blooms. They found that in the Chesapeake Bay and Coastal Bays of Maryland, USA, urea was positively correlated with the outbreaks of several harmful algae bloom species. Sources of the urea were largely ascribed to the increase in use of urea in fertilizers on farms within the catchment area.

The following study is an attempt to examine experimentally the response of natural phytoplankton assemblages in a brackish water system to nutrient enrichment by urea and the other dissolved N forms. Understanding phytoplankton community
growth response to nutrient loading is important since phytoplankton biomass and species dominance can be used as a bio-indicators for aquatic system health (Bianchi et al., 2003; Lepisto et al., 2004; Verlecar et al., 2006). This has implications for interpreting existing phytoplankton and water quality data sets and for establishing appropriate management strategies for sensitive water bodies in developed catchments.

The study was conducted using phytoplankton collected from the Myall Lakes, a near pristine but nutrient-sensitive brackish water body on the coast of New South Wales, Australia. We asked the following questions:

1) Do different forms of Nitrogen (NH₄⁺, NOₓ, urea) lead to similar growth patterns of individual taxon and to the development of similar phytoplankton assemblages in the lower Myall Lakes region (The Broadwater)?

2) Over what time frame do phytoplankton taxa respond to nutrient enhancement?

3) How does the phytoplankton response to urea and other N sources vary spatially within the lake?

2. Methods

2.1 Field sampling and experimental design

The nutrient response study was conducted during autumn and early summer in 2005, with two experiments conducted in each season. Phytoplankton samples were collected from surface waters (0-1 m) at three sites in Bombah Broadwater, Myall Lakes System, New South Wales Australia. The study utilized phytoplankton collected from a site near the mouth of the upper Myall River, a site in the middle section of Bombah Broadwater and a site in the northeast area of Bombah Broadwater, near Bombah Point.

During April and May (autumn), surface water samples (0-1m, depth integrated), from the three sites (River Mouth, RM; Mid Broadwater, MB and Bombah Point, BP) were collected in 22 L carboys (two for each site). The carboys were transported under dim light to the laboratory at the Ourimbah Campus and held overnight at room temperature (about 20°C). The following morning, the contents of the carboys for each site were gently mixed and transferred to 12 x 2L incubation containers. Nutrient treatments differed slightly between autumn and early summer
experiments. In autumn, nutrient treatments included a control group (no additional nutrients), and treatments enhanced with nutrients: NH₄+P, NO₃+P and Urea+P. Nutrients were added once, just prior to incubation. Concentrations at the start of the incubations were 0.4 mg/L-N for nitrate, ammonia and urea; phosphate additions either met or exceeded the requirement for phytoplankton growth, based on the Redfield weight ratio. The analysis of the nutrient samples was conducted at the NSW EPA nutrient laboratory in Lidcombe. Water quality variables (temperature, conductivity, salinity, dissolved oxygen, pH and turbidity) were recorded at all sites in the field and at 2 day intervals over each 10 day incubation period, using a calibrated Yeo-Kal water quality analyser.

All treatment replicates were incubated for 10 days in a large box fitted with a set of 10 Osram 58W Biolux light tubes and a sheet of fly screen to simulate sunlight at approximately 250 μmol photons m⁻² s⁻¹ (or 250 PAR, Photosynthetically Active Radiation). Daily photoperiod was set at 10 hours light and 14 hours darkness and the temperature in the incubator was maintained within 2°C of field temperatures.

In vivo chlorophyll a fluorescence was measured daily in all samples using a Turner Designs SCUFA®(Self-Contained Underwater Fluorescence Apparatus) submersible fluorometer, calibrated for chlorophyll a concentration. Samples for analysis of acetone-extracted chlorophyll a (50 ml) and phytoplankton counts (110 ml) were removed prior to incubation and at 2 day intervals during incubation.

2.2 Phytoplankton biomass

Fifty ml water samples were filtered into 0.22 μm polycarbonate filters (Osmonics) and the filters stored in 6 ml vials –20°C in a freezer for 24 hours. Then later, chlorophyll samples were extracted with 5 ml of 90% acetone and frozen for over 48 hours. Extracted chlorophyll a (chl a) was then measured spectrophuorometrically (Hitachi F-3000). The chlorophyll fluorescence was measured daily and prior to incubation using SCUFA submersible fluoro meter.

Water samples of 110 ml from each treatment replicate were preserved using approximate 1 mL Lugol’s iodine solution for later identification and enumeration. Similar with another variable (chlorophyll a), cell abundance and dominant taxa were determined prior to incubation and for 2 days interval. The upright light microscope was used to count phytoplankton with methods as described by (Lund and Le Cren, 1958; Hotzel and Croome, 1999). Identification guides used to identify phytoplankton
include (Prescott, 1978; Entwisle, 1997; Sonneman, 2000). Identification were made to genus level in most cases as identification of all individuals to species was often impossible and/or excessively time consuming.

2.3 Data analysis

All variables (chlorophyll fluorescence, extracted chl a, cell abundance and total dominant groups) were tested by two-way ANOVA for repeated measures to determine the effect of nutrient treatment to phytoplankton growth. When Levene’s tests revealed variance were heterogeneous, so raw data for all variables were transformed to log (n+1) (Underwood, 1997). To differentiate means, a Least Significant Difference (LSD) multiple comparison procedure was performed. To determine the pattern of variable (Chl fluorescence, chlorophyll a, and total cells and taxa abundance) between nutrient treatments among sites for each experiment event, overall means from LSD results were plotted. All analyses were performed using the SPSS version 11.0 software program.

To examine nutrient treatment effects on phytoplankton assemblages, multivariate statistical analyses were performed on log (x+1) transformed data using PRIMER V 5.2 non-metric multidimensional scaling (nMDS) procedures software. It is based on a Bray-Curtis similarity matrix, which is used to represent assemblages' composition in two-dimensional space. Points that are close together represent samples that are very similar in species composition, points that are very far apart correspond to very different communities (Clarke, 1993).

3. Results

3.1 Phytoplankton biomass (Chl fluorescence, extracted chl a and cell abundance)

Chlorophyll fluorescence and extracted chl a concentration initially were low but increased greatly (bloom conditions within in 2-6 days) in all nutrient treatments, except for control (no nutrients) (Fig 2a-f). Peak chl was reached on Days 6 – 8 in autumn experiment (Apr and May’05). In all experiment occasions, the peak values of chlorophyll fluorescence and chl a concentration in N+P treatments for all sites were a greater than 15 µg/L, with most experiments exhibiting peaks >40 µg/L. The range of peak values for chl fluorescence and chl a concentration were between 45 – 210 µg/L and 25 – 350 µg/L, respectively. This result indicated that urea and the
others dissolved N forms (with P in excess) in our experiment stimulated bloom condition (Fig. 2a-f). However, chl fluorescence and chl a concentration for most cases of the experiment decrease gradually after about 5-8 days until the incubation of samples was terminated (10 days).

Results of two-way ANOVA analysis showed that chlorophyll fluorescence varied significantly between nutrient treatments only in May’05, with LSD tests confirming that the treatment with Urea+P addition exhibited higher chlorophyll fluorescence than the other nutrient treatments (Table 1). Extracted chl a concs differed significantly with N form added during both autumn months (Apr and May’05), with chl a being significantly higher with Nitrate+P than Urea+P and NH4+P in Apr’05 (Table 1); in May Urea+P showed a significantly greater in chl a concentration than the other dissolved N forms.

Chlorophyll fluorescence and chl a levels were higher in N+P treatments than nitrate or urea-enriched treatments only (Fig 2). There were significant interaction of chl a concentration observed between nutrient treatments (N+P addition) and sites in Apr experiment only (Table 2). This result indicated that there was no consisting in response to N type with sampling site. One-way ANOVA analysis (Apr) showed that chl a concentration differed significantly between N+P treatments at BP and MB.

In Comparing sites, when phytoplankton biomass differed significantly, chlorophyll fluorescence and chl a were greater at MB in the autumn experiments than at the other sites (Table 1).

Cell abundance (Fig. 2a-f) peaked at Day 6 or 8 in autumn months and at Day 6 or 10 in early summer months. The average of peak value of cell abundance in early summer is higher than in autumn experiments, with value for autumn and early summer account for 132,000 cell/ml and 221,000 cell/ml, respectively. However, addition of different dissolved N forms did not result in significant difference of cell abundance for most experiment event, except for May experiment (Table 1), with Urea+P treatment exhibited higher response in total cell abundance than the other dissolved N forms. The dominant taxa in the May experiment was Microspora. The variability in cell abundance within site was large. Nutrient enrichment caused a significant difference of cell abundance between sites and treatments for autumn Fig 2 and Table 2 showed that BP has a consistently higher in cell abundance than the other sites. However, there was no significant interaction of cell abundance between
nutrient treatments (either N+P or N addition only) and sites for all experiment events (Table 1).
Figure 1. Chlorophyll fluorescence and chlorophyll a concentration (Mean±SEE, n=3) between nutrient treatments over period of incubation at all sites (note that Y-axis has a different scale).
Table 1. Mean square and significant value derived from ANOVA repeated measured of chl fluorescence, chlorophyll a conc total cell, Cyanophyceae, Chlorophyceae and Bacillariophyceae abundance (log (n+1) transformed) for autumn experiment (Apr and May/05). NB, df degree of freedom: * = P < 0.05; ** = P < 0.01; *** = P < 0.001. RM for Rivernouth, MB for MidBroadwater and BP for Bombah Point.

<table>
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<tr>
<th>Date</th>
<th>Source of variation</th>
<th>df</th>
<th>Chl fluorescence</th>
<th>Chlorophyll a</th>
<th>Total cells</th>
<th>Cyanophyceae</th>
<th>Chlorophyceae</th>
<th>Bacillariophyceae</th>
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<td>0.07***</td>
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<td>0.24 ns</td>
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<td>0.05***</td>
<td>0.05 ns</td>
<td>0.06 ns</td>
<td>0.07 ns</td>
<td>0.57 ns</td>
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<td>0.01</td>
<td>0.04</td>
<td>0.14</td>
<td>0.08</td>
<td>0.54</td>
</tr>
<tr>
<td>May</td>
<td>Treatment</td>
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<td>0.45*</td>
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<td>0.26**</td>
<td>0.36**</td>
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<tr>
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<td>2.13***</td>
<td>10.03***</td>
<td>27.09***</td>
<td>4.46***</td>
<td>4.29***</td>
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<td>Treatment * Site</td>
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<td>0.01 ns</td>
<td>0.25**</td>
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<td>0.062</td>
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Figure 2. Cell abundance (Mean±SEE, n=3) between nutrient treatments over period of incubation at all.
3.2 Phytoplankton class abundance

The main classes of phytoplankton showed varying responses to nutrient treatment. Cyanophyceae, Chlorophyceae and Bacillariophyceae phytoplankton classes were the most well represented for all experimental events. Significant changes in phytoplankton community composition were detected between nutrients treatments in the May experiment (Table 1). Urea+P addition significantly enhanced the abundance of Cyanophyceae compared to NO₃+P and NH₄+P in May (ANOVA, P<0.05). Furthermore, Cyanophyceae was the most abundant class at BP for all set of experiments (Table 1). However, there was no significant interaction between nutrient treatments and sites for Cyanophyceae during any experiment occasions.

There was no consistent trend in Chlorophyceae abundance between nutrient treatments among sites. A two-way ANOVA analysis showed that Chlorophyceae abundance differed significantly between nutrient treatments only in May and Dec, with Urea+P and NH₄+P exhibiting a higher Chlorophyceae abundance than NO₃+P in autumn (May). In May, there was a significant interaction between nutrient treatments and sites for Chlorophyceae. One-way ANOVAs result (data not shown) indicated that Chlorophyceae abundance was significantly difference between nutrient treatments in BP and MB. NH₄+P and Urea+P showed a greater of Chlorophyceae abundance than NO₃+P at BP and MB (data not shown).

There was significant interaction between nutrient treatments and sites, with MB being the only site showing a significant difference in Bacillariophyceae between nutrient treatments.

When the significant difference occurred, addition Phosphate stimulate the variation of phytoplankton class abundance between nutrient treatments (Fig. 2), however there is no significant difference of main phytoplankton class abundance (Cyanophyceae, Chlorophyceae and Bacillariophyceae) with nitrate or urea addition only for all experiment events (Table 1).

3.4 Phytoplankton community structure

Non-metric multidimensional scaling (nMDS) ordination of species assemblages between nutrient treatments was prepared for each experiment and site for day 6 (represents condition at near peak cell abundance) using all available replicate samples. These ordinations can be seen in Fig 3. The ordination shows
that there is a clear separation of species assemblages between nutrient treatment for most months sampled and sites, except for BP in May and RM in Apr.

For RM in May there was a distinct clustering of species assemblages; with clear separation between nutrient treatments (Fig. 3 b-d). ANOSIM result gave a Global R value ranged between 0.7 and 0.8 indicating a highly significant difference (P<0.05). In contrast, there was no clear clustering of species assemblages between nutrient treatments for BP in autumn experiment (May only), with the sample points was dispersed. The Global R value of 0.4 indicated that there is no significant difference of species assemblages between nutrient treatments (P>0.05). While species assemblages in MB for all experiment events showed a clear separation with N treatment with a significant difference of species assemblages (P<0.05) and ANOSIM result gave a the Global R value ranged in between 0.7 and 0.9.

Dissimilarity percentage of species assemblages varied between pair of nutrient treatment. However, SIMPER result showed that nutrient treatment in autumn (Apr and May) had less effect on dissimilarity of species, with percentage of species dissimilarity ranging between 12 and 36% (Table 2).

SIMPER result showed that each site and experiment event tends to have different dominant taxa, e.g. Palmella and Coelastrum (Chlorophyceae) were dominant taxa for RM, while Ulothrix and Gloeocystis (Chlorophyceae) were abundant at MB. Furthermore, Merismopedia (Cyanophyceae) was predominant taxa in BP (Table 3). In May'05 Microspora (Chlorophyceae) was the most abundance taxa and present at all nutrient treatments and all sites.
Figure 3. nMDS ordination plots for species of phytoplankton between nutrient treatments for each site at Day 6 of
Table 2. Three most of taxa common for all nutrient treatments at Day 6 for all sites and all experiment events. *** = > 20,000 cells/ml, ** = 10,000-20,000 cells/ml, * = < 10,000 cells/ml. Bolded genera has cell abundance more than 50,000 cells/ml. RM for Rivermouth, MB for MidBroadwater and BP for Bombah Point.

<table>
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<th>Season (Date)</th>
<th>Nutrient treatments</th>
<th>RM</th>
<th>MB</th>
<th>BP</th>
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<td>Autumn (Apr)</td>
<td>NO₃-P</td>
<td>Oocystis *</td>
<td>Ulothrix***</td>
<td>Merismopedia***</td>
</tr>
<tr>
<td></td>
<td>Coelastrum*</td>
<td>Microspora**</td>
<td>Chroococcus***</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Palmella*</td>
<td>Chroococcus*</td>
<td>Aphanocapsa***</td>
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</tr>
<tr>
<td></td>
<td>NH₄-P</td>
<td>Palmella *</td>
<td>Ulothrix ***</td>
<td>Merismopedia***</td>
</tr>
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<td></td>
<td>Cryptomonas *</td>
<td>Chroococcus ***</td>
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<tr>
<td></td>
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<td>Microspora*</td>
<td>Coelasperaerium***</td>
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</tr>
<tr>
<td>Urea+P</td>
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<tr>
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<td>Gloeocapsa*</td>
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Table 3. ANOSIM Pairwise tests and SIMPER results for phytoplankton assemblages showing significant nutrient treatment effects (P<0.05) on Day 6. Number of permutations was too low to detect significant differences between pairs of samples. Bolded genera are more numerous in the 1st treatment of the listed pairs. Global R values > 0.800 are considered to show strong differences between treatment pairs.

<table>
<thead>
<tr>
<th>Season (Date)</th>
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<th>Dissimilarity from SIMPER Results</th>
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<td>Global R Statistic</td>
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<td>NO₃-P vs U-P</td>
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4. Discussion

The nutrient response experiments were conducted to examine natural phytoplankton community growth and assemblage structure under conditions of enrichment with urea and other forms of dissolved nitrogen. In this bioassay experiment, water samples were enriched with dissolved nitrogen and phosphorus only. It was assumed that micronutrients (eg. Vitamins, Si, Mn, Fe, Mg, Ca) in the Myall Lakes system were not a limiting factor for phytoplankton growth, at least during the first week of incubation. This approach is comparable to numerous other phytoplankton growth studies; in these, micronutrients were not supplied (D'Elia et al., 1986; Dodds et al., 1993; Ayukai, 1996; Bernhard & Peele, 1997; Ault et al., 2000; Blomqvist, 2001; Cadee, 2003; Ornolfsdottir et al., 2004a; Carter et al., 2005; Dzialowski et al., 2005).

4.1 Water quality and chlorophyll growth response

Bombah Broadwater exhibits highly variable water quality, in particular salinity and nutrient concentrations, due to the influence of salt-water inflow and nutrient loading from the catchment area. In this set of experiments, we simulated various nutrient conditions and quantified the response of the natural phytoplankton assemblage over a 10 day incubation period.

The phytoplankton of Bombah Broadwater showed a significant response in biomass (chlorophyll fluorescence and chlorophyll a) to the addition of urea and other dissolved nitrogen forms, when phosphorous was in excess. During all experimental occasions, phytoplankton biomass was significantly higher in dissolved N+P treatments than in control and N-only treatments. Ornolfsdottir et al. (2004a) observed that phytoplankton growth rate with N+P addition was double and triple that of control treatments (without N or P) following nutrient addition.

The chlorophyll growth response was slow with chlorophyll a increasing only after 24 hours following nutrient addition. Slow response of phytoplankton growth to nutrient enrichment in this study was possibly due to the dominance of small-celled cyanobacteria. The current study indicates that when the phytoplankton community is dominated by large-celled phytoplankton (e.g. Microspora in May experiment), the growth response of phytoplankton to nutrient enrichment is faster. This finding supports previous studies which report strong correlations between size and growth rate of algae, with large uni-cellular algae (e.g. diatoms), showing a greater growth
rate per unit volume than small-celled taxa (Irwin et al., 2006; Nielsen, 2006), in particular when nutrients are in surplus (Ornolfsdottir et al., 2004a).

The current study demonstrated that urea-only and nitrate-only enrichments in the early summer experiments yielded significantly different responses for chlorophyll fluorescence and chlorophyll a concentration, but not for cell abundance. Graziano et al. (1996) noted that the main response of cyanobacteria to nitrogen addition was an increase in chlorophyll a while cell numbers remained relatively low. The Broadwater study also showed that Cyanophyceae chlorophyll responded positively to N-only treatments, compared to other phytoplankton groups (Classes). Enrichment with both urea and P resulted in greater phytoplankton growth than with N-only treatments. This was not surprising as it is well known that simultaneous N and P enrichment in lakes nearly always produces greater growth responses of phytoplankton than with single nutrient (N or P) enhancement (Elser et al., 1990; Dzialowski et al., 2005).

Although urea treatments exhibited higher abundances of some taxa, at some times, there was no consistent phytoplankton growth response to urea compared to other dissolved nitrogen forms. This finding indicates that phytoplankton in the Myall Lakes are able to use all DIN (Dissolved Inorganic Nitrogen) forms efficiently. Other factors may be responsible for site-to-site differences observed in the response to the different treatments. Ornolfsdottir et al. (2004b) report that the variability in phytoplankton community structure and biomass in Galvestone Bay, USA, was in response to nutrient pulses, which are modified by the physical (temperature and light), chemical and biological (grazing and phytoplankton community) characteristics of the Bay.

### 4.2 Cell response

The only significant differences observed in cell abundance, among the various nutrient treatments, occurred in May, when Urea+P addition mediated a greater response in phytoplankton growth than NO₃+P or NH₄+P. At this time, the phytoplankton assemblages at all three sites were dominated by Cyanophyceae (Merismopedia, Gloeocapsa) and Chlorophyceae (Microspora, Ulothrix). This finding is in line with Kristiansen (1983) who found that urea stimulated the highest maximum uptake rate by marine phytoplankton at Oslofjord, and that algae showed better growth on urea than on NH₄. The current study showed significantly higher cell abundance with urea than with nitrate, and may suggest that the N in urea is
assimilated at a faster rate than the N in nitrate. Ammonia might be preferentially taken up over nitrate because less metabolic energy is required to assimilate the already-reduced ammonium form (Richardson, 2001).

The variability in phytoplankton growth shown by N form was less than that shown for the different sites, and was reflected by the significant differences among sites in both biomass and assemblage structure, in all experiments. Spatial variability is partly affected by rainfall events and site-specific differences in water chemistry, including light availability and salinity. Nutrient availability at each site would naturally vary due to relative distances of sites from the upper Myall River, and their proximity to saline intrusion (Lower Myall River). In addition, it is known that phytoplankton growth response to nutrient enrichment varies with season, river flow regimes and the prior nutritional status of phytoplankton (D’Elia et al., 1986, Richardson et al., 2001). Salinity also contributes significantly to phytoplankton biomass and assemblage structure for Mid Broadwater samples (Redden and Rukminasari, 2008). Flamer et al. (1998) showed that in Perdido Bay, Florida, the response of phytoplankton to experimental N and P addition was correlated with both season and salinity gradient.

4.3 Phytoplankton assemblages

Tilman et al. (1982) and Sommer (1989) demonstrated that increases in nutrients in natural waters results in changes in the community structure of phytoplankton. The current study revealed that enrichment of different forms of dissolved nitrogen stimulated the growth of phytoplankton taxa in ways that resulted in significant differences in species assemblages among treatments for most sites. For the six phytoplankton classes that were most common, cell abundance was higher with urea and ammonia enrichment than with nitrate addition, on most occasions. This result indicates that there may be group-specific preferences for uptake of urea and ammonia. While the mechanism by which algae assimilate urea is still poorly understood (Siuda & Chrost, 2006), the current study indicated that most, if not all, of the phytoplankton groups (classes) assimilate and utilise urea. Siuda and Chrost (2006) report that cyanobacteria can utilize urea as NH₄⁺ following hydrolyzation of urea via enzymatic processes, and that some algal species (especially green algae) produce ATP-dependent urea amidolyase; some diatoms and dinoflagellates produce intracellular urease. Although, we did not investigate the physiology of urea uptake and assimilation by phytoplankton, this would be an
interesting direction for further research in coastal systems which receive large urea inputs from the catchment area.

Olden (2000) and Richardson et al. (2001) report that phytoplankton have different capabilities for nutrient uptake, which may result in species-specific or group-specific responses to different nitrogen forms and concentrations. Phytoplankton responses to differing forms of nitrogen may be expressed as differential growth rates, that are manifested in the prevailing phytoplankton assemblage structure (Stolte et al., 1994; Pickney et al., 1998; Richardson et al., 2001).

Large-celled phytoplankton tend to be more abundant and to dominate under high nutrient conditions, in contrast to small cells, whose relative abundance tend to decrease with increasing nutrient supply (Irwin et al., 2006). The current study similarly found that nutrient enrichment leads to changes in community structure and to the dominance of large cells over small cells. The dominant taxa in the May experiment were large-celled Microspora (Chlorophyceae), which replaced small-celled taxa (e.g. Merismopedia, Chroococcus) in dominance. Chlorophyceae appeared to be more competitive than Cyanophyceae when nutrients were in surplus. This finding is in agreement with Mitrovic et al. (2001), who reported that Chlorophyceae under nutrient-rich conditions tend to replace Cyanophyceae in dominance.

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O-PD16

STUDY OF HIGH ENERGY DIET ON CYNOMOLGUS MONKEYS (Macaca fascicularis) TO DEVELOP OBESE

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Obesity is a primary predisposition for diseases such as metabolic syndrome (such as insulin resistance, blood lipid abnormality, and hypertension), type 2 diabetes, and cardiovascular disease. The effort to overcome obesity is needed by understanding develop of obesity. Therefore, it is urgent to carry out preclinical trials by using an obese animal model. The objective of this research was to study the high energy feed with animal fat and carbohydrate to produce obese cynomolgus monkeys (Macaca fascicularis). Animals used in this study were 15 adult males divided equally into three treatment groups; 1) receiving feed A consist of beef tallow without egg yolk (energy 4,207 cal/g, fat 19.62%, and starch 60.34%); 2) receiving feed B consist of beef tallow and egg yolk, (energy 4,207 cal/g, fat 19.62%, and starch 60.34%) 3) receiving feed C, monkey chow as control (energy 4,330 cal/g, EE 5.55%, and NFE 51.38%). Measurement were carried out every four weeks for body weight gain, sitting height gain, hip diameter, abdominal thickness, body mass index, feed and nutrient consumption, and feed digestibility. Results showed that there was significant (p<0.05) body weight gain in week- 4 and 8. Based on the average difference test, it was known that feed B was resulting the highest (p<0.05) body gain compared to feed A and C. For the body measurement, the monkeys with feed B were bigger (p<0.05) than feed A and C. Therefore, it was considered as obese in terms of body mass index. Protein digestibility in feed A and B was lower (p<0.05) compared to C. However, digestibility in dry matter, fat, starch and energy were about 90%, meaning that the rations were considered good in making obese monkey. The feed formula containing tallow and egg yolk could increase body weight resulting in a positive correlation toward body measurement and body mass index.

Key words: obesity, cynomolgus monkeys, monkey chow, body measurements, and digestibility

not presented
O-PD17

DEVELOPMENT AND DISTRIBUTION OF LATICIFERS IN PHYSIC NUT (*Jatropha curcas* L.)

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Nowadays, physic nuts (*Jatropha curcas* L.) are widely growth. Its due to seed oil is used for biodiesel. Moreover, the plants contain secondary metabolites accumulated in secretory structure. Physic nuts belong to Euphorbiaceae which have articulated and non-articulated laticifers. The articulated laticifers consist of longitudinal chain of cells, while non-articulated laticifers are initiated as a single cell which elongate into tube-like structures. The laticifers contain latex, rubber, starch, protein, alkaloid, terpenoid or resin. Laticifers often form a network just behind the phloem and extend into the cortex, pith and epidermis. This research was to analyze the growth and distribution of laticifers at various ages of physic nut.

Methods used in anatomical preparation was free-hand section (semi permanent). Samples used were 7, 9, 12, 14, 18, 23 and 27 days old. The data taken from longitudinal and transverse sections of hypocotyls and stems. The development of laticifers were analyzed descriptively, while the distribution of laticifers were analyzed using ANOVA followed by LSD with 5% significance rate.

Longitudinal section of hypocotyls (7 and 9 days old) showed non-articulated laticifers, short and dark-red color. At the age of 12 and 14 days, laticifers has longer form, non-articulated structure and bright-red color. The laticifers start to extend into the cortex. In the stem of 18, 23 and 27 days old, laticifers have longest form, articulated and non-articulated structures and transparent color. The transverse sections of hypocotyls and stems show that the laticifers distributed in the cortex and phloem, while the number increase up to the age of 18, then decrease along with the increase of ages (27 days old).

**Keyword:** Laticifer, Articulated, Non-articulated, *Jatropha curcas*.

**INTRODUCTION**

*Jatropha curcas* (physic nut) is a drought-resistant shrub or tree belonging to the Family Euphorbiaceae. It is well adapted to arid and semi-arid condition and often used for prevention of soil erosion. The plant is native of North America but now thrives well in Africa and Asia (1). The seeds are black, oval in shape and rich of fixed oil, which can be used as a diesel substitute (biodiesel). The oil content of jatropha seed is reported to be in the ranges from 30-50% by weight of the seed and
ranges from 45 to 60% weight of the kernel itself. They are used also in medicines, soap and cosmetics manufacture in various tropical countries (8).

The jatropha tree has several beneficial properties such as its stem is being used as a natural tooth paste and brush, latex from stem is being used as natural pesticides and wound healing, and leaves show antileukemic activity (2). Chemically, leaves contain α-amyrin, stigmasterol, campesterol, β-sitosterol and its derivatives. Stem, fruit and its latex contain of glycoside, tannin, phytosterol, flavonoid and steroid sapogenin (3). Various above substances accumulate in a mixture and secreted in specialized structures or cells termed secretory structures or secretory cells. There are three kinds of secretory structure, i.e. external structures, internal structures and laticifers (4).

Laticifers are specialized cells with exudates, a latex or milky sap. Although most often milky in color, this sap may also be yellow, orange, red, brown or even colorless (9). The latex consist of various chemical substances, which are useful economically and biomedicinally, such as starch, protein, cardenolides, alkaloids, terpenoids and natural rubber. Laticifers provide an effective location to store defense metabolites, although not all latex-bearing plants accumulate bioactive natural products. Ecophysiological studies have shown that latex are vital for the defense of plants against insects (6).

There are two phylogenetically unrelated forms of laticifers are known, non-articulated and articulated laticifers. The non-articulated laticifer is initiated as a single cell in the plant embryo, which elongate into tube-like structures and never develop the cross wall, while the articulated laticifers are consist of longitudinal chains of cells in which the walls separating the individual cells, become perforated or completely removed (5). Non-articulated laticifers are classified into branched and unbranched types depending upon whether the elongation, growing cells develop lateral extension. Similar to non-articulated, articulated laticifers also classified into branched and unbranched types, and may be either non-anastomosing or anastomosing. The activity of the vascular cambium may contribute additional laticifers (4).
The earliest appearance of laticifer primordial cells is in the immature embryo at a stage when the developing cotyledons have grown. The primordial first arise in the cotyledons and in the cortex of the hypocotyls. Laticifers can be distinguished from the adjacent tissues by their smaller size and more darkly stained cytoplasm (5). The laticifers occur in all parts of the plant. They occur in very large numbers in the cortex, pith, xylem and phloem regions of the stem. In the leaf, they occur mostly in the midrib region and near the veinlets. Laticifers are more concentrated near the vascular elements (10). *J. curcas* belong to the Euphorbiaceae which its vegetative organs have articulated and non-articulated laticifers (4). The purpose of this research was to analyze the growth and distribution of laticifers at various ages of *J. curcas* stem, from 7 to 27 days old.

**MATERIALS AND METHODS**

Seeds of *J. curcas* used were grown in the soil and placed in a greenhouse. Samples for the development of laticifers were collected from 7, 9, 12, 14, 18, 23 and 27 days old seedlings.Slides were prepared using *free-hand section* method (11). The slides were examined under the microscope and photographed using digital camera.

The data taken from the slides of longitudinal and transverse sections of stems. The development of laticifers were analyzed descriptively, while the distribution of laticifers were analyzed using ANOVA followed by LSD with 5% significance rate.

**RESULTS AND DISCUSSION**

Laticifers were observed from the slides of 7 up to 27 days old seedlings. Fig. 1. showed that laticifers occur in all slides, particularly in cortex region and near the vascular element (phloem).
Fig. 1. Longitudinal sections of *J. curcas* seedling. A). 7 days old seedling, B). 9 days, C). 12 days, D). 14 days, E). 18 days, F). 23 days and G). 27 days (arrow showed the location of laticifer).

Longitudinal section of seedlings (7 and 9 days old) showed non-articulated laticifers, short and dark-red color, located at the near phloem and cortex. At the age of 12 and 14 days, laticifers getting longer, non-articulated structure and brighter-red color. In the age of 18, 23 and 27 days, laticifers have longer form, articulated and non-articulated structures and orange color. Laticifers in the young stem (hypocotyl) has more dark color than the old one. According to Lee & Mahlberg (1999), the dark color of laticifers in the young seedling because of the density of cytoplasm which contained large nuclei with euchromatin and various organelles, including ribosomes, rough endoplasmic reticulum (RER), mitochondria and proplastids. Metabolism at the young tissue (apical region) was in a highly active state, supported the growth of laticifer tips. Differentiation of non-articulated laticifers is accompanied by nuclear divisions resulting in a coenocytic condition.
Evert (2006) added that the non-articulated laticifer initials appear in the plane of embryo cotyledons. When an embryo develops into a plant, the laticifers grow continuously penetrate the meristematic tissues formed by the apical meristems. At the nodes of stem, laticifers would enter the leaf and pith via the leaf trace gap. Laticifers continued to elongate by intrusive growth following the growth and differentiation of tissues. On the other hand, the initials of articulated laticifers occur after the seed begins to germinate. The initials appear in the protophloem region of the procambial tissue or in both the cotyledons and the hypocotyls axis. When the cotyledons are well developed at this stage, the initials are arranged in more or less discrete longitudinal rows and the formation of lateral protuberances results in a anastomosing system.

All samples of *J. curcas* showed that laticifers found in the greater number in cortex and near the phloem than the other tissues. According to Pickard (2007), it is seems that laticifers has been attributed to symplastic transport of nutrients from phloem to parenchymal cells adjacent to the laticifer, and then followed by apoplast. The nutrients are taken up into the laticifer by sugar symport system and turned into latex.

Besides the development of laticifer, this study analized the distribution of all secretory cells in transverse sections of stems. The data were analyzed using ANOVA followed by LSD with 5% significance rate. Result showed that the secretory cells were distributed in the cortex and phloem. The number of secretory cells increase up to the age of 18, then decrease along with the increase of ages (27 days). Fig 2. showed the secretory cells number in each development stages. Stem was divided into three sections, there are proximal, medial and distal regions.

![Graph showing mean of secretory cells in different stem jatropha age](image)

**Fig. 2.** Secretory cells of *J. curcas* stem at the age of 7, 9, 12, 14, 18, 23 and 27 days old plant.
It seems that the reduction of secretory cells number in the stem after the ages of 27 due to the secondary growth of the stem affected the reduction of cortex region. From the results and discussion, it can be concluded that the development of laticifers are started by the changes of color and shape (getting dark, bright and longer form) with the increase of ages. The number of secretory cells increased up to the age of 18 and then reduced with the increase of ages.

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O-PD18

2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN (TCDD) INCREASE DESMIN EXPRESSION IN MOUSE FETUSES PALATAL TISSUE

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The pollutant, 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) induce cleft palate in mouse embryo. In general, the toxic responses generated by TCDD are based on its ability to modulate the expression of multiple genes. In the present study, we investigated the effect of TCDD on desmin expression in mouse fetuses palatal tissue. Swiss-Webster pregnant mice were dosed with either 10 µg TCDD /kg body weight or placebo on gestation day (GD) 11-12. Immunohistochemistry technique was used to examined the expression of desmin protein on GD 13 and 15. The result showed, TCDD significantly increased desmin expression in palatal tissue, especially in the area of medial edge epithelium (MEE). It seems that accumulation of desmin protein in MEE before palatal fusion may caused tightening of epithelial junction through the formation of more desmosomes, so as to prohibit MEE cell dissociation which is prerequisite for the palatal shelves fusion.

Key words: TCDD, desmin, palatogenesis, cleft palate

INTRODUCTION

TCDD (2,3,7,8-Tetrachlorodibenzo-p-dioxin) belongs to dioxin group, an unwanted byproduct chemicals generated by many industries and combustion processes. Dioxin is highly toxic, chemically persistent, and widely distributed pollutant that is teratogenic in mice, where it induces cleft palate.¹ ² Cleft palate is congenital fissure in the roof of the mouth, resulting from incomplete fusion of palatal shelves during embryonic development.

Mechanism of secondary palate development, or palatogenesis, has been described elsewhere.³ ⁴ ⁵ In brief, palatogenesis consisted of three stages: stage 1, formation of bilateral palate primordia from the maxillary processes and their subsequent vertical growth on either side of the tongue; stage 2, reorientation of palatal shelves growth from a vertical to a horizontal orientation toward their last position above the dorsum of the tongue (palatal shelves elevation); and stage 3, contact of opposing palatal shelves with each other and their fusion in the midline of
mouth roof. Cleft palate may be resulted from disturbance of processes at any stage of palatogenesis.3

TCDD induces cleft palate either by altering palatal shelves growth,6 delaying shelves elevation,7 or interfering palatal fusion.8,9 Several molecular mechanisms have been proposed for TCDD-induced cleft palate, including the effect of TCDD in modulating the expression of growth factor genes in palatal shelves. Abbott and Birnbaum10 suggested that modulation of TGF-α expression play an important role in TCDD-induced cleft palate. TGF-α suggested to not allow medial edge epithelial (MEE) cells to migrate, death, and transdifferentiate according to their normal developmental program. In this study, we examine the effect of TCDD on desmin expression in mouse embryo palatal shelves. Desmin has been reported as an intermediate filament protein which in embryonic development involved in cellular differentiation, its synthesis regulated by TGF-α.11

MATERIALS AND METHODS

TCDD (CAS#1746-01-06) was obtained from Neosyn Lab., USA, in > 98 % purity. Doses were prepared by dissolving TCDD in Dimethylsulfoxide (DMSO), and the solution was then diluted with sesame oil to adjust the concentration. Pregnant Swiss-Webster mice were obtained by overnight mating. The day of vaginal plug was designated as day 0 (Gestation Day 0; GD 0). Animals were maintained during pregnancy in animal house facilities (Unit Pengembangan Hewan Percobaan UGM) with Japfa Comfeed chicken par G pellet and ad libitum tap water. Two dams were treated with 10 μg/kg b.w. TCDD on GD 9-10, while two others only received sesame oil (control). Treatments were administered orally by gavage. On GD 13 the animals were anaesthetized with ether and then killed by cervical dislocation. Fetuses were removed from the uterus. Two fetuses from each dam were selected for immunohistochemistry preparation. They were decapitated, fixed in neutral formaline and embedded in paraffin according to routine histological procedure.12 Sections of 5 μm thickness were cut with a microtome and mounted on poly-L-lysine-coated glass slides for immunohistochemistry staining.

Paraffin embedded section were cleared with xylene and washed in Posphat Buffer Saline. To quenched endogenous peroxidase, the section were incubated in 3% H2O2 for 7 minutes. After washing with PBS, section were further blocked with 5% Bovine Serum Albumin for 15 minutes at room temperature (RT), and incubated
firstly with 1/100 rabbit anti-desmin (Sigma D8281) for overnight at 8 °C, then with biotinylated goat anti-rabbit IgG (Sigma B8895) for 30 minutes at RT, and finally with Extravidin-conjugated peroxidase (Sigma E-2886) for 20 minutes at RT. The sections were developed in 3,3'-Diaminobenzidine (Sigma D-5637) under microscope control, and counterstained with Meyer's haematoxylin. Positive cells in each section were visually evaluated, and the result were expressed as proportion of stained cells in palatal shelves area. Sections were scored according to the following criteria: 1) low expression, proportion of positive cells < 10%, 2) moderate expression, proportion of positive cells more than 10% up to 25%, 3) high expression, proportion of positive cells more than 25% up to 50%, 4) very high expression, proportion of positive more than 50%. Differences of desmin expression score were tested by Kruskal-Wallis test, a probability of less than 5 % was considered significant.

RESULTS AND DISCUSSION

Result showed that desmin protein was expressed in mouse fetuses palatal shelves on GD 13. The expression was found not only in mesenchyme compartment but also in epithelial area, especially in MEE. Expression in control mouse showed a predominantly low level, whereas expression in TCDD treated mouse showed a predominantly moderate level. Data analysis revealed, TCDD significantly increased desmin expression in mouse fetuses palatal shelves (Figure 1, control versus TCDD treated, Kruskal-Wallis test, P < 0.05).

Figure 1. Effect of TCDD on desmin expression in palatal shelves of mouse fetuses
Expression of desmin protein found in mesenchymal area of palatal shelves is not surprising, because desmin is one of the first muscle-specific protein to be detected in the mammalian embryo as a sign of initiation of myogenesis. Desmin expression can be detected for the first time in mouse embryo on GD 8.5, and later in palatal shelves of mouse on GD 11. Higher expression of desmin found in this study indicates that the differentiation of mesenchyme to myoblast accelerates in palatal shelves in TCDD treated mouse. This early differentiation may reduces the rate of cells proliferation in palatal shelves area, because cells that undergo differentiation will leave mitosis and enter resting G0 phase in cell cycle. Altered cell proliferation will lead to slower palatal shelves growth. This may explain why in some of the cleft palate induced by TCDD the feature of palatal shelves is small and diminutive.

Expression of desmin protein in epithelial area, especially in MEE indicates that the role of desmin in palatal shelves is not limited to myogenesis. Desmin expression in MEE may related to its role as structural component of desmosome, the cell junction which facilitates tight epithelial sheet of MEE cells. Desmin involve in anchoring other desmosomal proteins to their cytosolic microfilament network.

MEE of palatal shelves before contact consist of two layers, the inner layer and superficial periderm. Prior to contact, periderm is lost either by sloughing or migrating, while the cells of inner layer form a transitory midline epithelial seam (MES) which is also disappear resulting in direct apposition of mesenchyms. The disappearence of MES was proposed to occur by apoptosis, cell migration, and epithelial to mesenchyme transdifferentiation. In normal development, the final result of palatogenesis is the complete fusion of palate with the continuity of mesenchyme across the opposing palatal shelves. Before MES disappear, epithelial cells must dissociates by a complex process involving extracellular matrix change and desmosome degradation. Accumulation of desmin protein in palatal shelves of TCDD treated mouse may prevent desmosome degradation. If desmosomes stabilize, instead of undergo migration, apoptosis, or transdifferentiation, MEE cells will remain in their position and grow as epithelial cells. This is agree with a study which reported that the etiology of TCDD-induced cleft palate appears to involve hyperplasia and keratinization of MEE cells.

The biological and toxic responses associated with TCDD are mediated by a cytosolic aryl hydrocarbon receptor (AhR). AhR is a ligand-activated transcription
factor which dimerized and translocated to nucleus by aryl hydrocarbon receptor nuclear translocator (ARNT). After binding to TCDD the new heterodimer, the complex form of TCDD-AhR-ARNT, interacts with dioxin-responsive enhancer element (DRE) and activates transcription of target genes located downstream of DRE. Hundreds of gene have been characterized as TCDD target genes. Higher desmin expression in palatal shelves found in this study indicates that desmin may be directly affected by TCDD as one of its target gene. Another possibility is that TCDD indirectly increase desmin expression by modulating the expression of growth factors, especially Transforming growth factor alpha (TGF-α). TGF-α have been reported up-regulates desmin expression during embryonic mouse tongue myogenesis. It is concluded that increasing desmin expression in palatal shelves may be another pathogenesis of TCDD produce cleft palate, either by triggers early myogenesis or by inhibition of palatal fusion through medial edge epithelial sheet stabilization.

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O-PD19

COMPARISON OF NUMBER OF TILLERS BETWEEN THREE HYBRID RICE DIFFERING IN SEEDLING METHODS

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Abstract

Hybrid rice is very important to increase rice production in Indonesia, but it must meet the country's conditions and farmer's cultivation behavior needs. There are many of factors influencing or contributing in rice yield, and one of them is number of bearing tillers. During vegetative development of hybrid rice, number of tiller is varied due to many factors. One of them is methods of planting which is important to get high yield. Basically, there are two types of rice planting, direct seeding of seeds and seedling transplantation. Both methods are used in this research to compare tiller growth of three types of hybrid rice, Cp, Cs and Is. Transplanting rice seedlings has been commonly known to generate an increase in grain yield as a result of high tiller production. The objectives of this research were 1) to compare tiller number of three hybrids rice 2) to compare direct seeding and transplanting method based on number of tillage during vegetative development. Rice seeds were planted in a series of plastic containers filled with wet/muddy soil by direct seeding of seeds. Each type of hybrids was put in six plastic baskets/containers Two weeks later, prepared hybrid rice plantlets were transplanted into the basket according to each type with 15 cm distance from the previous plantlet planted by direct seeding of seeds. Number of tillers was being counted for each of hybrid rice and each planting method. The result showed that there are differences in number of tillers of three hybrids rice. The overall highest number of tiller was Cp with average of 32, whereas the least number was Cs with average of 11. As for number of tillers based on planting methods, direct seeding of seeds gave a higher tiller compare to transplanting method.

Keywords: Hybrid rice, tiller, direct seedling, transplanting
Introduction

Hybrid rice, new plant types and transgenic rice have received a lot of attention, particularly its higher yield of more than one tone of paddy per hectare over the best breed varieties. Many rice producing countries, including Indonesia have showed their interest of developing hybrid rice in order to fulfill the increasing demand of rice. With the increased population each year by ± 2 %, the government of Indonesia has been trying to enhance rice yield.

There are two growth stages are distinguished in rice plant development -- vegetative and reproductive. Tiller development occurs during the vegetative period, and commonly starts within 3 weeks after emergence and continues for about 3 weeks. One of the factors in yield determination is tiller number, thus tiller number is important in rice development particularly to get high yield. High tiller number in rice vegetative development is expected to produce high yield.

As for planting methods of rice plant, there are two: transplanting and direct seeding. Seedlings are prepared by any of the three methods described in Methods of Raising Seedlings. The seedlings are now ready for transplanting in the field. Transplanting is done in one of two methods: random or straight-row. Most publication stated that transplanting is widely used to grow rice, but it is not appropriate for very wide area with limited labours.

To estimate the influence of planting methods of hybrid rice on the tiller development, this present initial experiment investigated the tiller number. In this experiment, tiller number of three hybrids rice were compared and the impact of planting method by direct seedling and transplanting on number of tiller during rice vegetative development were also compared.

Materials and Methods

Materials

Hybrid rice identified as CS, Cp and Is, Urea, NPK, Sidamethrine (pesticide) and muddy soil in plastic container with a diameter of 40 cm were used for this research. Some tools and equipments such as Merck pH paper, pesticide sprayer, weighing scale, garden trowel and fork, thermometer, and hygrometer were also utilized.
Methods of Experiment

1. Plantlet preparation
Three types of hybrid rice identified as Cp, Cs and Is were used in the experiment. Rice seeds were cleaned in running water then soaked in solution of 20 g salt/litter water for 5 minutes to determine good and damaged seeds (Badan Penelitian dan Pengembangan Pertanian, 2008). The good seeds are submerged on the solution, while the damaged seeds are floating on the surface. The seeds then were cleaned in running water to dispose of salted water. Finally, the seeds were placed on wet cloth in a plastic container, which covered with plastic wrap and were incubated in a room temperature for 12 hours. Later, some germinated seeds were planted by direct seeding in the prepared containers filled with muddy soil, while some others were grown as plantlet in 25 cm by 40 cm with 5 cm height plastic containers.

2. Muddy soil preparation
The muddy soil was taken from paddy field in Serpong area. The soil was mixed with urea and NPK and placed in plastic containers. 18 plastic containers with the size of 40 cm in diameter and 60 cm in height were prepared for the experiment. The muddy soil in the containers was put in the containers and was soaked with water until about 5 cm above the soil surface and let it stand in a room temperature for 2 days. Prior to its use, the water must be thrown away, yet the soil must be kept wet.

3. Direct Plantation Method
Prepared germinated seeds are placed on the surface soil in series plastic containers filled with wet/muddy soil. Three seeds of each type of hybrids were planted in each container at about 5 cm from the container’s edge (see Figure 1). The plants were not being irrigated for about three days or until the leaves emerged. After the third leaf was developed, the soil must be soaked with water gradually. The water level must be maintained at 5 cm above the soil, especially when the plant height was above 10 cm (approximately the plant was two weeks old).

4. Transplanting Method
Two weeks after direct plantation, prepared hybrid rice plantlets of 14 days were transplanted into the containers according to each hybrid type with 15 cm distance from the previous plantlet planted by direct seeding (see Figure 1). Number of tillers was being counted for each of hybrid rice and each planting method.
Analysis

When the plants planted by direct seeding method were 46 days and by transplanting method was 32 days, the tiller number was counted manually. Descriptive analysis was made by comparing tiller number of three different hybrid rice and by comparing the average of tiller number from two planting methods.

Result and Discussion

Tiller Number of Hybrid Rice Planted by Direct Seeding Method

The result showed that there are differences in number of tillers of three hybrids rice planted by direct seeding. The highest number was Cp type from container 6 with 32 tillers, whereas the least number was Cs type the same container with 24 tillers. Comparison among the three hybrid rice showed that in average Cp type was highest with average of 32, followed by Is type with average of 29 and Cs type with average of 28. It can be inferred from Figure 2 that the difference in tiller number among three hybrid rice was not considered as substantial since there was only 4 tillers different at the maximum.
Figure 2. Tiller Number of Three Hybrid Rice Planted by Direct Seeding Method

According to Suprihatno et.al.(2009), average tiller number of hybrid rice, Maro and Rokan legalized by Indonesian authority is 18-30. Since the result showed that the average of tiller number was closed to 30 for Cs and Is and slightly above 30 for Cp, then it can be inferred that planting those hybrid rice by direct seeding method produced good result.

**Tiller Number of Hybrid Rice Planted by Transplanting Method**

As for tillers number of hybrid plants planted by transplanting methods, the highest tiller number was Is from container 1 with 19 tillers, whereas the least tiller number was Cs from container 5 with 11 tillers. It is showed in Figure 3 that the average of Is type is higher compare to the other two types. Because of only by 1 tiller difference, it can be considered that the different among the hybrid rice is not significant.
As previously stated, the average tiller number of hybrid rice, Maro and Rokan legalized by Indonesian authority is 18-30, but other type, Hipa 3 was 16-21. Comparison between the result of experiment to Maro, Rokan and Hipa 3 (as a standard), shows that tiller number of the present experiment was lower than the standard for Cp and Cs, but the same tiller number for Is. Thus, it can be assumed that transplanting method was not produced high number of tiller for the hybrid rice.

**Comparison between Direct Seedling Method and Transplanting Method**

The result of comparison based on tiller number between Direct Seedling Method and Transplanting Method of three hybrid rice is presented in Figure 4. It is clear that there is dissimilarity in tiller number of the three hybrid rice planted by Direct Seedling Method and Transplanting Method. In average, tiller number of Cp planted by Direct Seedling Method is doubled the Transplanting Method, whereas tiller number of Cs and Is planted by Direct Seedling Method are almost doubled the Transplanting Method.

![Tiller Number from Transplanting Method](image)
Although Pasquin et al., 2007 stated that transplanting rice seedlings 20 days old or older has been commonly reported to generate an increase in grain yield as a result of higher tiller production, the result showed differently. It is understood that the earlier the germinated seed is planted on the soil the quicker the plant adapted to its environment. The direct seeding method gives the opportunities for the plants to adapt themselves sooner, thus they will develop quicker than those planted by transplanting methods. Figure 5 shows the early development of plantlet, which already demonstrated the vigorous growth of plantlet planted by direct seeding compare to transplanting.
Since the direct seedling method produce higher tiller number for all the three hybrid rice compared to transplanting method, it is recommended to use direct seedling method rather than transplanting method.

Acknowledgement

The author would like to thank Soedjatmiko PhD. and Ir. Edward SPT, MM from CSS for helpful technical discussion and also Herlina Roseline, STP for her technical assistance. Appreciation also goes to unknown reviewer from ICBS for the suggestion and recommendation.

References

O-PD20

THE CARRYING CAPACITY OF GUABANA (*Annona muricata* L.)
TO THE FIFTH INSTAR OF ATAKAS (*Attacus atlas* L.) LARVAE IN THE FIELD

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Faculty of Biology UGM

The aim of the carrying capacity of guabana (*A. muricata*) to the fifth instar of atakas (*A. atlas*) in the field research is to determine the carrying capacity of guabana as the host plant of fifth instar of atakas silk. This study was used two factorial designs. The two factors were food availability of guabana leaves per larva and the rearing treatment by using screen and without screen. The food availability treatments per larva were 60 and 70 leaves. Parameters observed on this study were survivorship, cocoon weight, and cocoon shell weight.

The results of the study showed that although food availability is important, the pupae's survivorship, adult's survivorship, and cocoon shell weight were affected by the using of screen. The survivorship of atakas in the screen was higher than without screen. In unscreened rearing, 60 leaves per larva can support atakas's survivorship till 38.4±17.44 % and cocoon shell weight 2.50±0.19 g, while on screen rearing were 80.0±20.0 % and 1.80±0.69 g.

**Key words:** Carrying capacity, *Annona muricata* L., *Attacus atlas*, screen

INTRODUCTION

Natural silk production is not limited to silkworm of mulberry only, there are many other sericigenous species which produce non-mulberry silk or wild silk. (Jolly, 1980). *The tropical Asia Attacus atlas* L. is the most abundant wild-silk moth and widely distributed in Indonesia, particularly in Sumatra, Kalimantan, Jawa, Bali, Halmahera, Aru and West Papua. (Jolly, et al.1979). These insects are reared on food plants like, *Erythrina lithosperma*, Mig; *Nauclea orientalis* L., Guabana/Sour-soup, (*Annona muricata* Linn ), *Barringtonia asiatica*, Kurtz., under natural condition. But, because of the influence of various bio-factors, the cocoon production was quite low (Situmorang, 1996; Widyarto, 2001; Wahyudi, 2000; Kalshoven, 1981; Dally et al. 1981). This insect species produced silk which called non-mulberry silk, endowed very high quality silk because these silk have numerous vacuoles, more porous, sleek feel, cool, anti allergic and anti bacteria. (Akai, 1997)

Attempts were made to increase the cocoon production by implementing various methods of indoor rearing, especially for early instars larvae. Previous studies on indoor rearing of *A. atlas* indicate that this species feed only fresh leaves. The use of *A. muricata* leaves to feed *A. atlas* on indoor rearing for the first stage larvae up to the fourth stage larvae
were not adequate, besides the production of cocoon was low. (Widyarto, 2001, Wahyudi, 2000).

Therefore, the present study was undertaken to find an alternative method for outdoor rearing of A. atlas, particularly for the late instars larvae up to the pupa stage. The study was also to find out the effect of the use of screened or un-screened on rearing of the A. atlas outdoor and to study the carrying capacity of the A. muricata as a fodder plant to rear the A. atlas on outdoor rearing.

MATERIALS AND METHODS

Rearing of A. atlas was carried out on outdoor with screen and without screen condition with temperature 28-31 °C, and humidity 80-90 %. A. muricata (Guabana/Sour-soup) plants were maintained at Sawitsari Station of the Faculty of Biology, University of Gadjah Mada Yogyakarta, Indonesia. The plants were pruned according to the normally traditional rearing schedule to provide leaves of required maturity for Atakas rearing. The experiment was conducted from August to March 2003.

Healthy Attacus cocoons were collected from mahogany and Barringtonia trees in Yogyakarta. A total of 271 larvae were used for experiment on outdoor with screen and 267 larvae were used experiment on for out door without screen. The larvae were come from previously prepared disease-free laying eggs.

The first instars larvae stage (after eggs hatching) were placed directly on the leaves of the A muricata trees outdoors as done on the traditional rearing. The plants were pruned to get leaves of required maturity. The newly hatched larvae up to the fourth instars larvae were allowed to crawl on the leaves. The fifth instars larvae were reared by the method of rearing which was the same as that the fourth instars larvae except that leaves of A muricata were accounted for their diet. 60 and 70 leaves were given to feed each larva for developing the larvae up to cocoon stage. Daily observation were made in the morning, day light time. Feeding larvae behavior and the number of leaves fed by the larvae were observed and counted.

The carrying capacity was calculated by counting leaves were eaten by the each larva to grow to become cocoon stage. The pupae and the cocoon were weighted and the all data were statistically analyzed by using the Analysis of Variance Test. (Wilkinson, et.al.2002).
RESULTS AND DISCUSSION

Comparative result of outdoor rearing with screen and without screen of A. atlas (Table 1) indicate that the use of screen or without screen for outdoor rearing and the amount of leaves of A muricata to fed the fifth instars larvae were significantly affected to the survivorship of the larva. The outdoor rearing with screen and fed the larvae with 70 of A muricata leaves showed improvement of the survivorship of the larvae. The full grown larvae, cocoon and shell weight of larvae reared out door with screen and fed with 60 or 70 of A muricata leaves have a better result then the larvae reared outdoor without screen. It was also observed that larvae reared out door without screen appeared unhealthy, particularly in the time before and after the molting stages. Even though, the larvae could grow up to cocoon but the pupa usually died be caused of the cocoon shell to thin so it may infected easily by diseases (Sulistiaawati, 2003, Rahmawati, 2003). Daly et al. 1981, reported, even though food played the important role in insect population rearing but predators, parasites and diseases are also may effected to insects population density in field. This experiment indicated that there was no any different result of the survivorship of the larvae, whether they fed with 60 or 70 leaves of A muricata for each larva.

Table 1: Result of rearing of A. atlas outdoor with screen and without screen fed with 60 or 70 leaves of A muricata.

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<th>Characters</th>
<th>Reared with screen outdoor</th>
<th>Reared without screen outdoor.</th>
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<tr>
<td></td>
<td>60 leaves</td>
<td>70 leaves</td>
</tr>
<tr>
<td>Survival ship larva up to pupa (%)</td>
<td>80.00 ± 20.00 a</td>
<td>73.33 ± 11.55 a</td>
</tr>
<tr>
<td>Survival ship larva up to imago (%)</td>
<td>66.63 ± 11.55 a</td>
<td>88.87±10.72 a</td>
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<tr>
<td>Av. Cocoon weight (g)</td>
<td>8.94 ± 0.13 a</td>
<td>8.57±0.27 a</td>
</tr>
<tr>
<td>Av. Shell weight (g)</td>
<td>2.50 ± 0.19 a</td>
<td>2.32 ±0.28 a</td>
</tr>
</tbody>
</table>
CONCLUSION

The present study concluded that the use of 60 or 70 leaves of the *A. muricata* were enough amount to feed the fifth instars larvae up to cocoon stage. The outdoor rearing with screen for the fifth instars of *A. atlas* fed with 70 of *A. muricata* leaves may be feasible for commercial purposes.

REFERENCES


O-PD21

HOW DO PLANTS RESPOND TO NITROGEN-SHORTAGE BY REGULATING NITRATE UPTAKE?

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Nitrogen (N) is one of the most important nutrients for plants; its availability often limits plant growth and productivity both in natural and agricultural systems. Therefore, plants have evolved a set of responses to adapt to N-limiting conditions. These include biochemical and morphological changes to enhance N acquisition and allocation. However, little is known about the molecular basis of these responses. To address this issue, we performed an in silico screening using publicly available gene expression profiles deposited into the Gene Expression Omnibus (GEO) and identified 234 genes that are up-regulated specifically under N-limiting conditions in Arabidopsis.

Amongst these genes we are currently focusing on AtNRT2.4, which encodes a high-affinity nitrate transporter. Expression analyses revealed that AtNRT2.4 is induced as nitrogen availability getting scarce and repressed by supplement with various nitrogen species (NO$_3^-$, NH$_4^+$, ASN and GLN). Transgenic plants harboring promoter-GFP fusion gene revealed that AtNRT2.4 is preferentially expressed in epidermis of lateral roots, indicating that AtNRT2.4 plays a role in acquisition of nitrate under N-limiting conditions. To further investigate the role of AtNRT2.4, we characterized a T-DNA insertional mutant. The mutant showed reduction in nitrate uptake by up to 14 % and this was evident only in plants grown under the N-limiting condition. Together, these results suggest that AtNRT2.4 is involved in adaptation to N-limitation in Arabidopsis.

Keywords: Nitrogen, NRT2, high-affinity nitrate transporter, N-limitation
Introduction

Nitrogen (N) is one of the most important nutrients for plants; its availability often limits plant growth and productivity. In agricultural system, the use of nitrogenous fertilizers, have increased crop yield over the past 50 years in U.S. (Figure 1) and undoubtedly across the globe. However, excess nitrogenous fertilizer use has created unintended human and environmental problems, including surface and groundwater pollution (1), biogenic greenhouse gas emission (2) and increasing cost of production. To solve these problems, it is required to curve nitrogenous fertilizer use.

In natural system, plants frequently face N-limiting growth conditions because excess N can easily be leached by rainwater and/or consumed by microorganisms (3). Thus, plants have evolved elaborate responses to live through the N-limiting conditions. These responses are highly complex ranging from gene expression to morphological changes (4-6). A comprehensive understanding of the molecular network underlying these responses would lead to generating a crop with improved N use efficiency or N-limitation tolerance, which needs less fertilizer to produce similar yields.

In this study, we describe the identification of N-limitation inducible genes in Arabidopsis. An in silico screening using publicly available microarray data identified 234 genes that are up-regulated under N-limiting conditions. We confirmed N-limitation specific and inducible nature of several genes, including genes encoding putative high-affinity nitrate transporters AtNRT2.4 and AtNRT2.5, by semi-quantitative reverse transcription PCR (RT-PCR). Analysis of AtNRT2.4 promoter::GUS transgenic plants revealed root hair and lateral root specific expression of the gene. Collectively, these results suggest that the genes identified by in silico screening might play important roles in plant adaptation to N-limiting conditions.
Result and Discussion

Identification of nitrogen-limitation inducible genes in Arabidopsis

To gain insight into the molecular basis of N-limitation response, we exploited publicly available microarray data. The data were obtained as the supplemental materials on journal websites (8-10) or from AtGenExpress database (7). First, the microarray data of nitrogen-starved seedlings were compared with those of nitrogen-replete seedlings (N-limitation induced genes) (8). Secondly, the microarray data of nitrogen-starved seedlings were compared with those of nitrogen-replenished seedlings (N-supplement repressed genes) (8). Finally, the microarray data of phosphate-starved (9), sulfate-starved (10) and methyl viologen treated seedlings (AtGenExpress, TAIR accession 1007966941) were compared with those of phosphate-replete, sulfate-replete and mock treated seedlings, respectively (P, S and oxidative stress induced genes). In each analysis, genes giving more than two-fold increase were grouped into N-limitation induced genes (1523 genes, blue circle in Figure 2), N-supplement repressed genes (705 genes, orange circle in Figure 2) and P, S and oxidative stress induced genes (1373 genes, green circle in Figure 2). To narrow down genes specifically induced by N-limitation, we focused on genes shared between the N-limitation induced genes and the N-supplement repressed genes but not commonly grouped with P, S and oxidative stress induced genes, and found 234 genes that meet the criteria (indicated by red letters in Figure 2).
Verification of in silico screening by semi-quantitative RT-PCR

Amongst 234 genes identified by microarray analyses, we selected 4 genes involved in nitrogen acquisition and metabolism for further analysis, namely genes encoding putative high-affinity nitrate transporters AtNRT2.4 and AtNRT2.5, a glutamine synthetase AtGLN1:4 (11), and a glutamate dehydrogenase AtGDH3 (12). Transcript levels of selected genes were examined by semi-quantitative RT-PCR with appropriate primer sets. All the selected genes were expressed at the highest level in both the root and shoot of plants grown without nitrogen source, except for At5g60770 whose expression was not detectable in shoot (Figure 3). The expression levels gradually decreased as concentrations of nitrate in the media increased, suggesting that these genes are up-regulated in response to N-limitation (Figure 3). Furthermore, the expression of these genes was not induced under phosphate- or sulfate-limited conditions, demonstrating N-specific nature of the expression (Figure 3). Note that the hallmark genes of phosphate-starvation (AtIPS1) (13) and sulfate-starvation (AtSULTR1:1) (10) were up-regulated in our phosphate- and sulfate-limited experimental conditions, respectively (Figure 3). Together, AtNRT2.4, AtNRT2.5, AtGLN1:4 and AtGDH3 were confirmed to show N-limitation specific and inducible expression patterns, suggesting that the 234 genes identified by in silico screening might include important genes involved in N-limitation responses.

Figure 3. Semi-quantitative RT-PCR of N-limitation inducible genes identified in the microarray data analyses. Plants were germinated and grown on MGRL agar plates containing 10 mM KNO₃ for 10 days and then transferred to MGRL agar plate containing 10 mM KNO₃ (LN), 2 mM KNO₃ (MN) or 0.5 mM KNO₃ (LN), or lacking nitrogen (+N), phosphate (+P) or sulfate source (+S). Plants were grown for another 10 days and roots and shoots were collected separately. Total RNA were prepared from each sample and analyzed by RT-PCR with appropriate primer sets. AtIPS1 (At5g19770), encoding alpha-tubulin in Arabidopsis, is a loading control. Genes analyzed are AtNRT2.4 (At5g60770), AtNRT2.5 (At5g29400), AtGLN1:4 (At5g16870), AtGDH3 (At5g09110), AtIPS1 (At5g19770), AtNRT2.1 (At5g50992) and AtSULTR1:1 (At4g06620).
Expression analysis of AtNRT2.4 in transgenic plants carrying AtNRT2.4::GUS construct

Semi-quantitative RT-PCR showed that AtNRT2.4 is expressed in roots (Figure 3). We further studied organ-specific expression of the gene using transgenic plants harboring an AtNRT2.4 promoter::GUS transgene (1886 bp upstream of the inferred initiation codon was fused with β-glucuronidase gene). Consistent with the Semi-quantitative RT-PCR analysis, the GUS activity was evident only in roots of N-starved seedlings (Figure 4). Interestingly, the activity was predominantly detected in the root hair and lateral root (Figure 4), both of which are relevant to N-acquisition, indicating that AtNRT2.4 plays a part in N-limitation responses. Taken together, these results suggest that analyses of genes identified by in silico screening would help us to better understand the molecular basis of N-limitation responses.

References


O-PD22

THE GROWTH OF Chlorella pyrenoidosa IN WASTEWATER OF MOLASSES ETHANOL FERMENTATION (VINASE)

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Abstract

Microalgae have many uses. They can serve as water bioremediation agents, as feed for aquaculture, as food for humans and animals, in pigment production, in bioremoval of heavy metals, and in agriculture.

A study of the growth Chlorella pyrenoidosa in the wastewater of molasses ethanol fermentation (vinase) is presently conducted in the Laboratory of Microbiology of the Faculty of Biology, Gadjah Mada University; since C. pyrenoidosa offers an interesting alternatives for serve as a potential source of single cell protein and other essential nutrients, and as a water bioremediation agent. The aim of this present study was to study the growth of the freshwater microalga Chlorella pyrenoidosa, in wastewater of molasses ethanol fermentation (vinase) within a confined environment of batch cultivation. The growth response of Chlorella pyrenoidosa was studied under varied concentration of vinase (0 %, 25 %, 50 %, and 75 %). The temperatur was continously fixed at room temperatur, and pH 7, with an illumination of 3000 lux. An additional aeration was also employed. The result showed that the optimum growth of C. pyrenoidosa was in the concentration of 25 % vinase. The growth rate of the culture in vitro (µ) is 0.521 cell/hour and the generation time (g) is 1.329 hour (79.74 minute).

Keyword : Vinase, Chlorella pyrenoidosa, mollases, ethanol

INTRODUCTION

The limitations of agricultural land and the impacts of global climate change on agricultural productivity are factors of increasing relevance in the decisions that must be taken on land use for food, feed, chemicals and energy. This increasing competition for land is driving the current consideration of the potential of the aquatic environment for the production of food, industrial feedstock and biofuels.

Presently, Chlorella is consumed as a health food, and is used in food additives to milk products, fermented soya beans, liquors and others drinks, noodles, and cakes (Prud’homme van Reine and Trono, 2001). Moreover, the microalgae offers an interesting alternative for serve as a potential source of food, because they does not compete for land
suitable for agriculture irrigation or consumption by humans or animals for food security, high productivity, great variety in species for great variety in products, and a possibility to steer metabolism to production of specific compounds.

*Chlorella is a member of Chlorophyceae which offers an interesting alternative for serve as a potential source of single cell protein. The nutritional value of Chlorella is protein (58.5 %), total fat (9.3 %), carbohydrates (23.2 %), total dietary fiber (0.3 %), ash (4.2 %), vitamin B, A and E, mineral calcium, magnesium phosphate and Zn. The essential amino acid (% of total protein nitrogen) which comprises the Chlorella are tryptophane, tryonine, leucine, lysine, methionine, sisteine, phenylalanine, tyrosine, valine, arginine, histidine, alanine, aspartate acid, glutamate acid, glysine, pralyne, serine, praline and another amino acid (Prud'hommé van Reine and Trono, 2001). Therefore, Chlorella can be consumed as an alternative source of single cell protein and nutritional supplements.*

*Chlorella cells are single spherical or ellipsoidal cell with a cup-shaped chloroplast and 3 – 8 μm in diam. Reproduction in Chlorella is asexual, producing 4, 8, or sometimes 16 autospores, which are released by rupture or dissolution of the parental walls. Both light and temperature affect cell division, high light intensity and high temperatures are favorable (Prud'hommé van Reine and Trono, 2001). Chlorella is ubiquitous and found in both aquatic (freshwater, brackish, and marine) and terrestrial habitat, including soil and concrete walls.*

*Chlorella grows well in most inorganic media (e.g. Bold's, Knop) using NO₃⁻, NH₄⁺, or urea as a nitrogen source. The cultures are bubbled with air enriched by 5% CO₂, to enhance growth. Growth and biomass production can be further enhanced by adding organic carbon sources such as glucose and acetate (Prud'hommé van Reine and Trono, 2001).*

*Sugarcane manufacturing industry produced inorganic compound (as a source of nitrogen and mineral) wastewater and organic compound as a source of carbon which useful for the growth of Chlorella. The wastes of the sugarcane manufacturing industry are sugar fiber, oil, and water which including from the result of juice sugarcane fermentation, evaporation process, and sulphitation. The wastewater of the sugarcane manufacturing industry contains molasses, sulphate, phosphate, and ammonium. Usually, the wastewater has a biochemical oxygen demand (BOD) of 1.700 – 6.600 mg/l and chemical oxygen demand (COD) of 2.300 – 8.000 mg/l. This wastewater sometimes also contains contaminated pathogenic material as a result from production process. The wastewater of the sugarcane manufacturing industry is utilized for another product as well as bioethanol and biospiritus as by-product (Anonim, 1998).*
A study of the growth *C. pyrenoidosa* in the wastewater of molasses ethanol fermentation (vinase) is presently conducted in the Laboratory of Microbiology of the Faculty of Biology, Gadjah Mada University. The aim of this present study was to study the growth of the freshwater microalga *C. pyrenoidosa*, in wastewater of molasses ethanol fermentation (vinase) within a confined environment of batch cultivation.

**METHOD OF RESEARCH**

A species of freshwater isolated *Chlorella* was obtained from the laboratory’s collection of Dra. Lely Pangabean, M.Sc. (LON LIPI, Jakarta). The isolated species was continuously grown at room temperature (27 °C) with continuous illumination provided by white fluorescent lamps, 4000 lux. The stock culture was cultured in the modified media of f/2 without an addition of NaSiO3.5H2O to the media. The stock culture was continuously re-cultivated at optimum growth conditions and introduced to the experimental systems at logarithmic phase.

The culture was cultivated within a confined environment of batch cultivation. The growth response of *C. pyrenoidosa* was studied under varied diluted vinase (0 %, 25 %, 50 %, and 75 %). The temperature was continuously fixed at room temperature (27 °C), and pH 7, with a continuous illumination at 4000 lux. An additional aeration was also employed.

The growth of *C. pyrenoidosa* was measured by microscopic examination using a counting chamber (haemacytometer). The growth rate of the culture in vitro (μ) and the generation time (g), can be represented by the expression of:

\[
\mu = \frac{\ln Nt - \ln No}{t}
\]

\[
k = \frac{\mu}{\ln 2}
\]

\[
g = \frac{1}{k}
\]

**RESULTS AND DISCUSSION**

The growth of algal cultures is expressed usually as the increment of biomass, number of cells, amount of protein, pigments, etc., over a given period. Generally,
populations of unicellular organisms may be measured in term either of the number of individual cells or of their mass. The former may be termed cell concentration, defined as the number of individual cells per unit volume, whereas the latter is called cell mass or cell density, defined as the weight of cells or biomass per unit volume.

Under the typical regime of a simple homogenous batch culture, where the food supply is limited and nothing is added or removed from outside, the algal growth passes through several different phases: lag phase, accelerating growth phase, exponential growth (log phase), decreasing log growth (linear growth), stationary phase, accelerated death, and log death (Becker, 1994).

1. The Growth Rate of C. pyrenoidosa

The growth of C. pyrenoidosa in wastewater of molasses ethanol fermentation (vinase) as a by-product of sugarcane manufacturing industry is presented in the Picture 1. The discussion of the growth of C. pyrenoidosa is only presented by the growth of C. pyrenoidosa in the concentration of 25 % of vinase.

The lag phase of an algal culture is an adaptation time of an algal to the new environment. During the lag phase, the algal culture adjusts itself to the altered conditions, and the specific growth rate is significantly lower than that in subsequent one and increases with cultivation time. High physiological activity is found during the lag phase, the cells being much more sensitive to temperature and pH. The lag phase of the culture of C. pyrenoidosa is during the first and second day of the six experimental days.

After a short time of adaptation phase, the algal culture had adapted to the given cultivation condition, the cells enter the phase of exponential (logarithmic) growth. In this phase, the number of cell increased, and this phase of the experimental culture is during the third day of the six experimental days.

In the batch culture, where nothing is added or removed from the medium after inoculation, proceeds through another phase where decreased logarithmic growth starts to occur. This effect, as well as the beginning of limitation of minerals and increasing accumulation of toxic wastes, reduce the specific growth rate and the increase in the algal biomass becomes almost linear. This phase of the experimental culture is during the fourth day of the six experimental days.

The end of the stationary phase is marked by reduced viability in the cell population; the algal cells begin to die releasing organic, often growth-inhibiting, materials to medium. This phase is caused by unfavorable environmental conditions, over-age of the culture and
limited supply of nutrient. This phase of the experimental culture is after the fifth day of the six experimental days.

Finally, the death rate becomes exponential, leading to the complete breakdown of the algal population. This phase of the experimental culture is during the sixth day of the six experimental days.

The measurement of the growth rate of the culture of *C. pyrenoidosa* in vitro (µ) is 0.93 cell/hour and the generation time (g) is 7.7 hour.

![The Growth of Chlorella pyrenoidosa](image)

*Picture 1. The growth of C. pyrenoidosa*

The growth of *C. pyrenoidosa* in the concentration of 50% and 75% of vinase have a longer lag phase than in the concentration of 25% of vinase, because the higher concentration of vinase contains higher concentration of nutrients, which have involved a longer adaptation of the *C. pyrenoidosa* to grow in these medium.

2. **The pH of the culture**

The culture were initial set at pH of 7.0 (neutral). Then, the pH of the culture showed that the control and the diluted-medium of 25% vinase were in average of 7.0. However, the pH of 50% and 75% showed a lower pH; and the growth rate of both cultures were continuously declining after two days of incubation time. It therefore could be concluded that the *C. pyrenoidosa* does not adapt to live in lower pH (acid condition).
CONCLUSION

The optimum growth of *C. pyrenoidosa* was in the concentration of 25 % of vinase. The growth rate of the culture in vitro (µ) is 0.93 cell/hour and the generation time (g) is 7.7 hour.

References


O-PD23

EPIDEMIOLOGY OF DHF INCIDENCE IN JEMBER (recent five years)

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2 Biologi Department, Mathematic and Natural Sciences of Jember University,

The research has been held in the aim to know the profile of dengue hemorrhagic fever (DHF) in Jember, East Java. It covered the value of sickness and mortality, the age distribution and the time of DHF cases occurred. The data were collected from September 1st 2005 until August 31st 2009. The results shows that in one year there were 3354 case of DHF with the 52 death (Case Fatally Rate or CFR 1.2%). During the DHF epidemic, there were 425 case of cluster and the range in age of victim varied from 2 month to 80 years old. Most of the DHF victims were in the productive age of 15 – 44 year with 1281 cases, but the death were mostly in the children age of 5-9 year with 31 cases. For following strategies, there are needed some more environmental modification to control the spread and habitat of Aedes aegypti as main vector of DHF with community base development.

Keywords: epidemioloty, dengue hemorrhagic fever
### TOPIC 5: BIOMEDICS

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O-BM01

DETECTION AND IDENTIFICATION OF INFLUENZA VIRUSES BY POLYMERASE CHAIN REACTION (PCR)

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¹Vırology Molecular, Vıral Dıseases Program, US NAMRU2 Jakahta

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ABSTRACT

Influenza is a viral respiratory pathogen which is responsible for frequent seasonal epidemics. Influenza virus is RNA virus and belongs to the family of Orthomyxoviridae that may cause most of influenza illness in human. Misidentification of the virus infections may cause fatal effect in human. In order to avoid the fatal case, a better diagnostic method for detection and identification of Influenza viruses need to be improved. The viral cultivation is commonly used as a standardized method (i.e., gold standard) for viral influenza detection. Instead of having less sensitivity, viral cultivation is usually time consuming and needs days to get the results. A technique of Polymerase Chain Reaction (PCR) was aimed to solve this problem because of its greater capability to give the faster result. An assay for detecting viral influenza was conducted by using 193-patient Nasal and Throat Swab samples that collected from 22 hospitals in Indonesia. Two PCR techniques of real time PCR (RT-PCR) and multiplex nested RT PCR were implemented in this assay. Specific primer sets for influenza virus were designed for both PCR technique assays. To assess the comparative sensitivity, both PCR results were compared with the result of isolated viruses. This study reported that the real time RT PCR showed a greater reliability compared to that the multiplex nested RT PCR in identification of viral influenza.

Keywords: Detection, influenza, human, PCR method

INTRODUCTION

Influenza is a viral respiratory pathogen which is responsible for frequent seasonal epidemics. Influenza viruses are segmented negative-sense RNA viruses that belong to the family Orthomyxoviridae. There are three types of influenza viruses, types A, B, and C, have been described on the basis of antigenic differences in the matrix (M) protein and nucleoprotein. Influenza viruses A and B are associated with seasonal morbidity and mortality, while influenza virus C causes mild upper respiratory tract infections in children and
adolescent. Influenza virus infection has rapid onset with symptoms that can include fever (temperature $\geq 37.8^\circ\text{C}$), headache, malaise, cough, chills, myalgia, and some sore throat$^{1,2,3}$.

Virological analysis usually takes the form of isolation and typing of virus from nose and throat swabs with tissue culture or embryonated chicken eggs and/or of antigen detection by immunoflourescence (IF) or enzyme-linked immunosorbent assay (ELISA). Another approach to the rapid detection and identification of influenza viruses is the application of reverse transcription-PCR (RT-PCR)$^{4,5}$.

Misidentification of the influenza virus infections may cause fatal effect in human. In order to avoid the fatal case, a better diagnostic method for detection and identification of Influenza viruses needs to be improved. Current diagnostic methods include virus isolation, antigen detection, and serology has limitation. Major limitations of these techniques include prolonged time to completion, subjective evaluation, low sensitivity, and low specificity. Use of nucleic acid amplification techniques has made sensitive diagnosis of influenza virus infection feasible, with the possibility of type determination$^6$. Rapid diagnostic is important not only for timely therapeutic intervention but also for the identification of a beginning influenza outbreak$^7$. PCR techniques have been developed for the specific detection and sub typing of influenza viruses. They have proven to be very sensitive and specific but are often difficult to implement in a routine diagnostic setting and still require time-consuming sample handling and post-PCR analysis. Moreover, better techniques are still needed$^{8,9}$.

Here, we conducted an assay for detection and identification of influenza virus by Polymerase Chain Reaction (PCR). Two PCR techniques of real time PCR (rRT-PCR) and multiplex nested gel based RT PCR were implemented in this assay. To assess the comparative sensitivity, both PCR results were compared with the result of isolated viruses.

**MATERIAL AND METHODS**

**Sample collections.** Nasal and Throat swab samples were collected by January-February 2007 from 22 hospitals which collaborated with US NAMRU2 for the Influenza Surveillance research in Indonesia. Swab nasal and throat were taken from 193 patients who had influenza symptoms. Those samples prepared in sterile vials with 2 ml sterile transport medium (VTM): HBSS (Hanks Balanced Salt Solution) buffers, contained of 0.35 gram/L Natrium Bicarbonate (NaHCO3), 0.5% Bovine Albumin/gelatin, and 50 mg/L antibiotic (gentamycin). Samples were placed in liquid nitrogen and sent to the NAMRU2 laboratories in Jakarta for analyzed.
RNA isolation. Nasal and Throat swab samples were thawed and homogenized by vortexing before RNAs were isolated. Viral RNA isolation was done based on the procedure and protocol from the QIAmp Viral RNA mini kit (QIAGEN). Viral RNA was saved in refrigerator 4°C and used as a template for RT-PCR amplifications. All the viral RNA isolation were conducted in Biological Safety Cabinet Class II (Nuaire), at the Molecular Virology laboratory, Viral Diseases Program, US NAMRU2 Jakarta.

Multiplex nested reverse transcription PCR. Viral RNA was amplified by multiplex nested gel based RT PCR. For the first step PCR, RNA was amplified by reverse transcription-PCR (RT-PCR). The RNA transcripted becomes cDNA by activities of AMV-Reverse Transcriptase enzyme, which is amplified together with Tfl polymerase enzyme in single PCR tube (provided by Access Promega RT PCR kit).

The RT PCR for 40 cycles was:

- Pre RT PCR 48 °C : 45 minute
- Pre Denaturation 94 °C : 2 minute
  - PCR cycle (40 cycles) :
    - Denaturation 94 °C : 30 seconds
    - Annealing 45 °C : 1 minute
    - Elongation 68 °C : 2 minute
  - Extention 68 °C : 7 minute
  - Stand by 4°C ∞

In the second round PCR, amplicons which are from RT PCR was used as a template for nested PCR. By the nested PCR, DNA amplification was conducted based on Perkin Elmer PCR reagents kits procedure (provided by Applied Biosystems) for 25 cycles.

The nested PCR for 25 cycles was:

- Pre Denaturation 94 °C : 2 minute
- PCR cycle (25 cycles) :
  - Denaturation 94 °C : 1 minute
  - Annealing 45 °C : 1.5 minute
  - Elongation 72 °C : 3 minute
- Extention 72 °C : 7 minute
- Stand by 4°C ∞
All the PCR amplifications were done with Thermocycler/PCR machine (Applied Biosystems type 9600/9700). The nested PCR amplicons were quantified by 2% agarose gel electrophoresis and visualized in 1% ethidium bromide (EtBr). The result was documented by using Gel-Doc (Bio-Rad Gel Doc 1000).

**Oligonucleotide primers.** Oligonucleotide specific primers which are used in the multiplex nested gel based RT PCR was: outer oligonucleotide primers used for the RT PCR and inner oligonucleotide primers applied for the nested PCR. Those primers were specific for detection of protein matrix (M), haemagglutinin (HA) and neuraminidase (NA), part of conserved gene of influenza virus reference. Oligonucleotide primers designed for the assay was taken from the reference of Zhang dan Evans\(^6\), as seen in table 1.

**Tabel 1. Oligonucleotide primers specific for influenza detection**

<table>
<thead>
<tr>
<th>GEN TARGET</th>
<th>OUTER PRIMER (untuk RT PCR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ID</td>
<td>Sequence (5' to 3')</td>
</tr>
<tr>
<td>A/MP</td>
<td>AMPA</td>
</tr>
<tr>
<td></td>
<td>AMPDII</td>
</tr>
<tr>
<td>A/N1</td>
<td>AN1A</td>
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<tr>
<td></td>
<td>AN1EII</td>
</tr>
<tr>
<td>A/N2</td>
<td>AN2A</td>
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<tr>
<td></td>
<td>AN2DII</td>
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<tr>
<td>A/H1</td>
<td>AH1A</td>
</tr>
<tr>
<td></td>
<td>AH1FII</td>
</tr>
<tr>
<td>A/H3</td>
<td>AH3A</td>
</tr>
<tr>
<td></td>
<td>AH3DII</td>
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<tr>
<td>B/MP</td>
<td>BMPA</td>
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<tr>
<td></td>
<td>BMPDII</td>
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<tr>
<td>B/HA</td>
<td>BHAA</td>
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<td></td>
<td>BHADII</td>
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<tr>
<td>GEN TARGET</td>
<td>INNER PRIMER (for nested PCR)</td>
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<td>------------------------------</td>
</tr>
<tr>
<td>ID</td>
<td>Sequence (5’ to 3’)</td>
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<tr>
<td>A/MP</td>
<td>AMPB</td>
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<tr>
<td></td>
<td>AMPCII</td>
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<tr>
<td>A/N1</td>
<td>AN1B</td>
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<td></td>
<td>AN1DII</td>
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<td>AH3B</td>
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<tr>
<td></td>
<td>AH3CII</td>
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<tr>
<td>B/MP</td>
<td>BMPB</td>
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<td></td>
<td>BMPCII</td>
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<tr>
<td>B/HA</td>
<td>BHAB</td>
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<td></td>
<td>BHACII</td>
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**One step assay real time RT PCR.** The same viral RNA used for multiplex nested gel based RT PCR was also amplified by real time RT PCR. Primer/probes are designed specifically for identification and detection of human influenza virus type A and B. For identification of influenza A, primers/probes were designed specifically for identified the virus influenza subtype H1 and H3 which are usually infected human. Real time RT PCR procedure was conducted by optimizing the protocol from one step assay RT PCR kits (provided by ABI Taqman). This assay used real time PCR-ABI systems machine (series 7000, 7500). Taqman probes were labelled at 5’-end with reporter 6-carboxyfluorescein (FAM) and quencher at 3’-end with blackhole quencher 1 (Biorsearch Technologies, Inc.). Positive result was identified if the PCR amplification of samples has ability to reach the limited value for threshold (CT) of the reference, which is ≤ 40. Primer/probes and real time RT PCR amplification designed for this assay is followed the procedure and protocol from the Center of Diseases Control (CDC), Atlanta (not published).
**Sensitivity and specificity measurements.** Sensitivity showed the proportion of positive samples which are really positive in sample population. The greater sensitivity values mean the lesser misdetection that might be happened. Specificity showed the proportion of negative sample which are really negative in sample population. Sensitivity, specificity, *Positive Predictive Value* (PPV), and *Negative Predictive Value* (NPV) were calculated by binary classification below:

\[
sensitivity = \frac{\text{truepositive}}{\text{truepositive} + \text{falsenegative}}
\]

\[
specificity = \frac{\text{truenegatives}}{\text{truenegatives} + \text{falsenegatives}}
\]

<table>
<thead>
<tr>
<th></th>
<th>(<em>Gold standard</em>)</th>
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<tr>
<td><strong>Result</strong></td>
<td><strong>True</strong></td>
</tr>
<tr>
<td><strong>Test</strong></td>
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</tr>
<tr>
<td>Positive</td>
<td>True Positive</td>
</tr>
<tr>
<td></td>
<td>(Type I error)</td>
</tr>
<tr>
<td>Negative</td>
<td>False Negative</td>
</tr>
<tr>
<td></td>
<td>(Type II error)</td>
</tr>
<tr>
<td>↓</td>
<td>Sensitivity</td>
</tr>
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</table>

**RESULT AND DISCUSSION**

This study compared the two of generally used PCR methods, which are *multiplex* RT PCR with *nested* PCR and *one step assay real time* RT PCR. Both PCR methods used specifics oligonucleotide primers for detection of haemagglutinin (H), neuraminidase (N) dan matrix protein (M) of influenza viruses, type A and B.
By using the *multiplex nested gel based* RT PCR, the result found that there were 12 sample was positively infected by influenza type B, 6 sample shown negative PCR results, and another 175 sample was positively infected by influenza A, as seen in picture 1. From 12 sample, detected with *multiplex nested gel based* RT PCR, which has positive results infected by influenza type B, there are 6 sample shown negative result by *realtime* RT PCR detection.

![PCR results](image)

*Picture 1. PCR results of nasal swab and throat swab. Lane 1 swab nasal, Irian, negative. Lane 2 throat swab, Irian, negative. Lane 3 swab nasal, Bandung, H3N2. Lane 4 throat swab, Bandung, H3N2. Lane 5 swab nasal, Jakarta, H1N1. Lane 6 throat swab, Jakarta, H1N1. Lane 7 nasal swab, Bandung, H3N2. Lane 8 throat swab, Bandung, H3N2. Lane 9 nasal swab, Sumatera Utara, H1N1. Lane 10 throat swab, Sumatera Utara, negative. Lane 11 nasal swab, Kupang, flu A. Lane 12 nasal swab, Tangerang, Flu B. Lane 13, throat swab, Mataram, negative. Lane 14, control positive flu B (RT-PCR). Lane 15 kontrol negative (H2O). Lane 16 kontrol positive flu H1N1 (nested). Lane 17 kontrol positive flu H3N2 (nested). Lane 18 kontrol positive flu B (nested).*

Generally, both nasal and throat sample from patients who have influenza symptoms will shown positive results which clearly known viral subtype (picture 1). But after visualized by gel based PCR, there are some differences. Sometimes positive results found only in nasal swab, meanwhile the other sample shown negative results (Picture 1, lane 9-10). Sometimes the PCR could only shown matrix protein (picture 1, lane 11-12) instead of clearly viral subtype. By this condition, repeated nested PCR was needed. Those differences appeared because lack of viral concentration in swab samples. This will affect to the result of PCR amplification.

As Hayden, et al.\(^\text{10}\) said that viability of total of viral concentration will decrease only a few days after their infection. This condition will affect to the result of virus cultivation. If viability decreases, it may become harder to detect the viral type. Hindiyeh\(^3\) said that negative result of viral cultivation was happened because virus has been inactivated; as an effect of the unstandardized procedure of sample collection, storage, and transport. As said
by van Elden⁹ that for viral cultivation could only done within 1-2 days after viral infection, for a better result.

By this study, total 193 samples were tested, 56 samples were showed positive result by viral cultivation and 137 were shown negative result. Compared the result from both PCR methods and viral cultivation as standard, will found the value of specificity, sensitivity, positive predictive value and negative predictive value. As shown in Tabel 3.

Tabel 3. Sensitivity, specificity, PPV and NPV from both RT PCR methods for detection and identification of influenza virus type A and B, compared with viral cultivation as a gold standard.

<table>
<thead>
<tr>
<th></th>
<th>Flu A</th>
<th>Flu B</th>
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<tbody>
<tr>
<td></td>
<td>Real time RT PCR (%)</td>
<td>Multiplex nested gel based RT PCR (%)</td>
</tr>
<tr>
<td>sensitivity</td>
<td>84.9</td>
<td>92.5</td>
</tr>
<tr>
<td>specificity</td>
<td>52.3</td>
<td>12.9</td>
</tr>
<tr>
<td>PPV</td>
<td>44.1</td>
<td>28.7</td>
</tr>
<tr>
<td>NPV</td>
<td>74.8</td>
<td>81.8</td>
</tr>
</tbody>
</table>

From table 3 above, generally shown that both of PCR methods, real time RT PCR and multiplex nested gel based RT PCR, have sensitivity and specificity almost equally the same for detection of the influenza B (90-100%). Even though the PPV for real time RT PCR method was higher than multiplex nested gel based RT PCR (50%) for influenza B detection. By this result, it is said that real time RT PCR technique may give a better way to shown the sample which are real negative for flu B.

From table 3 also shown that real time RT PCR techniques has greater specificity for flu A detection (52,3%) with PPV 44,1%, higher than multiplex nested gel based RT PCR.
However, generally both PCR techniques have almost equal value of sensitivity for influenza A detection (80-100%) with a very common NPV (70-90%). It is wisely said that the real time RT PCR technique have the ability to shown the sample which are real positive for flu A.

This assay told us that the multiplex nested gel based RT PCR for detection and identification of influenza virus will decreased the possibility of unspecific reaction which usually caused of misdetection. It is because nested PCR will remove the cross contamination. Mis detection usually happened because of the unspecific primers or some inhibitor occurred in the PCR reaction. By this chance, an improved and developed of PCR techniques will greatly increased the value of sensitivity and specificity for detection and identification of influenza virus A and B.
CONCLUSION

Real time RT PCR technique have greater capability for detection and identification of influenza virus and shown lesser possibility for virus misdetection, compared with multiplex nested RT PCR technique.

ACKNOWLEDGEMENTS

Many thanks for staff in the virology department, US NAMRU2, Jakarta for their briefly help to perform this research.

REFERENCES


O-BM02

PRODUCTION AND DETECTION OF XYLOOLIGOSACCHARIDES: INTRODUCING THE USE OF PREBIOTIC COSMETICS

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Production and characterization of xylooligosaccharides has been performed, and in line with determination of several parameters that support on its production. These parameters were optimum condition of hydrolysis (incubation time and substrate concentration) of oat-spelt xylan by xylanase based on enzyme activity which exhibited, affinity of xylanase on xylan as substrate and hydrolysis velocity, as well as product detection of hydrolysis of oat-spelt xylan by xylanase via HPLC. Optimum condition of hydrolysis has occurred at 15 hours incubation and substrate concentration was 8.195 mg.ml^{-1}. Affinity of xylanase on xylan as substrate (Km) was 8.195 mg.ml^{-1} and hydrolysis velocity (Vmax) was 0.169 \mu mol.min^{-1}.mg^{-2}. Hydrolysis product exhibit formation of xylooligosaccharides, xylose, and arabinose. Antibacterial activity of xylooligosaccharides was assayed by supplementing of pure xylooligosaccharides and xylooligosaccharide-crude extracts to tryptone soya broth as medium of *Penicillium acnes* (skin problem-causing bacteria). Crude extracts of xylooligosaccharides were tested against *P. acnes* at concentrations of 0, 25, 50, and 75% (w/v). When crude extracts was used, it is seen that there was formation of inhibition zone only at concentration of 75%. Among the concentrations were used, 75% of xylooligosaccharide-crude extracts showed strong inhibitory effect. The number of colonies were also investigated, which the concentration of 75% was found to be most effective as antibacterial agents. Seen that at 75%, grows of *P. acnes* decrease significantly. It is assumed that xylooligosaccharide-crude extracts have the ability to decrease the growing of *P. acnes* isolates. The ability is akin to presence of acidic xylooligosaccharides which exhibits an antibacterial activity.

**Keywords:** oat-spelts xylan, hydrolysis parameters, xylooligosaccharides, antibacterial agent, *Propionibacterium acnes*.

*not presented*
O-BM03

MOLECULAR GENOTYPING OF HBV BY USING NESTED PCR AND RFLP AMONG HEPATITIS B PATIENTS IN YOGYAKARTA AND SURROUNDING AREA

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Hepatitis B virus (HBV) can be classified into 8 genotypes, namely genotype A to H. Genotype of HBV is important for clinical and etiological investigations. Research for HBV genotyping and study of HBV transmission using nested PCR in endemic area in Camerun have been reported by Repp et al (1993). Moreover HBV genotyping based on RFLP using restriction enzymes also have been reported by Zeng et al., (2004). However, both of these methods have been not applied for genotyping study among hepatitis B patients in Indonesia yet. Molecular genotyping of HBV will describe epidemiology of HBV, pathogenesis and clinical implication of HBV. Combination of nested PCR and RFLP methods to determine genotype of HBV in Indonesia is still less information. This study was to develop a system for HBV genotyping by nested PCR combined with RFLP method for surface (S) protein encoding gene of HBV genom and also to determine HBV genotypes which predominantly found among hepatitis B patients in Yogyakarta and surrounding area.

Total of 149 sera samples from chronic hepatitis B patients from Yogyakarta and Central Java Province were used in this research. DNA of HBV was extracted from patients sera and used as template in first round amplification using outer primers set. Amplification products of first round PCR were used as template for second round amplification using inner primers set. Amplification products of second round PCR were restriction digested by Sty I and Bsr I enzymes. For HBV genotyping then the restriction products were RFLP analyzed based on length of nucleotides.

Nested PCR amplification using outer primer sets generated DNA fragments in size 1.233 bp. This DNA fragment are whole gene encoding S protein of HBV. Amplification using inner primer set generated DNA fragments in size 585 bp. Genotype analysis for all samples using RFLP methods by restriction digested of Sty I and Bsr I enzymes found only 2 HBV genotypes among hepatitis B patients, namely genotype B and C. Quantification showed that in Yogyakarta and surround area the most of hepatitis B patients infected by HBV genotype B (92.8%), and genotype C (3.6%). There is 3.6% unidentified genotype.

Keywords: HBV, nested PCR, RFLP, genotyping, Sty I and Bsr I enzymes
O-BM04

MOLECULAR EFFECTS OF METHOXYACETIC ACID ON HUMAN SKIN FIBROBLAST CELLS
IN VITRO

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Methoxyacetic acid (MAA) is a metabolite of ester phthalates and is a potent bio- toxin. Because of the wide use of ester phthalates in daily life and commercial products including, detergents, cosmetics, insecticides, plasticizers, gelling agents and food containers, it poses a risk to human health. Some studies have shown the presence of ester phthalate-metabolites in the milk, urine, saliva and serum of pregnant women. Hence there is an immediate and urgent need to study the molecular effects of MAA in living cells. To investigate the molecular effects of MAA, Human skin fibroblasts cultured at 50-60 % confluency were treated with MAA (10 mM) for 24 h followed by culture in normal medium for 1 h and subjected to molecular analyses. We found that MAA upregulated the expression of p53 and p21 proteins and downregulated proteosome activity in human skin fibroblasts leading to growth arrest that appeared similar to senescence phenotype as detected senescence associated β-GAL staining. We found that MAA treatment induced ROS production and DNA damage evoking early senescence in normal human fibroblasts.

Keyword: cellular, methoxyacetic acid, human skin fibroblast, in vitro
O-BM05

CLONING OF LARGE HEPATITIS B SURFACE ANTIGENS (L-HBSAG) FROM INDONESIAN ISOLATES : DEVELOPMENT OF THE THIRD GENERATION VACCINE

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Hepatitis B virus surface antigen (HBsAg) vaccination has been proven to be effective in preventing hepatitis B virus (HBV) infection. The S protein of hepatitis B virus is the principal component of virus envelope and the primary target of anti-HBs response. The extension polypeptides, namely large HBsAg (L-HBsAg), and they may play an important role in the process of virus infection and the induction of a defensive host response. Two isolates of large HBsAg were amplified by PCR from HBV genome which were obtained from Hasan Sadikin Hospital, Bandung, Indonesia. Subsequently the resultant fragments were then purified, ligated into pGEM®-T Easy vector, and cloned in Escherichia coli DH5α. Clones of L-HBsAg, designated as pGLHB. The DNA sequences of L-HBsAg were compared to the corresponding gene of HBV in the NCBI database and were found to have 98-99% similarity. One of pGLHB refers to wild type of L-HBsAg, was detected to be identical in “a” determinant area, in a major antigenic region in L-HBsAg. Another pGLHB was detected to have one substitution of amino acid T143M at “a” determinant area refers to vaccine escape mutant. Both of strains were detected as genotype B. The wild type and T143M escape mutant were also detected as adw2 and ayw1 subtypes, respectively. This research suggests the possibility of producing a new alternative wild type and escape mutant T143M third generation HBV vaccine to overcome hepatitis B problem in Indonesia.

Keywords : Large HBsAg, T143M, and Indonesia
O-BM06

PHYTOCHEMICAL CONSTITUENTS TOWARDS CONTRIBUTE AN ANTIOXIDANT AND ANTI-CANCER PROPERTIES OF DIFFERENT ZINGIBER SPECIES ENDEMIC TO BORNEO ISLAND

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Methanolic and aqueous extracts of rhizomes and stems of two Zingiber species (Zingiber vinosum and Zingiber pseudopungens) were evaluated for total polyphenols (qualitative and quantitative analyses) and antioxidant activity. The qualitative results showed flavonoid, alkaloids and saponin are present in the extracts. Quantitative analyses by HPLC showed gallic acid, caffeic acid, p-coumaric acid and rutin are presence in rhizome of Z.pseudopungens. The evaluation by spectrophotometric methods showed total phenolic and total flavanoid content in both rhizomes and stems of Z. vinosum and Z. pseudopungens were in the range of 4.46 to 41.70 gallic acid equivalent/g and 0.04 to 8.50 catechin equivalent/g, respectively. Evaluation of antioxidant activity was conducted using 1,1-diphenyl-2-picrylhydrazyl free radical-scavenging assay (DPPH), 2,2′-azinobis-3-ethylbenzothioazoline-6-sulphonate radical scavenging assay (ABTS) and ferric-reducing antioxidant power (FRAP). Among the results, methanolic extracted showed the higher result of all analyse than aqueous extracts. The results showed that the stem of Z.vinosum displayed the highest antioxidant properties in three different assays compared to other samples (p<0.05) for methanolic extracts. Z.vinosum have effected to MDA-MB-231 cells and arrested at G1 phase through cell cycle analyses. As conclusion, endemic gingers investigated in this study are shown to be a novel rich source of natural antioxidant and anti-cancer that was contributed by its phytochemical constituents.

Keywords : Z. vinosum, Z. pseudopungens, total phenolic, total flavanoid, antioxidant activity, anti-cancer activity.

not presented
O-BM07

ESSENTIAL OIL PROFILE OF TEMU LAWAK (Curcuma xanthorrhiza Roxb.) CALLUS AFTER HAVING PHOTOPERIOD AND SUCROSE VARIATIONS

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The level of sucrose and photoperiod variation affect secondary metabolites, especially essential oil content, in callus. The research aimed to analyze the effect of photoperiod and sucrose variation on essential oil contents in callus and its similarity with essential oil in the in vivo shoot. Temulawak shoots were grown on the MS medium without or with the addition of 1 μM NAA, 0.5 μM BAP and two levels of sucrose concentrations (20 and 30 g/l) then treated with four levels of photoperiod (0, 8, 16 and 24 hours/day). The callus growth were observed until fourth week after incubation. Essential oil were analyzed using gas chromatography. Based on the peak pattern and area, the chromatogram profiles of essential oil were compared. Data were analyzed descriptively and quantitatively. The similarity index between the samples were analyzed using simple matching measurement. The result showed that low sucrose level (20g/l) and short photoperiod decreased the callus growth and the quality (number of compounds) of essential oil. However its quantity (concentration of compounds) of essential oil was increased. The increase of photoperiod increased the quality of compound in the essential oil. MS medium with growth regulator, 20 g/l sucrose and 24 hours/day photoperiod produced the highest quantity of essential oil (14.387.700 AU), while MS medium with growth regulator, 30 g/l sucrose and 24 hours/day photoperiod produced the highest quality of essential oil. The highest similarity on the quality of essential oil was showed between shoot and medium with growth regulator, 30 g/l sucrose, 24 hour/day photoperiod and two weeks incubation with coefficient similarity of 62.5%.

Keywords: temulawak, callus, essential oil, gas chromatography.

INTRODUCTION

Temulawak is a member of Zingiberaceae family. The plant is Indonesian native, distributed from the costal area upto the hill. The plant rizhomes are used as a traditional medicine due to the cell secretary content known as assestial oil. The plants are propagated conventionally by planting the seedling. However, traditional propagation seems faced a lot of constrains such as requiring 7 months for growing the plant, large areas for planting should be provided and a lot of labour are needed for planting and harvesting the plant. To ensure the production, plant cell culture methodology provides an alternative approach to the production of plant secondary metabolites considering that each cell carries all the genetic information for all plant functions, including the biosynthesis of secondary metabolites [1]. In the past years, plant cell culture was shown to be technologically feasible on industrial scale.
However, in most cases the growth of the cell cultures is slow and the productivity is too low for an economically feasible production. Many strategies have been tried to improve productivity. For instance, screening and selection of high-producing cell lines and optimization of growth and production media. However, medium optimization only works if a basal level of the desired compounds is present in the cell culture. Hairy root cultures obtained by the transformation of plants with *Agrobacterium rhizogenes* are another possibility for productivity improvement. However, it is difficult to grow hairy root on a large scale. Abiotic and biotic elicitors induce the production of secondary metabolites but this approach is limited to certain type of compounds only, and in many cases these are not the desired compounds [2]. Therefore, the research aimed to analyze the effect of photoperiod and sucrose treatments on essential oil contents in *Curcuma xanthorrhiza* callus and its similarity content with essential oil in the *in vivo* shoot.

**MATERIAL AND METHODS**

*Temulawak* shoots were grown on the MS medium without or with the addition of 1 μM NAA, 0.5 μM BAP and two levels of sucrose concentrations (20 and 30 g/l) then treated with four levels of photoperiod (0, 8, 16 and 24 hours/day). The experiment used four replications. The growth of callus were observed at the second and fourth week after incubation. The growth of callus were assessed based on the size, calour and texture. Essential oil analyses were decided based on the results of callus growth. Essential oil were extracted using petroleum ether and analyzed using gas chromatography (GC). Based on the peak pattern and area, the chromatogram profiles of essential oil were analysed. Data were analyzed descriptively and quantitatively.

The similarity index between the samples were analyzed using simple matching measurement. To indetify the compounds, the analyses were continued using gas chromatogrophy-mass spectra (GC-MS).

**RESULTS AND DISCUSSION**

The quality of callus were displayed in Figure 1. From the table, it is known that the explants grown in medium with growth hormon gave better quality than those of medium without growth hormon. The results were in egreement with Dodds and Robert [3] who stated that the suplay of auxin and cytokinin in the medium will increase the growth of callus. Based on the results from Figure 1, the analyses of essential oil were selected from the
callus grown in medium with growth hormon (Figure 2). The callus were selected based on the exotic characters of the callus. Callus number 2, 6, 7, 8, 10, 14, 15, 16 and shoot were selected for essential oil analyses.

Figure 1. Temulawak callus quality after four weeks growing in medium with or without growth hormon based on the size, color and texture.

<table>
<thead>
<tr>
<th>Medium</th>
<th>Size*</th>
<th>Colour**</th>
<th>Texture***</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medium without growth hormon</td>
<td>2.54 ± 1.12</td>
<td>3.91 ± 0.34</td>
<td>2.11 ± 0.65</td>
</tr>
<tr>
<td>Medium with growth hormon</td>
<td>3.96 ± 1.54</td>
<td>4.62 ± 0.38</td>
<td>2.5 ± 0.44</td>
</tr>
</tbody>
</table>

* The size were graded from 1 upto 7 with the interval 0.5 cm

** The colour were graded based on Munsen Color charts plant tissues 2.5 Grey – Yellow

*** The texture were graded from 1 upto 3 based on the callus compacness

The data were collected without respecting two other treatments.

The results of essential oil analyses in intact plant and callus using chromatography gas can be seen in table 3. The varitions in peak number and compound concentration may caused by environment factors [4]. Two of them are photoperiod and sucrose concentration. The analyses were continued with simple matching measurement to know the best treatments which are the most similar with intact plant. The result of simple matching measurement is displayed in Figure 3.

Figure 2. Temulawak callus quality after two and four weeks growing in medium with growth hormon, two kinds of sucrose concentration, and four levels of photoperiod based on the size, color and texture.

<table>
<thead>
<tr>
<th>Callus number</th>
<th>Medium</th>
<th>Size*</th>
<th>Colour**</th>
<th>Texture***</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>S20C0W2</td>
<td>2.00 ± 1.00</td>
<td>4.67 ± 0.58</td>
<td>1.67 ± 0.58</td>
</tr>
<tr>
<td>2</td>
<td>S20C0W4</td>
<td>3.67 ± 0.58</td>
<td>5.00 ± 0.00</td>
<td>2.67 ± 0.58</td>
</tr>
<tr>
<td>3</td>
<td>S20C8W2</td>
<td>2.33 ± 0.58</td>
<td>5.00 ± 0.00</td>
<td>2.33 ± 0.58</td>
</tr>
<tr>
<td>4</td>
<td>S20C8W4</td>
<td>4.00 ± 1.00</td>
<td>5.00 ± 0.00</td>
<td>2.67 ± 0.58</td>
</tr>
<tr>
<td>5</td>
<td>S20C16W2</td>
<td>3.00 ± 1.00</td>
<td>4.33 ± 1.15</td>
<td>2.33 ± 0.58</td>
</tr>
<tr>
<td>6</td>
<td>S20C16W4</td>
<td>5.67 ± 1.53</td>
<td>4.00 ± 1.73</td>
<td>3.00 ± 0.00</td>
</tr>
<tr>
<td>7</td>
<td>S20C24W2</td>
<td>2.67 ± 0.58</td>
<td>4.00 ± 1.00</td>
<td>2.00 ± 0.00</td>
</tr>
<tr>
<td>8</td>
<td>S20C24W4</td>
<td>6.00 ± 1.00</td>
<td>4.00 ± 1.73</td>
<td>3.00 ± 0.00</td>
</tr>
</tbody>
</table>
Table 3. Peak numbers and peak area of GC chromatograms in *temulawak* intact plant and selected callus.

<table>
<thead>
<tr>
<th>Material</th>
<th>Shoot</th>
<th>Callus 2*</th>
<th>Callus 6*</th>
<th>Callus 7*</th>
<th>Callus 8*</th>
<th>Callus 10*</th>
<th>Callus 14*</th>
<th>Callus 15*</th>
<th>Callus 16*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>32</td>
<td>12</td>
<td>11</td>
<td>17</td>
<td>8</td>
<td>23</td>
<td>27</td>
<td>31</td>
<td>14</td>
</tr>
<tr>
<td>Total peak area (AU)</td>
<td>3,888.483</td>
<td>13,179.708</td>
<td>4,199,400</td>
<td>14,387,700</td>
<td>6,581.785</td>
<td>7,375,700</td>
<td>12,438,051</td>
<td>6,529,025</td>
<td>2,731,144</td>
</tr>
</tbody>
</table>

Callus 2: sucrose 20%, dark, 4 weeks growth; Callus 6: sucrose 20%, 16 hours photoperiod, 4 weeks growth; Callus 7: sucrose 20%, 24 hours photoperiod, 2 weeks growth; Callus 8: sucrose 20%, 24 hours photoperiod, 4 weeks growth; Callus 10: sucrose 30%, dark, 4 weeks growth; Callus 14: sucrose 30%, 16 hours photoperiod, 4 weeks growth; Callus 15: sucrose 30%, 24 hours photoperiod, 2 weeks growth; Callus 16: sucrose 30%, 24 hours photoperiod, 4 weeks growth.
Figure 3. Dendogram of similarity between shoot and selected callus based on essesntial oil content.

From the results, it can be known that low sucrose level (20g/l) and non photoperiod decreased the callus growth and the number of compounds of essential oil. However the quantity essential oil counted from total detected peak area was increased. The increasing photoperiod increased the number of compounds. MS medium with growth regulator, 20 g/l sucrose and 24 hours/day photoperiod (Callus 7) produced the highest quantity of essential oil (14.387.700 AU), while MS medium with growth regulator, 30 g/l sucrose and 24 hours/day photoperiod (Callus 15) produced the highest number of compound in essential
oil. The highest similarity on the kinds and the concentration of compounds was showed between shoot essential oil and the essential oil of callus grown in medium with growth regulator, 30 g/l sucrose, 24 hour/day photoperiod and two weeks incubation (Callus 7) with coefficient similarity of 62.5%. The chromatogram shows that four peaks were always accru in each reatment. Based on GC-MS anayses, it was known that two of them are curcumen and zingiberene.

REFERENCES


O-BM08

PHYTOCHEMICALS, ANTIOXIDANT AND ANTICANCER PROPERTIES OF BOESENBERGIA SPECIES (ZINGIBERACEAE) ENDEMIC TO BORNEO

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Boesenbergia, also known as Kaempferia is one of the genus from the family of Zingiberaceae. The species of Zingiberaceae family have been reported to possess both antioxidant and anti-inflammatory activity, and thus might be effective as anticancer agents. The present study was started with plants screening for the antioxidant, total phenolic and flavonoid content as well as cytotoxicity activities; further determination of the phytochemicals were conducted using High Performance Liquid Chromatography (HPLC) for the possible active compounds. In this study, the leaves and rhizomes from B. rotunda, B. pulchella, B. pulchella var attenuata and B. armeniaca were analysed separately. B. rotunda was used as a positive control. In overall, B. pulchella and B. pulchella var attenuata displayed better antioxidant activity compared to the positive control. Total phenolic content of the samples were in the range of 2.36±0.06 to 15.37±0.06 mg gallic acid equivalent/gram dry weight. MCF-7 (hormone dependent breast cancer), MDA-MB-231 (non-hormone dependent breast cancer), CaOV3 and HeLa (cervical cancer), HT-29 (colon cancer) were cultured for cytotoxicity activity. For MCF-7, B. rotunda, B. pulchella var attenuata (rhizome) and B. armeniaca (rhizome) displayed positive cytotoxic effects with IC₅₀ values of 51, 93±2.83 and 94.5±0.71 µg/ml, respectively. 13 standards of phytochemicals were run in HPLC. Quercetin was detected as the major active compound that contributed to anticancer activity. B. rotunda contained the major quercetin compound with 580.46 mg/kg dry matter followed by B. armeniaca (rhizome) with 498.43 mg/kg dry matter. The results revealed that extracts from certain Boesenbergia species displayed promising health-benefits properties.

Keywords: Boesenbergia species; antioxidant activity; phenolic; flavonoid; cytotoxicity

not presented
O-BM09

EFFECTS OF MONOVALENT AND BIVALENT VACCINES FROM Photobacterium damselae subsp. damselae AND Vibrio harveyi ON SERUM ANTIBODY RESPONSE OF THE ASIAN SEABASS, Lates calcarifer Bloch.

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The antibody response to monovalent and bivalent vaccines of Photobacterium damselae subsp. damselae and Vibrio harveyi, the gram negative bacterial pathogens correlated with diseases of Asian seabass, were evaluated for their effectiveness in developing bivalent vaccine. The Asian seabass were both vaccinated with monovalent and bivalent vaccines from formalin killed whole cells of Photobacterium damselae subsp. damselae and Vibrio harveyi. The sera from vaccinated and unvaccinated fish were collected every 7 days post vaccinations until 35 days post vaccination and determined the antibody titers which were analyzed by ELISA technique. A specific antibody response of seabass were detected at 7 days post vaccination that rose significantly (p<0.05) at 28 days and 21 days post vaccination for monovalent and bivalent group against Photobacterium damselae subsp. damselae antigen. Whereas, the antibody titers of monovalent and bivalent group against Vibrio harveyi antigen were detected at 7 days post vaccination and peaked significantly (p<0.05) at 21 days post vaccination. The vaccine efficacy in protecting fish from re-infection was evaluated in laboratory challenge. Similar level protection (p>0.05) was recorded in the group vaccinated with monovalent and bivalent vaccines. The results suggest that both monovalent and bivalent vaccines effectively triggered specific antibody production and protected the seabass against diseases caused by Photobacterium damselae subsp. damselae and Vibrio harveyi.

Keywords: Photobacterium damselae subsp. damselae; Vibrio harveyi; monovalent; bivalent; vaccine

not presented
O-BM10

PAPUAN DNA MITOCHONDRIAL DATABASE, A GENOMIC RESOURCE SUPPORTING POPULATION GENETICS STUDIES AND BIOMEDICAL RESEARCH

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Population genetics studies based on the analysis of mtDNA and mitochondrial bioetnoanthropology and biomedical studies have produced a huge quantity of sequence data and related information. These data, classified as mtDNA SNPs, HVS1 and HVS2 sequences, and D-loop mtDNA sequences, are at present distributed Papuan in differently organised databases and web sites. Several mitochondrial specialised databases and databases related with variability data have been designed and implemented, but generally they are structured as simple databases where data are stored, without the possibility to perform any analysis. Moreover it is not generally possible for the user to submit and contemporarily analyse its own data comparing them with the content of a given database and this is valid both for population genetics data, and for mitochondrial disease data. As far as population genetics data, for example, the problem of sequence classification in haplogroups is becoming more and more important as the improvement of sequencing technologies is increasing the availability of new complete D-loop genomes. Indeed, up to now, the pathogenicity of mtDNA mutations has been, in most cases, prevalently validated by their segregation with the disease and their consequent loss of function when the mutation involves a structural gene. Here we present the design of a Human Mitochondrial genome Database on Papuan Populations that will collect the D-loop human mitochondrial genomes publicly available interfaced to analysis programs, allowing the classification of newly sequenced human mitochondrial genomes, and the prediction, through site-specific nucleotidic and aminoacidic analysis, of the mutations potential of mitochondrial polymorphisms.

Keywords: Mitochondrial Database, Papuan Population, Genetics Studies, Bioetnoanthropology and Biomedical Research
Introduction

Population genetics studies based on the analysis of mtDNA and mitochondrial bioetnoanthropology and biomedical studies have produced a huge quantity of sequence data and related information. These data, classified as mtDNA SNPs, HVS1 and HVS2 sequences, and D-loop mtDNA sequences, are at present distributed Papuan in differently organised databases and web sites [1]. Several mitochondrial specialised databases and databases related with variability data have been designed and implemented, but generally they are structured as simple databases where data are stored, without the possibility to perform any analysis. Moreover it is not generally possible for the user to submit and contemporarily analyse its own data comparing them with the content of a given database and this is valid both for population genetics data, and for mitochondrial disease data. As far as population genetics data, for example, the problem of sequence classification in haplogroups is becoming more and more important as the improvement of sequencing technologies is increasing the availability of new complete D-loop genomes [2-3]. Indeed, up to now, the pathogenicity of mtDNA mutations has been, in most cases, prevalently validated by their segregation with the disease and their consequent loss of function when the mutation involves a structural gene.

Materials and Methods

These data, classified as RFLPs, mtDNA SNPs, pathogenic mutations, HVS1 and HVS2 sequences, and complete mtDNA sequences, are at present distributed worldwide in differently organised databases and web sites, not well integrated among them. Several mitochondrial specialised databases and databases related with variability data have been designed and implemented, but generally they are structured as simple databases where data are stored, without the possibility to perform any analysis. Moreover it is not generally possible for the user to submit and contemporarily analyse its own data comparing them with the content of a given database and this is valid both for population genetics data, and for mitochondrial disease data. However, no systematic statistical analysis of the mtDNA SNPs has been performed until now. Here we present the design of a Human Mitochondrial genome DataBase (HMDB) that will collect the complete human mitochondrial genomes publicly available interfaced to analysis programs, allowing the classification of newly sequenced human mitochondrial genomes, and the prediction, through site-specific nucleotidic and aminoacidic analysis[2][3], of the pathogenic potential of mitochondrial polymorphisms.
Results and Discussion

HMDB will allow the user to perform retrieval of data from the database, to analyse the query resulting genomes, to compare a newly sequenced genome with the stored data in order to classify it according to the haplogroup classification. Moreover this resource could contribute to estimate the pathogenetic proneness of a newly detected mutation. The project is in progress. The database and the whole resource have been completely designed (Fig. 1 shows some of the slides describing the whole project). The variability study of the available complete human mt genomes has been performed. Refinement of the resulting data and implementation of the resource will be performed in the present year. We hope to release the first version of the whole package at the end of 2010. Here we present the design of a Human Mitochondrial genome Database on Papuan Populations that will collect the D-loop human mitochondrial genomes publicly available interfaced to analysis programs, allowing the classification of newly sequenced human mitochondrial genomes, and the prediction, through site-specific nucleotidic and aminoaciddic analysis, of the mutations potential of mitochondrial polymorphisms.
References


O-BM11

ANTIFERTILITY ACTIVITY OF Flagellaria indica L. FRUIT EXTRACT ON FEMALE ALBINO MICE (Mus musculus L.) SWISS WEBSTER

Yohanes E. Gunawan, Meda, G. Sarahayu, A. Haryono, and Suatma

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Global search on anti-fertility agents is going on to tackle the problem of population explosion. Many hormonal drugs are available for the purpose but they are not free from side effects. Hence, the search for a suitable product from indigenous medicinal plants is proposed which could be effectively used fertility regulation. The traditional Kahayan Hilir people used uwei namei (Flagellaria indica L.) fruit as antifertility agent. The experimental work was evaluated for the effects of uwei namei fruit on female albino mice fertility regulation. The mice treated single dose of uwei namei ethanol extract fruit at 0.21, 0.42 and 0.84 mg/kg bw per oral. The result shows estrus cycle period be longer and dominate by estrus phase. The extract shows significantly decreasing preimplantation embryos, implantation embryos and totally no fetus found in all treated mice uterine. Result of this study suggested that the ethanol extract of Flagellaria indica fruit potentially used as anti-fertility agent.

Keywords: Antifertility activity
International Conference on Biological Science

ADVANCES IN BIOLOGICAL SCIENCE:

Respect to Biodiversity from Molecular to Ecosystem

for Better Human Prosperity

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P-MB01

SURVEY ON THE OCCURRENCE OF CUCUMBER GREEN MOTTLE MOSAIC VIRUS (CGMMV) INFECTING CUCURBITS IN YOGYAKARTA

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Abstract

Cucurbits including melon (Cucumis melo L.), watermelon (Citullus lanatus L.), cucumber (Cucumis sativus L.), angled loofah (Luffa acutangula L.) and bitter gourd (Momordica charantia L.) are grown throughout the Yogyakarta province of Indonesia as dry season crops. However, the cucurbits cultivation has been faced with virus infections which tend to reduce their production. Cucumber green mottle mosaic virus (CGMMV), a member of the genus Tobamovirus has been reported infecting cucurbits in Indonesia. To study the occurrence of CGMMV in Yogyakarta, a survey and samples collection were conducted in Sleman and Kulon Progo during March – June 2009. Double Antibody Sandwich Enzyme-Linked Immunosorbent Assay (DAS-ELISA) was applied to detect CGMMV in cucurbit samples. Results of survey showed that a total of 50 cucurbit samples have typical disease symptoms such as mosaic, mottling and malformation of leaves, fruit deformation and stunting of plants. DAS-ELISA result exhibited that cucurbits infected CGMMV in 2009 (4%) was lower than survey results conducted in 2000 and 2001 (12.8%).

Key words: CGMMV, Cucurbits, symptoms observation, DAS-ELISA

Introduction
Cucurbit crops, including melon (Cucumis melo L.), watermelon (Citrullus lanatus L.), cucumber (Cucumis sativus L.), angled loofah (Luffa acutangula L.) and bitter gourd (Momordica charantia L.), produced throughout Yogyakarta, a province in Indonesia, are important vegetable fruit crops. Java is one of the main cucurbit growing areas of Indonesia and more than 80% of national production is produced by five provinces in Java i.e. East Java, Central Java, Jakarta, West Java, and Yogyakarta (BSCI, 2003).

The yield of the crop of cucurbit plants depends not only on agricultural and meteorological condition, but also on the diseases including those caused by plant viruses. Plant viruses have become a problem in cucurbits production in Indonesia. Cucumber green mottle mosaic virus (CGMMV), a member of genus Tobamovirus has been reported for infecting cucurbits in Indonesia. Daryono and Natsuaki (2009) reported that melon and cucumber plants grown in Java Indonesia during 2000-2001 were severely affected by a new kind of mosaic disease. The infected plants showed growth retardation, mottle and mosaic on the leaves and water soaked spots on the surface of fruits. Sometimes malformation of fruits was also observed. A virus with morphological characteristics similar to CGMMV was originally isolated from melon plants, but not further characterized. Despite this information, there is no data about the incident and distribution of CGMMV in field grown cucurbits in Yogyakarta, Indonesia.

Materials And Methods

Surveys and samples collection. Samples were collected during March – June 2009 from 2 districts in Yogyakarta including Sleman and Kulon Progo. Fields were selected randomly from various locations in 5 villages in Sleman and 3 villages in Kulon Progo. Surveys were performed in 12 fields containing melon, watermelon, cucumber, angled loofah, and bitter gourd plants.

Surveys were preferably conducted during a mid developmental stage of the cucurbit crops. Plants were randomly evaluated base on symptoms though to be caused by virus infection such as chlorosis, mosaic, mottling and malformation of leaves, fruit deformation and stunting of plants. Young leaves from some symptomatic plants were placed on plastic bags and incubated in refrigerator at -20°C.

Serological testing. The Double Antibody Sandwich Enzyme-Linked Immunosorbent Assay (DAS-ELISA) method was performed according to Clark and Adams (1977). Melon, water melon, cucumber, angled loofah, and bitter gourd were ground in
mortar with carbonate buffer (pH: 9.6) added to wells which were pre-coated with *Cucumber green mottle mosaic virus* (CGMMV) specific polyclonal antisera respectively diluted in extraction buffer (PBS buffer). Plates were incubated at 4°C overnight and washed three times with PBS-Tween 20 buffer. Then, plates were coated with alkaline phosphatase conjugated antibody diluted in extraction buffer and incubated for 3 hours at 37°C. After washing, p-nitrophenyl phosphate in diethanolamine substrate buffer (1 mg/ml, pH: 9.8) was added to each well and incubated at room temperature for 1 hour. Absorbance values were read at 405 using a microplate reader. The dilution was 1:200 for the antisera to CGMMV and for the conjugate antibody.

Healthy cucurbit species were used in DAS-ELISA as negative control and the CGMMV infected melon plants were used as virus positive controls to compare absorbance values among plates. The average of absorbance values of three wells for each sample was used for evaluates virus infection. Samples were considered to be positive when the absorbance at 405 nm values exceeded more than 2 times of negative control.

**Results and Discussion**

**Results**

A total of 12 fields were surveyed and 50 plant samples were randomly collected from two cucurbit growing areas in Yogyakarta during March – June 2009. Amongst 50 plant samples, 42 were collected from Sleman while 8 samples were collected from Kulon Progo (Table 1).

During the survey, most fields contained plants with viral symptoms. Field surveys of the cucurbit crops, symptoms observation revealed a high incidence of leaf mosaic, mottling and malformation, fruit deformation and stunting of plants. The angled loofah shows mosaic symptoms during the survey (Figure 1).

![Figure 1. Angled loofah showing mosaic symptoms in Brebah-Yogyakarta.](image)

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The results of serological test Double Antibody Sandwich Enzyme-Linked Immunosorbent Assay (DAS-ELISA) showed that cucurbit plant were infected by CGMMV. Tests were conducted using several negative controls of cucurbit plants. Therefore, the range of absorbance values of negative controls varied from 0.631 to 0.834 at 405 nm, whereas positive samples gave absorbance value of 1.053 to 1.290 (Table 2).

Table 1. Numbers of fields surveyed and samples collected and tested in each district/village during survey in March – June 2009

<table>
<thead>
<tr>
<th>District, Villages</th>
<th>No. of fields surveyed</th>
<th>No. of samples collected</th>
<th>DAS-ELISA* CGMMV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sleman</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cangkringan</td>
<td>3</td>
<td>10</td>
<td>0/10</td>
</tr>
<tr>
<td>Ngemplak</td>
<td>1</td>
<td>1</td>
<td>0/1</td>
</tr>
<tr>
<td>Kadirojo</td>
<td>1</td>
<td>5</td>
<td>0/5</td>
</tr>
<tr>
<td>Tempel</td>
<td>1</td>
<td>6</td>
<td>0/6</td>
</tr>
<tr>
<td>Brebah</td>
<td>3</td>
<td>20</td>
<td>2/20</td>
</tr>
<tr>
<td>Kulon Progo</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Karang</td>
<td>1</td>
<td>3</td>
<td>0/3</td>
</tr>
<tr>
<td>Sewu</td>
<td>1</td>
<td>2</td>
<td>0/2</td>
</tr>
<tr>
<td>Congot</td>
<td>1</td>
<td>3</td>
<td>0/3</td>
</tr>
<tr>
<td>Glagahsari</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>12</td>
<td>50</td>
<td>2</td>
</tr>
<tr>
<td>% infection</td>
<td>-</td>
<td>-</td>
<td>4</td>
</tr>
</tbody>
</table>

DAS-ELISA reading (absorbance at 405 nm) of sample extracts

Table 2. Range of absorbance values of negative control, positive and negative samples

<table>
<thead>
<tr>
<th>Plant</th>
<th>Absorbance values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative controls</td>
</tr>
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<td></td>
<td></td>
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</tbody>
</table>
The incidence of these viruses in the sample of cucubit plants is shown in Table 3. The result shows that CGMMV was detected in 1 of 12 field or 2 of 50 plant samples. CGMMV was not detected in Cucumis melo, Cucumis sativus, Citrullus lanatus, and Momordica charantia and only detected in Luffa acutangula. In Luffa acutangula, CGMMV was detected in 2 of 7 plants respectively.

Table 3. The incidence of viruses on cucubit plants grown in Yogyakarta

<table>
<thead>
<tr>
<th>Species</th>
<th>No. of samples tested</th>
<th>CGMMV*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cucumis melo</td>
<td>24</td>
<td>0</td>
</tr>
<tr>
<td>Cucumis sativus</td>
<td>13</td>
<td>0</td>
</tr>
<tr>
<td>Citrullus lanatus</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Momordica charantia</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Luffa acutangula</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>50</strong></td>
<td><strong>2</strong></td>
</tr>
<tr>
<td>Percent (%) Infected</td>
<td>-</td>
<td><strong>4</strong></td>
</tr>
</tbody>
</table>

Discussion

CGMMV was detected for first time in Indonesia in 2000 (Daryono and Natsuaki, 2009). During the survey, CGMMV was detected only in angled loofah samples. Infected angled loofah showed mottle mosaic symptoms on the leaves and stunting of plants. Moreover, CGMMV infected only 4% of collected samples. This value was lower than survey results conducted by Daryono and Natsuaki (2009) in 2000 and 2001, that ELISA result 12.8% of CGMMV. CGMMV is a member of the genus Tobamovirus has been reported as a serious disease agent of cucubit crops and causing economical losses in several countries (Ugaki et al., 1991; Antignus et al., 2001; Shim et al., 2005). CGMMV was found in Europe, Japan, Saudi Arabia, Israel, Greece, Pakistan, and Korea (Ainsworth, 1935; Ugaki et al.,...
1991; Al-Shahwan dan Abdalla, 1992; Antignus et al., 2001; Ververi, et al., 2002; Ali et al., 2004; Shim et al., 2005). CGMMV is spread efficiently by mechanical transmission and infected seeds (Ryu et al., 2004 and Daryono et al., 2009).

In this study CGMMV was found in Yogyakarta province, Indonesia. Though in this time CGMMV only infected angled loofah, preventive needed to prevent spreading of virus more to other agriculture crops.

Acknowledgment

This study was supported by The Indonesia Toray Science Foundation (ITSF) Science and Technology research grant for 2009. We would like to thank Dr. Tri Joko, Plant Biotechnology Laboratory, Faculty of Agriculture, Gadjah Mada University for providing melon samples.

References


P-MB02

EXPRESSION OF SUCROSE PHOSPHATE SYNTHASE IN TRANSGENIC SUGARCANE AFTER FIVE ROUNDS OF VEGETATIVE PROPAGATION

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ABSTRACT

Sucrose Phosphate Synthase (SPS; EC 2.4.1.14) is a key enzyme for sucrose biosynthesis in plants. It catalyzed the formation of sucrose from fructose-6-phosphate (F – 6 – P) and uridine-5-diphospho glucose (UDPG). The over-expression of SPS in sugar cane had led to increase the synthesis and accumulation of sucrose. In this research, the expression of SPS gene have been investigated in the transgenic sugar cane after grown for five rounds of that were grown in the research field of PTPN XI at Jatiroto-Lumajang. The agronomic parameters, SPS activity, sucrose, and PCR analysis were investigated. RT-PCR analysis showed that about 69% of sugarcane was positively expressing the SPS gene under control of CaMV 35S promoter. The SPS activity and sucrose content were higher than those of wild type in some clones of sugarcane. The number of secondary shoot of transgenic sugarcane was difference compared with the wild type; it had more secondary shoots than that of wild type. Regarding to the morphological and physiological characteristics, the results show that transgenic sugarcanes have genetic stability after five rounds of vegetative propagation in the field planting.

Keywords: Sucrose Phosphate Synthase (SPS), transgenic sugarcane, over expression

INTRODUCTION

Sucrose Phosphate Synthase (SPS; EC 2.4.1.14) is a key enzyme for sucrose biosynthesis in plants. It catalyzed the formation of sucrose from fructose-6-phosphate (F – 6 – P) and uridine-5-diphospho glucose (UDPG). The over-expression of SPS in sugarcane had led to increase the synthesis and accumulation of sucrose (Miswar et al., 2007). In this research, have investigated the expression of SPS gene in the transgenic sugarcane after five rounds of that were grown in the research field of PTPN XI at Jatiroto-Lumajang under
control of biosafety risk assessment. The SPS activity and sucrose content were higher than those of wild type in some clones of sugarcane. The number of auxiliary shoot of transgenic sugarcane was difference compared with non-transfromant sugarcane; it had more number of auxiliary shoot than that of wild type. Regarding of the morphological and physiological characteristics the results showed that transgenic sugarcanes have genetic stability after five rounds of vegetative propagation in the field planting.

MATERIAL AND METHODS

Plant Growth Condition

Transgenic sugarcane plants were grown in the field experiment station of PTPN IX at Jatiroto-Lumajang for 1 year. Fully expanded youngest leaf (the first visible dewlap) was harvested from 4 lines of 3 months old of transgenic sugarcane (27 individual plants) at day time. The leaf samples were divided into three parts along middle lamella for isolation of DNA, enzymes and sucrose analysis, and plunged into liquid nitrogen.

Protein extraction and Measurement of SPS activity

Frozen leaves were ground in a mortal with liquid nitrogen and after thawing, grinding was continued in extraction buffer (3 mL/g fresh weight) containing 50 mM 4-morpoline-propanesulfonic acid (MOPS)-NaOH (pH 7.5), 10 mM MgCl₂, 1 mM EDTA, 5 mM DTT, 2% PEG6000, 0.5 mM phenylmethanesulfonyl fluoride (PMSF), and 10% polyvynil polypirrolidone (PVP). After centrifugation at 10,000 rpm for 10 minute at 4°C, supernatant was desalted on Sephadex G-25 column. Activity of SPS was measured according to the method described by Sugiharto et al. (1995).

Western blot analysis

Extracted total leaf protein (30 µg) was separated by Sodium Dodecyl Sulfat-Polyacrylamide Gel Electrophoresis (SDS-PAGE) on 12.5% gel and transferred to nitrocellulose sheet. The filter was washed by TBS, and then blocked with 0.5% milk protein (low fat). After incubation with first antibody against recombinant-SPS protein (Sugiharto et al 1997), then incubated with second antibody, Goat Anti-Rabbit IgG AP Conjugated. The
membrane was washed again with TBS solution, followed by visualization of signal with BCIP and NBT.

Measurement of sucrose contents

Sugars were extracted from 4 lines of transgenic sugarcane by blended steam of 1 year planted transgenic sugarcane. Extracted sugars were then diluted with water and measured with spectrophotometer as mentioned in Sugiharto et al. (1995).

RESULT AND DISCUSSION

Activity and level of SPS protein.

The SPS activities was varied between lines of the transgenic sugarcane as commonly observed with transgenic plants, but the activity was higher compared to the control non-transformant (Fig. 1). To analyze whether the increase of SPS activity was due to the increase of expression of SPS gene, SPS protein levels were detected by Western Blot analysis. The level of SPS protein corresponding with 128 kDa was clearly detected in the transgenic sugarcane, but was undetectable in the control of non-transgenic sugarcane (Fig. 2). Significant increased the level of SPS protein was found in the leaf of transgenic sugarcane line 7. The results confirmed previous observation that over-expression of SoSPS1 gene increase SPS activity in the transgenic plants.

![Figure 1. Variation of SPS activity (mg sucrose minute-1 mg-1 protein) in leaf of transgenic and control non-transgenic (K) sugarcane. Proteins were extracted from the leaf of 3 months sugarcane plant and SPS activity was measured spectrophotometrically in the extracts.](image1)

![Figure 2. Detection of SPS protein by western blot analysis using antibody against sugarcane recombinant-SPS protein. Total proteins were extracted from leaf of transgenic and non-transgenic sugarcane and subjected for western blot analysis. Lin 1, 2, 3, 4, 5 are presenting proteins extracted from non-transgenic, transgenic line 4, 5, 6, 7, respectively.](image2)
The sucrose content.

The increased in the activity of SPS would increase sucrose biosynthesis and content in plants. Measurement of sucrose content in steam of 1 year old transgenic sugarcane showed that the sucrose content was significantly increased SPS in all of the transgenic lines (Fig. 3). However, the reduction sugars contents seem to be lowered compared to the non-transgenic sugarcane. Furthermore, morphological observation showed that number of auxiliary shoot of transgenic sugarcane was difference compared with non-transfromant sugarcane; it had more number of auxiliary shoot than that of wild type. Collectively, the results indicated that expression of SPS is stable increased in the transgenic sugarcane after five round vegetative propagation.

Figure 3. Concentration of sucrose and reduction sugars in steam of transgenic and non-transgenic sugarcane. The sugars were extracted from steam of 1 year cultivated sugarcane and measured with spectrophotometer. Presented data is means from two experiment replications.

Acknowledgements

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REFERENCES


P-MB 03

DIVERSITY ANALYSIS OF INDIGENOUS Bacillus thuringiensis ISOLATES PATHOGENIC TO Crocidoloma binotalis BY USING NUMERICAL SYSTEMATIC APPROACH

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Abstract

Diversity of B. thuringiensis (Bt.) isolates pathogenic to C. binotalis was determined by using Numerical Systematic Method. Ten isolates were taken to represent 43 pathogenic isolates along with two reference strains namely B. thuringiensis serovar kurstaki HD1 and B. thuringiensis serovar israelensis H14. The test isolates were examined for 89 phenotypic characters by using conventional method for colonial and cell morphology (37 characters) as well as physiological characteristics (3 characters) but biochemical characterization (49 characters) was conducted by using commercial API-50 CHB procedures. All phenotypic characters existed in one of two mutually exclusive states and were either scored plus (1) of minus (0). The binary data were prepared in Programmer’s File Editor (PFE) software. The data were analysed by using the Multi Variate statistical Package (MVSP) Plus-Version 2.0 using the Simple Matching Coefficient (SSM). Clustering was achieved using the UPGMA algorithm. The results were presented as dendrograms. It was obtained that the test isolates were clearly assigned to two distinct multimerbar clusters defined by 79.6 similarity level (S-level) in the SSM, UPGMA analysis. The two distinct clusters represented by each of two widely known different group of Bt. strains, namely serovar israelensis and serovar kurstaki. The first cluster contained reference strain of B. thuringiensis serovar israelensis, and two of the isolates (Slk2.3, and YPPA1) and the second cluster contained another reference strain of B. thuringiensis serovar kurstaki, and 8 of the isolates. Therefore, it strongly suggested that the application of numerical-fenetic analysis could provide a powerful tool to unravel the strain diversity belong to species B. thuringiensis.

Keywords: diversity, indigenous Bacillus thuringiensis, isolates, pathogenic, Crocidoloma binotalis, numerical systematic.
P-MB04

THE 45 kDa FIMBRIAE PROTEIN OF PROTEUS MIRABILIS AS HEMAGLUTININ AND ADHESION MOLECULE

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ABSTRACT

Proteus mirabilis is opportunistic and nosocomial pathogen that usually found in clinical specimen from patients with catheter. The pathogenic mechanism of the bacteria are not fully elucidated especially its potential activity of the protein as hemaglutinin and adhesion molecule. The aim of this study is to evaluate the role of 45 kDa fimbriae protein from P. mirabilis. The fimbrial preparation was analyzed by poly acrilamide gel electrophoresis in the presence of sodium dodecyl sulfate (SDS-PAGE) in slab gels by standart methods (Smeds et al. 2001). The dominant bands were cut and elucidated for furthermore analysis. The dialisat solution is hemaglutinized and invtro adhesion test. The study showed that the 45 kDa fimbria protein of P mirabilis was a hemaglutinin protein that agglutinated mice erythrocytes, rabbit erythrocytes, and human group O erythrocytes. Hemaglutination test were negative on erythrocytes human blood group A,B, and AB. The 45 kDa fimbriae protein was also adhesion protein showed by its activity to adherence to rabbit vesica urinaria epithel receptor. The increase dose of 45 kDa fimbriae protein will decrease the mount of P mirabilis bacteria to adherence to rabbit vesica urinaria epithel (p< 0,05).

Keywords : Proteus mirabilis, fimbriae, hemaglutinin protein, adhesion molecule.

INTRODUCTION

Adherence to specific epithelial element of the human urinary tract is an important factor for the Gram negative bacteria( Salyer and White, 2002). This adhesion is mediated by specific fimbrial types .Analysis of the tissue binding specificities of the various of uropathogenic E. coli in the human kidney and bladder has demonstrated that the virulence potential carried by a fimbrial type depend of two factor : the present of receptor molecules
on the uroepithelia and the lack of soluble inhibitors of adherence in urine (Vircola et al, 1988). The uropathogenic strain of P. mirabilis also adhere to exfoliated epithelial cells of human urine and frequently express fimbriae. Fimbriae of P. mirabilis are expressed in kidneys of infected rats and are presumably important in the pathogenesis of pyelonefritis caused by these bacteria. The pathogenic mechanism of the bacteria are not fully elucidated especially its potential activity of the protein as hemaglutinin and adhesion molecule. The aim of this study is to evaluate the role of 45 kDa fimbriae protein from P. mirabilis.

METHODS

Bacteria was isolated from the urine patient which is grown in biphasic medium BHI-TCG (Ehara, 1992). After 2 X 24 hours bacteria was harvested and isolated fimbriae by an omnimixer. The fimbrial preparation was analyzed by poly acrilamide gel electrophoresis in the presence of sodium dodecyl sulfate (SDS-PAGE) in slab gels by standart methods (Smeds et al. 2001). The dominant bands were cut and elucided for furthermore analysis. The dialisat solution is hemaglutinized with Lie method (1999). The protein inhibiting erythrocytes agglutination, with the largest dilution is then continued with adhesion test from the bladder epithelia of a rabbit using Weiss method. (Nagayama et al.1995).

Results

After the pili are cut gradually, until a supernatant color equally PBS, electrolysis with SDS-PAGE is done to the fimbriae to find out the molecule weight of each protein, specifically the 45 kDA protein.
Fig 1. SDS-PAGE result from various fragments *P mirabilis* fimbriae. Lane 1, protein marker, lane 2 fimbrial fragment 1, lane 3 fimbrial fragment 2, lane 4 fimbrial fragment 3, lane 5 fimbrial fragment 4.

Afterwards, the protein is cut, electroelucided, and dialysed, resulting in a protein solution. Furthermore from this result, a hemaglutination test is executed on the erythrocytes of rat, rabbit and human with A,B,O,AB blood type and the outcome is that the 45 kDa fimbriae protein is able to inhibit agglutination rat, rabbit and O blood type erythrocytes.

Table 1. Hemaglutination test result of 45 kDa fimbriae protein from *P. mirabilis* with gradual dilution different types of erythrocytes.

<table>
<thead>
<tr>
<th>Sample erythrocytes</th>
<th>Dilution 45kDa protein 1x</th>
<th>2x</th>
<th>3x</th>
<th>4x</th>
<th>5x</th>
<th>6x</th>
<th>7x</th>
<th>8x</th>
<th>9x</th>
<th>10x</th>
</tr>
</thead>
<tbody>
<tr>
<td>rat</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>rabbit</td>
<td>+</td>
<td>+</td>
<td>-</td>
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When an adhesion inhibition test using 45kDa fimbriae protein is conducted, the results were a reduction of bacteria quantity attached to bladder epithelia compared to those which is not coated with the protein. The amount of bacteria attached to the epithel decreases in accordance with the increased dosage of 45kDa protein.

REFERENCE


P-MB05

Transfer of pbinsRNAlics plasmid into Musa acuminata (AAA Group) cv. pisang ambon plant tissues using Agrobacterium tumefaciens

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Abstract

Agrobacterium tumefaciens is known to be able to genetically transform monocotyledon tissues including banana. The aim of this research was to transform pbinsRNAlics plasmid into banana (Musa acuminata AAA group cv. pisang ambon) leaf tissues using A. tumefaciens harboring pbinsRNAlics plasmid, having kanamycin resistance gene as selection marker and the ACC (1-aminocyclopropane-1-carboxylic acid) synthase gene RNAi construct as gene of interest. The research was performed by several steps, including compatibility test of A. tumefaciens, optimization of phytohormone concentration to induce tissue growth in vitro, sensitivity test of banana leaf tissue toward kanamycin antibiotic, plant genetic transformation mediated by A. tumefaciens using vacuum infiltration method, and transgenic leaf selection. Compatibility test of A. tumefaciens between A. tumefaciens strain GV3101 and GV3850 showed that A. tumefaciens strain GV3101 was the most compatible strain to infect banana leaf tissue. In vitro tissue growth in form of leaf enlargement was shown in the combination of 1.8×10⁻⁵ M of 2,4-D and 4.4×10⁻⁶ M of kinetin with an average increase on diameter of 0.22±0.12 cm in the average. Kanamycin concentration which inhibited banana leaf tissue growth was 60 mg/L, this concentration was used in the selection medium. Banana leaf tissues, which have been transformed using Agrobacterium, cultured in leaf enlargement media added with 60 mg/L of kanamycin for 7 days. Among the leaf discs tested for kanamycin resistance, several leaf discs (20%) showed tissue growth. This finding indicated that 20% of leaf discs were resistant to kanamycin.

Keywords: Agrobacterium tumefaciens, transformation, leaf disc, pisang ambon, vacuum infiltration

INTRODUCTION

Pisang Ambon (Musa sp. AAA group) is an edible banana cultivar exclusive from Indonesia. As food source, pisang ambon is affordable food for its inexpensive price besides its good taste. When traded in local market, it provides income source for rural population (FAO, 2003). Development using conventional breeding program have not been successful
due to long life cycle, sterility, and polyploidy of most edible cultivar (Gosh et al., 2009). Genetic manipulation through transfer gene become common to introduce important single gene into banana cultivar. Basically there are two methods of gene transfer, direct and using vector (indirect). Indirect gene transfer into plant cells can be done using plasmid vector from Agrobacterium utilized its T-DNA (Gelvin, 2003) and this method become the most common used method. Primary advantage of Agrobacterium-mediated transformation over direct gene transfer is it reduces the copy number of transgene and then it will lead to fewer problems with co-suppression and instability because of the existence of the transgenes (Tripathi, 2005). Several cultivars such as Rasthali (Ganapathi et al., 2001), Cavendish & Lady finger (Khanna et al., 2004), Gain nain (Acereto-Escofie et al., 2005), and Agbagba (Tripathi et al., 2005) have been successfully transformed. Until now, genetic transformation of triploid banana varieties, especially the 'pisang ambon' AAA variety, has not been reported. In this research pisang ambon leaves tissue were transformed using Agrobacterium tumefaciens GV3101 pbinSRNAIcS. pbinSRNAIcS is a binary vector, which has a kanamycin resistance gene (neomycin phosphotransferase III nptIII gene). Kanamycin resistance gene is a marker gene that will be used for transformation selection step.

MATERIALS AND METHODS

The plantlets of the banana cultivar pisang Ambon (AAA) were obtained from PT. Dafa Teknoagro Mandiri, Bogor, West Java and were sub cultured every 4 weeks. A. tumefaciens was maintain in yeast extract peptone/ YEP (10 g/L pepton, 10 g/L yeast extract, dan 5 g/L NaCl) media contain with 50 g/L rifampicin and growth at room temperature. Optimization of phytohormone combination for banana leaves in vitro growth were done using six (6) different 2,4–dichlorophenoxyacetic acid (2,4–D) and kinetin concentration combination (table 1). Agrobacterium-mediated transformation in banana leaves was done using vacuum infiltration method (Acereto-Escofie et al., 2005). Transformed banana leaves were incubated in media contains 60 mg/L of kanamycin for 7 days to obtain transgenic leaves.

Table 1. Concentration combination of 2,4–D and kinetin added in MS salt media to obtain optimum combination to induce tissue growth in vitro
RESULTS AND DISCUSSION

Banana leaves transformation was done using *A. tumefaciens* GV3101. The *A. tumefaciens* GV3101 gave higher transformation efficiency than *A. tumefaciens* GV3850 (data not shown). The optimum combination of 2,4–D and kinetin for banana tissue growth is DK6 media (see table 1). Growth response was shown as the diameter of the leaves disc increasing (2,2±1,2 mm) after 2 weeks of incubation period (Picture 1). In the selection step, there are 12 from 60 explant (20%) which survive in the presence of 60 mg/L kanamycin in the media (Picture 2). Banana leaves tissue was increasing 1,75±0,8 mm after 7 days of selection. Compare to Acereto-Escofie *et al.* (2005) transformation efficiency was higher than transformation efficiency in this research. Several factors that may influence this result are *Agrobacterium* compatibility, duration of co-cultivation time, age of explants, media form (liquid or solid), pre-culture time (Jaiwal *et al.*, 2001).

[Picture 1. Leaves growth in vitro indicated by diameter increasing while incubate in MS salt media added with 1,8 × 10⁻⁵ M 2,4–D dan 4,4 × 10⁻⁶ M kinetin.]

[Picture 2. Transformed banana leaves explants underwent selection for 7 days. (a) positive control, (b) negative control, (c) leaves explants which show growth response/ kanamycin resistance (red arrow) and explants which didn’t shows growth response (yellow arrow), (d) non-transformed leaves explants in growth media (DK6).]

REFERENCES


P-MB06

Screening of AZF Gene Deletion on Javanese and Chinese Infertile Men

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ABSTRACT

Idiopathic male infertility related with AZF (Azoospermic Factor) gene deletions in the long arm Y chromosome (yq), but the study results still had variation. The aim of research was to get prevalence of AZF gene deletion on Javanese and Chinese infertile men and to know the differences of deletion between both of the population. Samples of the research were DNA that extracted from peripheral blood of primary infertile men with abnormal sperm category. The analysis of deletion was conducted by amplification DNA with PCR (Polymerase Chain Reaction) method used AZF gene from subregion AZFa (sY84, sY86), subregion AZFb (sY127, sY134 and RBM/E355), and subregion AZFc (DAZ/sY254, sY255). Amplification results of each gene locations were sequenced and aligned with the same gene from NCBI (National Center of Biotechnology Information), to get the percentage of homology. The Result showed that the deletion of AZF genes was different between Javanese and Chinese (p < 0.05). The highest prevalence of gene deletion was sY86 gene, on the other hand the most rarely deletion gene was DAZ/sY254 gene. The prevalence of deletion on Javanese infertile men was sY86, sY84, sY255, sY134, RBM, sY127 and sY254, respectively. Meanwhile, the prevalence of deletion on Chinese infertile men was sY86, sY84, sY255, sY134, sY127, RBM, and sY254.

Key Words : male infertility, azoospermic factor, gene deletion, sperm abnormality

INTRODUCTION

The primary caused of men infertility was abnormality of sperm count, that were oligozoospermia (sperm count < 20 × 10\textsuperscript{6} / ml ejaculate) until azoospermia (sperm absent in ejaculate). Most of genetic study on infertile men indicated that abnormality of sperm quantity and quality related with gene deletions in the AZF (Azoospermic Factor) region in
long arm of Y chromosome (Yq). That region consisted of three subregions were subregion AZFa, AZFb and AZFc.\(^{(1)}\) Nevertheless, frequency and location of the gene deletions in each of sperm abnormality groups still have variation. In Indonesia, genetic screening in infertile men have not been a standard procedure, whereas at infertility department of one of hospitals in Surabaya there were average hundreds of infertile men with oligozoospermia until azoospermia that were making effort to get children. They were healthy and the only probability that caused infertility was genetic factor. The aim of research was to get prevalence of AZF gene deletion on Javanese and Chinese infertile men and to know the differences of deletion between both of the population. Result of this research was expected to be an consideration for androlog in diagnose patients so that it will thrifty time and cost.

MATERIALS AND METHODS

Infertile men were those have not succeed to make pregnant their copule at least 2 years after intercourse without used contraception. Ethnicity was determined from marriage lineage, that was man and three generations over him married with the same ethnic. Sperm analysis was observed to three categories; count, motility and morphology. Sperm count was classified in normal (sperm count ≥ 20 x 10\(^6\) / ml ejaculate), azoospermia (sperm absent in ejaculate), oligozoospermia (sperm count between 5 x 10\(^6\) / ml and 20 x 10\(^6\) / ml ejaculate) and severe oligozoospermia (sperm count < 5 x 10\(^6\) / ml ejaculate). Motility was classified in (a) sperm moved fast and straight forward (b) sperm did not move straight (c) sperm did not move at all. Normal motility was defined that at least 50 % of the sperm moved with (a) and (b) mentioned above category. Morphology of the sperm was classified to normal morphology if sperm had oval head, good servix and straight tail. Classification of normal morphology was at least 15 % of the sperm had normal morphology. For analysis of deletion, genomic DNA was extracted from peripheral blood of infertile men used DNA extraction kit. DNA amplification was amplified by PCR method for each of primer pairs. In this research we used 7 genes-specific markers, that were sY84, sY86 (in subregion AZFa), sY127, sY134 dan RBM/E355 (in subregion AZFb), dan DAZ/sY254, sY255 (in subregion AZFc) and SRY gene for control of testis factor. All of PCR products were sequenced at Eijkman Institute Jakarta and aligned with the same gene from NCBI (National Center of Biotechnology Information).

RESULTS AND DISCUSSION

Frequency of gene deletion for Javanese shown in Figure 1. Based on the gene locations that have deletion, there was different between Javanese and Chinese (p < 0,05).
The prevalence of deletion on Javanese infertile men was sY86, sY84, sY255, sY134, RBM, sY127 and sY254, respectively. Meanwhile, the prevalence of deletion on Chinese infertile men was sY86, sY84, sY255, sY134, sY127, RBM, and sY254. This case was different from previous research results that indicated the genes in subregion AZFa rarely deleted than genes in the subregion AZFb and AZFc. It was the opposite with genes in subregion AZFc, if the previous research results indicated that the genes were the most often deleted than genes in subregion AZFa and AZFb. This research showed the genes were the most rarely deleted. This difference may be caused by samples background, Javanese (Indonesian) was mixing from three races, there were Mongolid, Weddid (black) and Caukasid that came from Iran and migrated to Indonesia through South East Asia. Whereas Chinese was part of Mongolid. Different race caused different haplotype Y, it was group of men that involved Y chromosome alteration. So that, if analysis of the AZF gene deletion previously was be done in infertile men not came from Indonesia (most of European), because of that trend of gene deletions in this research different from previous research.

Different race has different haplotype and related with the origin of samples geography. Haplotype Y distribution in population related with specific interference that affected reproductive capability of men. Haplotype Y was different between one country and the others, indeed in the one country. Some of development factor likes as diet and pollutant can affect to different Y kromosom group, it was supported by epidemiololgy data about the low sperm quantity and quality. So that different of AZF gene deletion between Javanese and Chinese, besides of caused by different race also related with diet in each of the ethnics.
REFERENCES


P-MB07

ISOLATION AND CHARACTERIZATION OF LACTIC ACID BACTERIA FROM AN INDONESIAN TRADITIONAL FERMENTED FISH SAUCE “BAKASANG”

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Abstract

Forty-five strains were isolated from an Indonesian traditional fermented Fish Sauce “Bakasang”. These isolates were screened based on phenotypic character such as Gram stain appearance, catalase-test, endospore-forming, motility-test and gas production from glucose. Twenty-seven strains among forty five strains were identified as lactic acid bacteria (LAB). These isolates were characterized further using morphological, physiological and biochemical tests and were identified by profile matching method. The result showed that 27 lactic acid bacteria separated into three group. Group I consist of five strains, three among them were represented as rod-shape heterofermentative and the others being rod-shape homofermentative bacteria. They were identified as member of Genus Lactobacillus. Group II consist of fifteen strains were represented as homofermentative-cocci (tad) and putatively identified as member of Genus Pediococcus. Seven homofermentative-cocci strains include in group III were identified as Genus Leuconostoc.

Keyword: Bakasang, Fermented Fish Sauce, Lactic Acid Bacteria, Profile Matching.

INTRODUCTION

Lactic acid bacteria (LAB) commonly utilized in the production and preservation of various fermented foods for example bakasang, such an Indonesian traditional fermented fish sauce. Bakasang are generally high in protein and amino acid compounds besides its potential source of wide variety of LAB species because low-pH product with an added carbohydrate source, will support the predominance of LAB. Some of the genera that play an important role in fermentation foods commonly are Lactobacillus, Pediococcus,
*Enterococcus, Leuconostoc, and Weissella*.[1,2] Isolation and characterization of LAB from *bakasang* is scarce still.

**MATERIALS AND METHODS**

*Isolation method:* LAB were isolated from *bakasang* using MRS agar medium plus 1% CaCO₃ and supplemented with 0.02% sodium azida by pour plate method. Lactic acid bacteria were picked up from colonies with clear zone surround grown on MRS+CaCO₃. Pure cultures were maintained both as frozen stock held at -80°C in 10% glycerol and 20% skim milk (1:1).[2]

*Phenotypical characterization of isolates:* LAB strains used as reference strains for the characterization and identification of *bakasang* LAB isolates include *Pediococcus acidilactis* FNCC 0110, *Pediococcus pentosaceus* FNCC 0019, *Pediococcus halophilus* FNCC 0032, *Lactobacillus fermentum* FNCC 0104, *Lactobacillus brevis* FNCC 0021, *Lactobacillus plantarum* FNCC 0026 and *Leuconostoc mesenteroides* FNCC.....before being tested, the isolates were subcultured in MRS broth at 37°C. The following phenotypical tests were conducted: gram-reaction; gas from glucose; catalase reaction; endosphere forming; motility-test; the effects of temperature (10; 45; 50°C); different starting pH (3.5; 7.5; 9.6); different concentration of NaCl (6.5; 18%) and finally morphological character including cell form and cell arrangement.

*Fermentation type of isolates:* cultures were grown in 5 ml of MRS broth for 2 days. The amount of lactic acid accumulation in these cultures were calculate from titration value of 0.1 N NaOH solution.

**RESULT AND DISCUSSION**

A total number of 45 isolates of LAB in which production clear zone around theirs colonies were obtained from *bakasang*. The clear zone appearance is due to the dissolution of CaCO₃ on MRS medium by acid agent[4]. Among the 45 isolates were rearrange and confirmed as LAB in amount of 27 isolates. All these isolates were gram positive, rods or cocci, appeared singly, in pair, chain, tetrad. Cell were non motile and non sporing, they gave negative reaction for catalase. These strains were then classified into genus level using profile matching method. Based on the profile matching method (Table 1.) showed that 27 isolates separated into three groups. Group I consisted of 5 isolates, 2 among them were
rod-shape heterofermentative and others were represented as rod-shape homofermentative. These groups were putatively identified as genus *Lactobacillus*. Sixteen isolates were include in group II. They were represented as cocci (tetrad) homofermentative which were identified as genus *Pediococcus*. Finally, group III comprised of six cocci-heterofermentative LAB were identified as genus *Leuconostoc*. It was concluded that lactic acid bacteria isolated from *bakasang* are dominated by *Pediococcus*, *Leuconostoc* and *Lactobacillus*.

Table 1. Identification of lactic acid bacteria isolates into genera level by profile matching method.

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<th>Characteristics</th>
<th>I</th>
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<th>Pediococcus</th>
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REFERENCES


P-MB08

PHYLOGENETIC ANALYSIS OF THE MALEO (Macrocephalus maleo) BASED ON THE FIRST INTRON OF RHODOPSIN NUCLEAR GENE SEQUENCES

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ABSTRACT

The phylogenetic relationships of the Maleo were analyzed based on the first intron of rhodopsin (RDP1) nuclear gene sequence data obtained from 15 individuals, along with those of 22 species obtained from GenBank. The phylogenetic trees were constructed by Neighbor-joining (NJ) method by using five species as outgroup and were compared with existing phylogenies based on morphological data. Result indicated that from 954 bp of RDP1 sequence the base composition for all species analyzed in this research were as follows: T 25.3%, C 26.3%, A 18.5%, and G 29.9%. The G + C contents of the third, second, and first codon positions of the RDP1 fragments in this study were found 55.8, 57.7, and 55.2% respectively. Analysis of RDP1 sequences produced trees that were remarkably well resolved and had topologies at the genus level. The phylogenetic analysis showed that maleo was monophyly of Macrocephalon and closely related to Aepypleodes, Talegalla, Leipoa, and Alectura. Molecular Phylogenetic is a powerful tool for bird phylogeny, not only at a small scale (i.e., relationships between, intraspecies, species) but also at the family level.

Keywords: Macrocephalon maleo, phylogenetic, genetic diversity, RDP1
INTRODUCTION

The maleo (*Macrocephalon maleo*) is an endemic bird of Sulawesi, classified as an Endangered Species on the International Union for Conservation of Natural Resources (IUCN) and has been the subject of conservation project.

One of the most important methods to secure the bird is to examine its genetic diversity, which is a fundamental component for conserving maleo. The partitioning of genetic diversity within and among populations is a major contribution that conservation geneticists can make towards evaluating population viability. Genetic issues have gone from relative obscurity to a significant emphasis in conservation research as modern molecular techniques revolutionized our ability to delineate relationships among individuals, populations, species and intraspecies.

The aim of the research is to examine the genetic diversity of maleo based on the first intron of rhodopsin (RDP1) nuclear gene sequences, to implement it as a consideration to recommend conservation action of maleo, as well as for other Megapodes.

MATERIALS AND METHODS

Genomic DNA was extracted from blood samples using *Qiaamp DNA Blood Mini Kit* (Qiagen). Fragment of the first intron of rhodopsin gene (RDP1) were amplified via polymerase chain reaction (PCR) using the following primers U1 (5’-GTAACAGGGTGCATACATCGA-3’) and L1 (5’ ACAGACCAC CACATATGGT-3’) (Birks & Edwards, 2002).

Fragment were amplified in 50 ul reactions under the following conditions : free denaturation at 96°C for 50 min, followed by 35 cycles of 94°C for 35 s, 52°C for 30 s, and 72°C for 30 s; followed by a final extention of 7 min at 72°C. PCR products were purified using a *QiaQuick PCR Purification Kit* (Qiagen, USA) and were sequenced using an ABI PRISM 3100-Avant Genetic Analyzer (Applied Biosystems, USA). Multiple sequence alignments were obtained using the Clustal X. The Phylogenetic relationships of the maleo were analyzed based on the first intron of rhodopsin (RDP1) nuclear gene sequence data obtained from 15 individuals, along with those of 22 species obtained from GenBank. The phylogenetic trees were constructed by Neighbor-joining (NJ) method by using four species as outgroup and were compared with existing phylogenies based on morphological data. The bootstrap test (1000 repeats) was use to determine reliability of different branches in Neighbor-Joining dendogram.
RESULTS

The total data set consists of 954 bp including 419 (43.9%) variable sites and 321 (33.6%) phylogenetically-informative nucleotides. The base compositions of this RDP1 fragment for all species analyzed in this research were as follows: T 25.3%, C 26.3%, A 18.5% and G 29.9%. The A + T contents of the third, second, and first codon positions of the RDP1 fragment were 55.8, 57.7, and 55.2%, respectively.

The sequence characteristics of the first intron of rhodopsin are similar to those of other phylogenetically informative nuclear markers, including the R 35 introns (Fujita et al., 2004), ovomucoid (Armstrong et al., 2001), and b-fibrinogen (Prychitko & Moore, 1997). In general, such characteristics include homogeneity in base pair composition, low ti:tv ratio, and low among-site rate heterogeneity (Fujita et al., 2004).

This study showed the phylogenetic signal provided by RDP1 for comparisons among Macrocephalon species was weak. Neighbor-Joining analysis of RDP1 sequences produced trees that were remarkably well resolved and had topologies at the genus level.

Nuclear intron accumulate substitutions at slower rates than mtDNA (Birks & Edwards, 2002) and often do not offer sufficient resolving power to determine the relationships among closely related species (Peters, et al., 2005).

The phylogenetic analysis showed that maleo was monophyly of Macrocephalon with bootstrap values of 100 and closely related to Aepypodeus, Talegalla, Leipoa, Alectura with the bootstrap support for this node was >50 for this analysis.
Figure 1. Phylogenetic tree based on the first intron of rhodopsin gene sequences using Neighbor-Joining algorithm (Saitou & Nei, 1987).

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EFFICIENCY OF AGROBACTERIUM-MEDIATED TRANSFORMATION FOR TOMATO USING CAMV35S AND RICE UBIQUITIN2 PROMOTERS

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Abstract

An efficient procedure of genetic transformation ultimately can accelerate the been produced from Agrobacterium-mediated transformation. Promoter is a key DNA that control roles thread and number of gene expression, it have main role on succesful control of genetic transformation. A Gene cannot be activated without the presence of promoter. CaMV promoter is one of strong promoter and usually use in all plant transformation. Ubiquitin promoter, rice ubiquitine2 reported has highest expression on sugarcane transgenic plant. The aim of this research to evaluate transformation efficiency on tomato plant after infected by using Agrobacterium LBA4404 with different promoter, rice ubiquitine2 and CaMV35S. After confirmation that putative transformant contained PKyS plasmids, CaMV35S and PCL4 plasmids with rice ubiquitine in sugarcane SPS gene. Tomato cotyledon was 14 days from the germination tissue culture that was cut off with two sides. Explants infected use Agrobacterium LBA4404; and cultured in Co-cultivation medium, Ceftotaxin medium, selection medium, and regeneration medium. Based on five times selection of the putative transformant plants were (14 days per selection); we found 87% transgenic plantlet in the first selection, 30% in second selection, 9% in third until fifth selection. PKyS plasmid that contained CaMV35S can be detected in promoter transgenic plantlet was 91% at the first selection, 39% at second selection, 11% at third until fifth selection of Agrobacterium PCL4 plasmid contained rice ubiquitine promoter.

Keywords: transformasion, Agrobacterium tumefacien, CaMV35S promoter, rice ubiquitin promoter, tomato.

INTRODUCTION

Tomatoes were the famous crop in the world and tomatoes had many functions among them as vegetables, the table, the drink, in fact as the cosmetic material and the
medicine (Duriat 1996). The development of biotechnology offered the DNA transformation technique to put the important gene in the improvement of the characteristics of the most cheap and proven Method crop effective for the crop dicot was the transformation used Agrobacterium. tumefaciens (Hatanaka et al., 1999) but also succeeded in being carried out to the crop monocotil like rice (Raineri et al., 1990; Park et al., 1996).

Promoter was DNA the key that arranged the pattern and the number that were exact from gene expression in the special method or constitutive, because that played the important role in the success of the transformation (Liu et al., 2003). Without promoter the generation was inactive but could be replicated. Promoter Cauliflower Mosaic the Virus 35S (CaMV 35S) usually was used in the transformation of several crops dicotil and monocotil. However the activity promoter CaMV 35S this was low to the sugarcane crop (Chowdhury et al., 1992). RUBQ2 can be expressed in sugarcane (Liu et al., 2003).

METHODOLOGI

Tomatoes cotyledon as a explants for transformation. Tomato seeds was growth in sterile media for 11 until 13 days. The cotyledon was cut off in base side as a explants for transformation.

Plasmid vectors and Agrobacterium strains. Plasmid of pCL4 and pCAMBIA1301 were used as plasmid vectors containing gene gus driven by rice ubiquitin and CaMV35S promoter, respectively. A single colony of the Agrobacterium containing the plasmid was inoculated in 3 mL liquid YEP medium containing 50 mg l⁻¹ kanamycin and 50 mg l⁻¹ rifamphenicine and incubated at 28°C on shaker for 2 days. One ml of the culture was added to 50 ml of liquid YEP medium containing the antibiotics and incubated in same condition until the culture reached an OD₆₀₀ 0.8-1.0. The culture was centrifuged at 4000 x g for 10 min and suspended in fresh 2 ml LB medium for infection.

Co cultivation and selection. Cocultivation was done for 2 days in the cocultivation media. Then wash the explants in 500 mg l⁻¹ ceftotaxin for tree times. It was take the explants and was regenerated into cefttaxin media for 7 days. Finally, the explants was subcultured in selection media for 75 days.
RESULTS

The number of explants, callus and shoot (Fig A) and shoot development in the selection media (Fig B)
It can be seen Fig 1, show that the number of explants around 200 explants. The callus formation was found around 78.5% and 74% using CaMV and RuBq2 promoters, respectively.

![Graph showing callus formation](image)

The result for shoot formation around 28.7% for RuBq2 promoter and 27.7% for CaMV promoter. The putative transgenic tomato only 34 plants and 39 plants for CaMV and RuBq2 promoters, respectively (fig 2)

![Graph showing shoot formation](image)
CONCLUSION

The result of transgenic tomato was higher 22.5% with LBA4404-PCL4 plasmid and rice Ubiq2 promoter then LBA4404-PkYS plasmid with CaMV35S promoter around 20.5%.

REFERENCES


P-MB10

ISOLATION AND SCREENING BACTERIA DEGRADING POLYCYCLIC AROMATIC HYDROCARBON (PHAs) IN DISTRICTS IN RAJA AMPAT PAPUA WAGEO

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Abstract

Polycyclic aromatic hydrocarbons (PAHs) are important environmental contaminants in soil and water. These compounds have a potential risk to human health, as many of them are carcinogenic and toxic to marine organisms such as diatome, gastrophodophore, mussel, and fish. PAHs are composed of fused aromatic rings in linear, angular, or cluster arrangements and are produced during the pyrolysis of organic material. Although some PAHs are toxic, carcinogenic, or teratogenic, a variety of bacteria can degrade certain PAHs completely to CO₂ and metabolic intermediates, enroute gaining energy and carbon for cell growth. Phenanthrene is one of the hazardous hydrocarbon compounds. The purpose of this research was to characterize microbial strains from Waigeo district, Raja Ampat Papua Island and their ability to remove phenanthrene. An artificial sea water mineral salt medium (ONR7a) used for sublimation test to see the ability of pure culture to degrade certain PHAs. The isolate which degrade the PHAs showed by clear ring zone on ONR7a medium. Five isolates were identified their characteristics based on the composition of nitrogen base. Molecular identification based on 16S rRNA gene sequences indicated that bacteria had the highest similarity with Pseudomonas stutzeri, Caulobacterium sp, Rhodobacteraceae bacterium, Rhodovulum adriaticum, and Roseobacter sp. RW37 16S.

Keyword: Phenanthrene, PHAs, sublimations, molecular identification, Raja Ampat

INTRODUCTION

Nearshore marine environment receive a wide variety of recalcitrant organic compound, including hydrocarbons from shipping activities, terrestrial and freshwater runoff and pollution, and accidental spillage of fuels and other petroleum products. The aliphatic
hydrocarbons tend to be more easily degraded than the aromatic compound. Polycyclic aromatic hydrocarbons (PHAs) are of particular concern because of their persistence and toxicity. Most of these compounds are known to be mutagenic and are suspected carcinogens. Because of their relatively poor solubility in water and hydrophobic nature, PHAs strongly adsorb to particulate material, settle to the seafloor, and accumulate in marine sediment (2). High concentration of toxic contaminants in sediments have been correlated with a high incidence of histopathological abnormalities in bottomfish, toxicity to sediment in fauna and other adverse effect (6). Some marine bacteria have been reported to be PAH degraders, such as members of the genera *Cycloclasticus*, *Marinobacter*, *Pseudomonas*, and *Sphingomonas* (4,7). The genera *Marinobacter* and especially *Alkanivorax*, seem to play a major role in the crude oil biodegradation in marine environment. Raja Ampat Island, Papua reported have a high biodiversity of organisms including marine bacteria. Besides that is still very limited data or information about the marine microorganisms in this area, especially bacteria-degrading hydrocarbon. The objective of this research are isolation and screening bacteria which have potential ability to degrade Polycyclic Aromatic Hydrocarbon, especially phenanthrene and dibenzothiophene.

**MATERIALS AND METHODS**

**Enrichment and isolation of the PAH-degrading bacteria.**

Seawater samples were collected from Raja Ampat Island, Papua. Each 1.0 ml of seawater was placed into sterile 2.0 ml eppendorf tube containing 1.0 ml of an artificial seawater salt medium (ONR7a) and supplemented with 1000 ppm of PHAs (Phenanthrene and Dibenzothiophene) as the sole source of carbon and nitrogen (1; 2). The cultures were incubated at 30ºC on a rotary shaker for several weeks until a reddish or brown color developed. Bacteria capable of degrading phenanthrene, dibenzothiophene were isolated from the enrichment cultures containing the PAHs. Enumeration of PHAs-degrading bacteria were accomplished by spreading a serial dilution of the cultures onto the surface of ONR7a agar medium by using sublimation technique of phenanthrene and dibenzothiophene onto the agar (1, 2, 5) and incubated at room temperature until bacteria grew (Fig 1). After incubation, the ability to metabolize the PAHs was indicated by the presence of clearing zones. Colonies with clearing zones in the PAHs layer were picked and streaked for isolation (1)
Confirmation Test

Purified isolates that were collected should be checked by sublimation to confirm that isolate were capable of degrading PAHs. Control plate without sublimation was also inoculated. Molecular identification based on 16S rRNA gene sequence.

RESULTS AND DISCUSSION

Five isolates of bacteria-degrading PAHs were obtained from seawater collected in Raja Ampat Island, Papua. Four isolates have potential abilities to degrade phenanthrene and the others has ability to degrade dibenzothiophene. The presence of clearing zones around the colonies and color changing from colourless to brown for phenanthrene bacteria degrading and red radish for dibenzothiophene bacteria degrading during the initial isolation. Color changing after incubation indicated that these isolates were able to degrade phenanthrene and dibenzothiophene (Fig.2A&2B). Color change was also an indication of ring cleavage of the aromatic compounds (4). This can be explained with the accumulation of several intermediates, particularly 1-hydroxy-2-naphtoate (5).
Fig 2. Clearing zones can be seen around colonies. Sublimation with

Phenanthrene (A) and Dibenzothiophene (B). Confirmation test (C) and Control (D)

Although some PAHs are toxic, carcinogenic, and teratogenic, a variety of marine bacteria reported can degrade certain PAHs completely to CO$_2$ and metabolic intermediates, enroute gaining energy and carbon for cell growth (3, 4). Five isolates of bacteria-degrading PAHs were obtained from Raja Ampat Island. Molecular identification based on 16S rRNA gene sequences indicated that bacteria had the highest similarity with Caulobacterium sp., Pseudomonas stutzeri, Rhodobacteraceae bacterium, Rhodovulum adriaticum, and Roseobacter sp. RW37 16S. These isolates were preserved for further analize and might prove to be useful candidates for applying to biodegradative process.

REFERENCES


P-MB11

SELECTION OF THE POTENTIAL BACTERIAL POLY-\(\beta\)-HYDROXYBUTYRATE (PHB)
PRODUCER FROM SAGO STARCH PROCESSING AREA

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Abstract

The best poly-\(\beta\)-hydroxybutyrate (PHB) producers of bacterial isolates were selected from the twelve potential isolates obtained from sago starch processing area in Kendari, Southeast Sulawesi based on PHB production ability. Determination of qualitative production of PHB was done by Sudan black staining and quantitative production of PHB was done by the spectrophotometrical method. The best of PHB producer were identified further using phenotypic characteristics by profile matching method. The result of the study showed that two isolates of bacteria, namely PSA10 and PPK6 were found to be the best of PHB producers which were able to produce PHB using sago starch as substrate as much as 52.28 % and 30.90 % (g PHB/g cell dry weight), respectively. PSA10 and PPK6 were identified as member of genus Bacillus. Therefore, both of isolates showed a good prospective as bioplastic producing bacteria from sago starch substrate for industrial purposes.

Keywords: Selection, Poly-\(\beta\)-hydroxybutyrate (PHB), Bacteria, Sago Starch.

INTRODUCTION

Poly-\(\beta\)-hydroxybutyrate (PHB) is aliphatic polymer naturally produced via a microbial process where it acts as carbon and energy storage material in bacteria. It was first biodegradable polymer to be utilized in plastics. PHB is accumulated inside a variety of microorganisms in the presence of excess carbon, when either oxygen, nitrogen or phosphorus becomes a limiting factor\(^1\). The main advantage of PHB is that since they are of biological origin, they degrade naturally and completely to CO\(_2\) and H\(_2\)O under natural environment by enzymatic activities of microbes\(^1\). So, using of PHB as the raw material
bioplastic to substitute of synthetic plastic is the effective way to reduce the environmental impact of plastics. This study focused on the isolation and selection of the isolates for PHB producing abilities from environment to obtain a potential PHB producing bacteria.

MATERIALS AND METHODS

Screening of PHB producing bacteria. Twelve potential isolates from sago starch processing area in Kendari, Southeast Sulawesi were screened for PHB production qualitatively and quantitatively. Determination of qualitative PHB production was done by staining with Sudan black B stain$^2$ and determination of quantitative PHB production was done by N-hexan acetone-dietil eter method$^3$. The PHB content was measured by U.V. spectrophotometer at 235 nm$^3$.

Identification of PHB producing bacteria. The selected isolates were then identified based on their phenotypic characteristics as described in Bergey’s Manual of Determinative Bacteriology$^4$.

RESULTS AND DISCUSSION

Table 1 showed that 12 isolates produced PHB in their cells more higher than reference strain i.e. Bacillus megaterium FNCC 0083 and only two isolates shown significant of high PHB production. Both strains namely PSA10 and PPK6 were able to produce PHB in the shake flask experiment using sago starch as much as 52,28 % and 30,90 % (g PHB/g cell dry weight), respectively. These isolates were selected as the potential PHB producers for further identification based on phenotypic characteristics.

Identification was done based on phenotypic characteristics and compared with the standard description of the Bergey’s Manual of Determinative Bacteriology$^4$. The result of phenotypic properties of PSA10 and PPK6 isolates are summarized in Table 2.

Based on the result at Table 2, PSA10 and PPK6 isolates were identified as a member of genus Bacillus because their characters were compatible with key characters for genus Bacillus, those are i) rod cell shape, ii) gram positive, iii) motil, iv) catalase positive, and v) formed endospora.
Table 1. PHB production using sago starch as a carbon source by 12 selected isolates

<table>
<thead>
<tr>
<th>Isolates</th>
<th>PHB (g/L)</th>
<th>CDW (g/L)</th>
<th>PHB % (W/W)</th>
<th>Isolates</th>
<th>PHB (g/L)</th>
<th>CDW (g/L)</th>
<th>PHB % (W/W)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LCA8</td>
<td>0.090</td>
<td>1.11</td>
<td>8.10</td>
<td>PSA10</td>
<td>0.669</td>
<td>1.28</td>
<td>52.28</td>
</tr>
<tr>
<td>ASD6</td>
<td>0.067</td>
<td>0.49</td>
<td>13.75</td>
<td>PSL1</td>
<td>0.064</td>
<td>0.84</td>
<td>7.62</td>
</tr>
<tr>
<td>ASA7</td>
<td>0.174</td>
<td>1.04</td>
<td>16.68</td>
<td>PPK6</td>
<td>0.213</td>
<td>0.69</td>
<td>30.90</td>
</tr>
<tr>
<td>ASA13</td>
<td>0.087</td>
<td>1.21</td>
<td>7.21</td>
<td>PPB3</td>
<td>0.100</td>
<td>1.10</td>
<td>8.99</td>
</tr>
<tr>
<td>ASA17</td>
<td>0.061</td>
<td>0.84</td>
<td>7.30</td>
<td>PPN1</td>
<td>0.071</td>
<td>0.98</td>
<td>7.24</td>
</tr>
<tr>
<td>ASA21</td>
<td>0.082</td>
<td>0.99</td>
<td>8.29</td>
<td>TA8</td>
<td>0.124</td>
<td>1.21</td>
<td>10.25</td>
</tr>
</tbody>
</table>

Table 2. Phenotypic characteristics of PSA10 and PPK6 isolates

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>PSA10</th>
<th>PPK6</th>
<th>Bacillus</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Morphology</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cell shape</td>
<td>Rod</td>
<td>Rod</td>
<td>Rod</td>
</tr>
<tr>
<td>Motility</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Endospora</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Gram staining</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><strong>Cultural characteristic</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colony shape</td>
<td>Sirculair</td>
<td>Sirculair</td>
<td>Variable</td>
</tr>
<tr>
<td>Growth on nutrient broth pH 5.0-7.0</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Growth in NaCl 2.0-7.0 %</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Growth at 5, 15 and 60°C</td>
<td>+/-</td>
<td>+</td>
<td>+/-</td>
</tr>
<tr>
<td>Biochemical properties</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Catalase</td>
<td>-</td>
<td>+</td>
<td>+/-</td>
</tr>
<tr>
<td>Nitrate reduction</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
A number of genus *Bacillus* have been reported as PHB producers. They were able to produce PHB 9-48 % (g PHB/g DCW)\(^5\). When compared to related literatures, our results shown a higher PHB production, especially PSA10. Therefore, PSA10 and PPK6 were to be potent as a PHB producer.

**References**


P-MB12

THE CALCIUM-DEPENDENT PROTEIN KINASE (CDPK) GENE FAMILY
IN THE GREEN ALGA Volvox carteri

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Abstract
Calcium-dependent protein kinases (CDPKs) comprise a large family of serine/threonine kinases in lower and higher plants. Gene structure reveals that CDPK has a serine/threonine kinase catalytic domain and calcium binding domains (EF-hand). Therefore, plant CDPKs response to various environmental stresses in calcium signaling. Unlike in higher land plants, informations concerning CDPK genes in the lower plant, particularly multicellular green alga are still very limited in publications. Recently, genome sequence of the multicellular green alga volvox carteri has just been completed. In the present study, genome and cDNA data bases have been explored to identify CDPK gene family in Volvox carteri. By using bioinformatics approaches, 13 CDPK genes were identified. When CDPKs of Volvox were compared to the ones of Arabidopsis, it was revealed that Volvox CDPKs were not closely related to Arabidopsis. This suggests that the multicellular green algal CDPKs might play distinct roles with land plant CDPKs.

Keywords: CDPK gene, genome analyses, Volvox carteri

INTRODUCTION
Calcium-dependent protein kinases (CDPKs) comprise a large family of serine/threonine kinases in lower and higher plants. CDPKs primary structure consists of four domains: a N-terminal variable domain, a protein kinase domain followed by an auto-inhibitory domain and a calmodulin-like domain. Auto-inhibitory domain contains a pseudo-substrate, which is in the conditions without Ca$^{2+}$, binds the catalytic center and maintains the kinase in the inactive state. Binding of Ca$^{2+}$ with calmodulin-like domain causes conformational changes that lead to the release of the pseudo-substrate domain and
activating kinases\textsuperscript{1,2}. A number of biotic and abiotic stimuli trigger the increased concentration of cytosolic Ca\textsuperscript{2+} which then acts as a second messenger to transduce the signal. In \textit{Dunaliella}, a halotolerant unicellular green alga, CDPKs respond to osmotic stresses through calcium signaling pathways by phosphorylating target proteins\textsuperscript{3}.

\textit{Volvox carteri} is a multicellular green alga. \textit{Volvox} has been used as a laboratory model system. Recently, genome sequence of \textit{Volvox carteri} has just been completed. Unlike in higher land plants, information concerning CDPK gene family in the lower plant, especially algae is still very limited in publications. In the present study, therefore, \textit{Volvox} CDPK gene family was determined from genomic database and its structure was analyzed.

**MATERIALS AND METHODS**

CDPK-like genes were identified in \textit{Volvox carteri} f. \textit{nagariensis} genome database of Joint Genome Institute, JGI (http://genome.jgi-psf.org/Volca1/Volca1.home.html) by performing a keyword search. The deduced amino acids were analyzed for conserved domains using SMART program (http://smart.embl-heidelberg.de). The putative CDPK were selected base on the presence of serine/threonine protein kinase domain and EF-hand motif(s) in its structure. Molecular weight of each CDPK protein was predicted by using ExPASy program (http://us.expasy.org/tools/pi_tool.html). To determine the cellular localization of CDPK, a transmembrane protein analysis program, SOSUI (http://bp.nuap.nagoya-u.ac.jp/sosui/sosui_submit.html) was used. Relatedness of CDPK family from \textit{Volvox carteri} was compared with CDPK from \textit{Arabidopsis thaliana} by using phylogenetic tree analysis (http://clustalw.ddbj.nig.ac.jp/top-e.html).

**RESULTS AND DISCUSSION**

Domain structure of plant CDPKs which exhibit kinase domain and EF-hand motif is shown in a schematic representation in Fig.1.

![Fig.1: Plant CDPK structure](image-url)
In the present study, whole *Volvox* genome was analyzed, and has revealed 13 genes encoding putative CDPKs. As shown in table 1, *Volvox* CDPKs were designed as VcCPK1-13. It has a protein kinase domain, a N-terminal variable domain with vary in length and a number of EF-hand motif(s) in the C-terminus. Amongst *Volvox* CDPKs, only VcCPK8 has a long N-terminal variable domain and contains a C2 motif. C2 motif is supposed to be responsible for interacting with the membrane. This type of CDPK is also found in unicellular green algae *Chlamydomonas* sp and *Dunaliella tertiolecta*, and reported to play an important role in osmoregulation\(^3\) and wound healing in *Ventricaria ventricosa*\(^4\).

The completed *Arabidopsis* genome sequence has revealed 34 genes encoding CDPKs. These CDPKs have been characterized in responses to biotic and abiotic stresses\(^5\). When *Volvox* CDPK family was compared with *Arabidopsis thaliana* CDPK family in phylogenetic analysis, *Volvox* CDPKs seemed not to be similar with *Arabidopsis* CDPK. This suggests that the green algae CDPK might play distinct roles with known higher plant CDPKs.

<table>
<thead>
<tr>
<th>Name</th>
<th>Predicted protein</th>
<th>N-terminus</th>
<th>C-terminus</th>
<th>Soluble / membrane protein</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ID</td>
<td>MW (kDa)</td>
<td>Length (AAs)</td>
<td>Domain</td>
</tr>
<tr>
<td>VcCPK1</td>
<td>105941</td>
<td>87,7</td>
<td>40</td>
<td>-</td>
</tr>
<tr>
<td>VcCPK2</td>
<td>109867</td>
<td>54,2</td>
<td>26</td>
<td>-</td>
</tr>
<tr>
<td>VcCPK3</td>
<td>116471</td>
<td>102,7</td>
<td>53</td>
<td>-</td>
</tr>
<tr>
<td>VcCPK4</td>
<td>119030</td>
<td>48,7</td>
<td>54</td>
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<tr>
<td>VcCPK5</td>
<td>41333</td>
<td>49,0</td>
<td>21*</td>
<td>-</td>
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<td>VcCPK6</td>
<td>57999</td>
<td>49,3</td>
<td>42</td>
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<td>VcCPK7</td>
<td>62695</td>
<td>55,6</td>
<td>33</td>
<td>-</td>
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<tr>
<td>VcCPK8</td>
<td>74309</td>
<td>67,4</td>
<td>169</td>
<td>C2</td>
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<td>VcCPK9</td>
<td>80507</td>
<td>44,1</td>
<td>0</td>
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<tr>
<td>ID</td>
<td>MW</td>
<td>AAs</td>
<td>kDa</td>
<td>Status</td>
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<td>----------</td>
<td>--------</td>
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<td>-----------------</td>
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<tr>
<td>VcCPK10</td>
<td>81022</td>
<td>53.6</td>
<td>0</td>
<td>264</td>
</tr>
<tr>
<td>VcCPK11</td>
<td>82146</td>
<td>51.7</td>
<td>56</td>
<td>152</td>
</tr>
<tr>
<td>VcCPK12</td>
<td>84165</td>
<td>53.0</td>
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<td>270</td>
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<tr>
<td>VcCPK13</td>
<td>89054</td>
<td>70.7</td>
<td>28</td>
<td>334</td>
</tr>
</tbody>
</table>

ID : identity number in JGI  
MW : molecular weight;  kDa: kilo Dalton  
AAs: Amino acid residues  
* : partial protein

REFERENCES

P-MB13

AGROBACTERIUM-MEDIATED TRANSFORMATION AND REGENERATION OF THE TRANSFORMANTS EXPRESSING SUGARCANE SUCROSE PHOSPHATE SYNTHASE

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Abstract

Agrobacterium is one of soil bacterium that can be used as a tool for gene transformation in plants. The aim of this study is to transform sucrose phosphate synthase (SPS) gene of sugarcane into tomato (Lycopersicum esculentum) plant and analyze its expression. Sucrose biosynthesis in most plants depends on SPS activity. Therefore, SPS can be a target to increase sucrose of tomato plant. Agrobacterium tumefaciens LBA 4404 containing construct of SPS gene sugarcane (pKYS-SPS LBA 4404), SPS gene was controlled by promoter CaMV35S and of antibiotic resistance genes (NPT II). Construct confirmation of SPS genes was done by using PCR analysis is, the first step of all transformation process. Agrobacterium tumefaciens was cultured on medium YEP for 2 days. Bacterial density was determined by spectrophotometer in wavelength 600 nm = 0,8. Explants of germ or cotyledon were dip in those bacteria culture for 15 minutes. One hundred mg/ l of Acetosyringone in MS medium was used as cocultivation medium. Cefotaxim 500 mg/l and Kanamycin 50 mg/l was used as marker selection of transformed plantlet and the selection was done up to five times. Putative transformant were acclimated on steril media that composed of sand and compost (1:1). PCR analysis, SPS activity, and sucrose content used for determination of SPS gene expression.

Keywords: Sucrose Phosphate Synthase, Agrobacterium tumefaciens, sugarcane, tomato

INTRODUCTION

Tomato (Lycopersicon esculentum) is one of the most important vegetable crops and genetic models for improving other dicotyledonous crop plant (McCormick et al., 1986; Ling et al., 1998). In basic and practical studies for tomato improvement, successful transformation is essential. Tomato transformation using Agrobacterium tumefaciens has developed very rapidly. Factor such as plant variety, explants material, growth regulators, bacterial
concentration and Agrobacterium virulent genes inducers have an influence on transformation efficiency (Stachel et al, 1986). In the current study, we evaluated shoot regeneration for the developed of simple, rapid and efficient reproducible Agrobacterium tumefaciens genetic transformation methods for tomatoes.

**MATERIALS AND METHODS**

*Lycopersicon esculentum* seeds were sterilized by shaking in 5% Clorox for 2 min. To remove surfactants, sterilized seeds were rinsed three times with sterilized water and blotted on to sterile filter paper. Seeds were germinated at 25°C and 70% relative humidity for 14 days under fluorescent light. Explants germ or cotyledons of the sterile tomato seedlings were cut off, and transversely to bacterial suspension.

**Plant Transformation**

*Agrobacterium tumefaciens* strain LBA 4404 containing the transgenic *Sucrose Phosphate Synthase* gene (LBA4404SPS), carrying the neomycin phosphotransferase (NPTII) gene (Figure 1) was grown in YEP medium to 0.6 – 1 OD<sub>600</sub>. Tomato explant were removed from medium germination and transferred to the bacterial suspension for 15 min. Then 100 explants tomato (germ or cotyledon) were either placed on cocultivation medium MS supplemented with 100 (mg L<sup>-1</sup>) acetosyringone (CM1) for 2 days at 25°C in the dark. After 2 days on cocultivation medium, 100 explants were immersed in the washing medium (MS+ 500 mg L<sup>-1</sup> cefotaxime) dried on sterile filter paper and transferred to medium MS supplemented 500 mgL<sup>-1</sup> (CM2) for 5 days. After 5 days, explants transferred to shoot regeneration medium (MS+growth regulator+50mgL<sup>-1</sup> kanamisin, 500 mgI<sup>-1</sup> cefotaxim) at 25°C and 70% relative humidity under fluorescent light, 8 h dark and 16 h light. Three weeks later, plantlet sub culture in same medium. When regenerated plantlet reached 2-3 cm in height, they were cut off and placed on rooting medium (RTM) and then acclimatization on medium acclimatization (AM).

![Diagram](image)

Figure 1. Construct Agrobacterium tumefaciens LBA 4404 with SPS gene.
RESULTS AND DISCUSSION

Confirmation construct in Agrobacterium tumefaciens

Confirmation construct in Agrobacterium tumefaciens are shown in figure 2. Agrobacterium vector transformation containing the transgenic SPS gene, NOS : nopaline synthase promoter, NPT II : neomycin phosphotransferase gene, Nos ter : nopaline synthase terminator and CaMV35S : cauliflower mosaic virus 35S promoter.

Figure 2. Confirmation construct in Agrobacterium tumefaciens. 1;2;5;6;7;9 : Dna plasmid and 3,4,8 : Marker DNA 1 KB (fermentas).

Effect of concentration BAP on tomato explants survival

The effect of BAP concentration in shoot generation medium is shown in figure 3. Tomato cotyledon explants on MS medium in with BAP concentration is 2 mg L⁻¹ + IAA 0.5 mg L⁻¹ presented dramatic increase on shoot regeneration. In RM2, new healthy and green tissue developed survival prior shoot differentiation.

Figure 3. BAP effect on cotyledon explants survival. Cotyledon explants on MS regeneration medium containing 3 mg L⁻¹ BAP (benzyl amino purin);0.2 mg L⁻¹, Kinetin (A); and on MS modified regeneration medium containing 2 mg L⁻¹ BAP (benzyl amino purin) ; 0.5 mg L⁻¹ IAA (B).
DISCUSSION

MS medium (Murashige and Skoog, 1962) is the most commonly used commercial medium in tissue culture. Two explants sources i.e. germ and cotyledon were used. Germ and cotyledon showed variable response by use of different growth regulator. Their response was dependent on the different combinations of growth regulator. However, shoot regeneration of cotyledon explants in MS modified regeneration medium containing 2 mg L⁻¹ BAP (benzyl amino purin) ; 0,5 mg L⁻¹ IAA is developed green shoot.

To compare the invitro performance of explants, cotyledon appeared to be better exsplant source as compared germ. Cotyledon exsplant have high percentage plant tomato putative transman dependent exsplant germ. Plantlets lose five in medium selection antibiotic and acclimatize on growth chamber.

REFERENCES


P-MB14

GENETIC POLYMORPHISM IN DENGUE MOSQUITO Aedes aegypti (DIPTERA : Culicidae) BASED ON RAPD-PCR ANALYSIS

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ABSTRACT

The mosquito Aedes aegypti is serious threat to public health, dealing with its role as the vector in the Dengue Hemorrhagic Fever (DHF). In general the number of DHF cases in Indonesia tends to increase. One of the factors determining the high number of occurrence is the density vector. The research on the genetic polymorphism of Aedes aegypti must get a serious attention, since there is a correlation between the genetic polymorphism and the adaptation for survival. Random Amplified Polymorphic DNA (RAPD) molecular marker was used in the genetic polymorphism analysis. The mosquitoes were collected from Sumbersari, Patrang and Kaliwates (endemic area) in kabupaten Jember. Five oligonucleotida were used as RAPD’s primer. The result of RAPD qualitative analysis showed that the polymorphism level of Aedes aegypti from Sumbersari, Patrang and Kaliwates were 73.7%, 72.2% and 64.3% respectively.

Key word: genetic polymorphism, Aedes aegypti, RAPD-PCR

INTRODUCTION

Dengue Hemorrhagic Fever (DHF) is major public health problem in Indonesia. Dengue fever is most important viral disease transmitted to human by mosquito Aedes aegypti. Aedes aegypti is the principal vector for dengue virus, this vector is able to maintain the four serotypes of dengue viruses (DEN 1,2,3 and 4) in urban transmission cycle. More than 80.837 cases were reported through calendar year 2005 in Indonesia. One of the factors determining the high number of occurrences is the density vector (Aedes aegypti). The research on the genetic strain variability of Aedes aegypti must get a serious attention, since there is a correlation between the genetic strain variability and the adaptation
for survival (Wallis et al. 1984). Organism with higher variability will survive and regenerate more easily than those with lower variability. Vector insects with higher change of survival will have higher population. The more densely populated the vector, the higher its chance to run into humans. This is what cases the increasing number of occurrence of DHF cases and the expanding width of the infected area.

Based on above illustrations, therefore, a research on the genetic phenomena dealing with genetic variability (genetic polymorphism) of the dengue mosquito (Aedes aegypti). The genetic polymorphism level can be revealed by conducting an analysis on the DNA polymorphism with RAPD (random Amplified Polymorphic DNA). RAPD technique detects randomly amplified polymorphic DNA fragments in PCR with single arbitrary primer of 8-10 bp (Williams et al., 1990). The number of fragments amplified and the degree of polymorphism in eukaryotic species depend on the nucleotide sequence, the secondary structure and the number of primers used for each RAD assay. These features of the RAPD assay make it possible to detect DNA polymorphism in the absence of specific nucleotide sequence information (Moreira, 2006).

Material and Methods

Mosquitoes larva and pupae were collected from endemic are in Jember, during April-July 2008. Mosquitoes were reared until adult stage under standart conditions. Genomic DNA was extrated from individual female mosquitoes using CTAB method. Legs were selected as the tissue of choice for the source of DNA. DNA was amplified in PCR reaction using single primer of arbitrary nucleotide sequence. Primer used in this analysis have the following sequences: OPE 16 : 5’ GGTGACTGTG 3’, OPE 17 : 5’ CTACTGCCGT 3’, OPE 19 : 5’ ACGGCGTATG 3’. OPF 4 : 5’ GGTGATCAGG 3’ dan OPF 2 : 5’ GAGGATCCCT 3’ ((Anggraeni, E, 1998). The RAPD products were analyzed by electrophoresis in 1.5% agarose. The sizes of DNA fragments were estimated by comparison with DNA marker (1 kb).
Result and Discussion

<table>
<thead>
<tr>
<th>Location</th>
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<th>Polymorphism</th>
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<td></td>
<td>OPF 2</td>
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<td>OPF 4</td>
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<tr>
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Based on this table, the analysis of all DNA bands based on all location in kabupaten Jember, indicates that polymorphism level of *Aedes aegypti* DNA from Sumber-sari is the highest compare the others sample location. The result of RAPD qualitative analysis showed that the polymorphism level of *Aedes aegypti* from Sumber-sari, Patrang and Kaliwates were 73.7%, 72.2% and 64.3% respectively. The *Aedes aegypti* which have high polymorphism level are indicated high genetic variability. Having high genetic variability, the *Aedes aegypti* will have high survive and cause more population. The sizes of DNA fragments of *Aedes aegypti* that are amplificated with five oligonucleotida (OPE 16, OPE 17, OPE 19, OPF 2 and OPF 4) were ranged from 163 bp-2622 bp.
References


P-MB15

IDENTIFICATION of opaque 2 (o-2) GENE IN CROSSING BETWEEN MADURA’S LOCAL MAIZE (Zea mays L. cv. Guluk-guluk) AND QUALITY PROTEIN MAIZE (Z. mays L. cv. Srikandi kuning)

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Abstract

Maize (Zea mays L.) is one of the main foods instead of rice in Indonesia. Generally, amino acid of lysin and tryptophan content in maize is low. Maize production in Indonesia has been increasing but its production is still low. Many efforts have been applied to enhance the maize production through breeding. However, breeding of a Madura’s local maize (Guluk-guluk) with a Quality Protein Maize (Srikandi kuning) has not been conducted. In maize, opaque 2 gene (o-2 gene) is a recessive gene and could be regulate a number of enzymes of glycolysis and tricarboxylic acid pathway, regulates and reduces zein gene expression, influencing ask1 and ask2 gene which encode of lysin and threonin synthesis. Hence, the gene has been increase of amino acid of lysin and triptophan particularly. The research was carried out to study the inheritance of o-2 gene in crossing between Guluk-guluk and Srikandi kuning. The research was started by crossing between Guluk-guluk and Srikandi kuning to obtain F1, F2 and back cross population. The DNA maize was isolated and its DNA quality was determined by electrophoresis on 2% agarose gel. Polymerase Chain Reaction (PCR) was applied to detect o-2 gene in maize. Based on the results of detection of o-2 gene, it could be revealed that the inheritance of o-2 gene in maize follows the Mendel law and controlled by a single recessive gene.

Keywords: o-2 gene, recessive gene, Guluk-guluk, Srikandi kuning.
P-MB16

STUDY OF SUGAR CANE SUCROSE TRANSPORTER BY FUNCTIONAL EXPRESSION IN YEAST

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ABSTRACT

The sucrose transports is essential process for carbohydrate distribution in plants. In order to distribute sucrose, specified transporter proteins have an important role to transport sucrose from source tissue to sink tissue. In this study sucrose transporter gene were isolated from Sugarcane and constructed to expression plasmid pYES2 for SUT1 and pYX112 for SUT2. The plasmids were transformed in yeast (Saccharomyces cerevisiae) and grown in minimal medium (SD – urasil) as selection medium. Polymerase chain reaction was used to confirm the transformed colony. To study the functional expression, yeast grown in YPD medium with 2% sucrose than the sucrose uptake were measured in some interval using resorcinol method. The result shows that yeast INVSc1-pYES2-SUT1 and BF264-pYX112-SUT2 have better ability to transport sucrose than the control-INVSc1 for SUT1 and control-BF264 for SUT2. In addition, the result shows that SUT1 have better ability to transport sucrose than SUT2.

Keywords: Sugarcane, Sucrose Transporter, Yeast

INTRODUCTION

The sucrose transports is essential process for carbohydrate distribution in plants. In order to distribute sucrose, specified transporter proteins have an important role to transport sucrose from source tissue to sink tissue. The study of sucrose transporter (SUT) was advanced developed since the first isolation of plant sucrose transporter in spinach (Reismeier et al., 1992). Yeast has been known as effective media of expression study especially in sucrose transporter study (Reismeier et al., 1992; Noiraud et al., 2000; Rae et
al., 2005). In these study sugarcane sucrose transporter genes which are isolated in former study were constructed into expression plasmid. The gene activities were analyzed by measurement of sucrose concentration which is regulates by the expression plasmid in the yeast cells. The over expression of SUT will increase the sucrose uptake in yeast. Resorcinol method is the general method to measure the sucrose concentration; even though this method not sensitive in small amount of sucrose concentration, it still can use as the first method to indicate the activities.

MATERIAL AND METHODS

Yeast Material
The yeast material used in this study is Saccharomyces cerevisiae from Invitrogen strain INVSc1 for SUT1 and strain PYX112 for SUT2. SUT1 and SUT 2 gene were constructed in plasmid pYES2 and pYX112. The plasmid transformed into Yeast INVSc1 and BF264 using LiCl method.

Confirmation of Transformed Yeast
First confirmation method is the yeast cells grown in minimal medium (- urasii) as selective medium. The second method is using PCR. The yeast inoculated in YPD medium contains 2% glucose and growth overnight, 30°C in incubator shaker. Centrifuged in 4°C 5000 rpm for 5 minutes to harvest yeast cells, take pellet then add 1 ml sterile H2O and centrifuged in 4°C 5000 rpm for 5 minutes to wash cells and repeated 3 times, take pellet. Add glass beads equal volume and vortex for 1 minute to lysis cells. Centrifuged in 4°C 5000 rpm for 5 minutes take supernatant into new sterile centrifuge tube. The DNA in those supernatant were treated to isolated DNA then specific primer used in PCR.

Functional Expression in Yeast
Yeast inoculated in 1 ml YPD medium contains 2% Glucose. Incubate 18 hours, 30°C in incubator shaker. 300ml starter inoculated into 15 ml YPD medium contain 2% Sucrose for expression study. Culture grown for 48 hours, sucrose concentrations was measured in some interval using resorcinol method.

RESULT AND DISCUSSION
The confirmation in selection medium showed that transformed cell have ability to live in media which is doesn’t contain urasii, the confirmation of transformed cell using PCR
method showed that transformation process was successfully inserting expression plasmid into yeast cells. In electrophoresis gel SUT1 plasmid examined in 1040 bp.

![Fig. 1: a. DNA Construct of pYES2-SUT1; b. pYX112-SUT2; c. Electrophoresis of PCR Product pYES-SUT1; d. electrophoresis of PCR](image1)

![Fig. 2: a. pYES2-SUT1 and b. pYX112 grown in minimum medium (-urasil)](image2)

![Fig. 3: Sucrose Concentration a. SUT1 compared with WT; b. SUT2 Compared with WT-BF (SUT1: Sucose in cell; MSUT1: in Medium of SUT1; WT: Sucose in wild type cell; MWT: in Medium of wild type)](image3)

From this study we can examined that the growth of all of transformed yeast strain (INVSc1-pYES2-SUT1 and BF264-pYX112-SUT2) are faster than control; these growth ability can be conclude as the role of over expressed SUT gene in transformed yeast cell. The over expressed gene influence the sucrose transport ability of the cells; the transformed
yeast cells can transport sucrose faster than control showed that the inserted genes have functional expression as SUT gene. In addition the general result showed that SUT1 have better ability to transport sucrose than SUT2.

REFERENCE
P-MB17

GENETIC VARIATION OF Liberibacter asiaticus FROM SOME REGIONS IN INDONESIA BASED ON 16S rDNA SEQUENCES

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ABSTRACT

This research had been conducted to uncover the genetic variation of huanglongbing (HLB) bacteria L. asiaticus from some regions in Indonesia those are from Bali, Bima, Cianjur, and Tulungagung based on 16S rDNA sequences, as well as to compare them with 16S rDNA HLB bacteria strain Poona (India) and Nelspruit (South Africa). The 16S rDNA fragments of L. asiaticus were amplified by PCR method using O11 and O12c primers. The results of PCR were sequenced and identified using the BLAST program. The research results showed that there were some the genetic variation among all of those bacteria. Based on the similarities and the differences of bacterial 16S rDNA sequences, there were some genetic variation, those are nucleotide insertions and deletions, base transversions, transitions, as well as inversions uncovered. It found too that there were some unique 16S rDNA sequences of L. asiaticus from Bali, Bima, Cianjur, and Tulungagung. Those unique sequences showed differences in 16s rDNA sequences compared to those of the Poona and Nelspruit strains.

Keywords: genetic variation, 16S rDNA sequences, Liberibacter asiaticus

INTRODUCTION

Huanglongbing (HLB), also known as CVPD (citrus vein phloem degeneration) disease in Indonesia is the most serious threat to citrus production due to the reduction of fruit quality as well as plant death. Currently, three major forms of the disease are recognized associated with three different Candidatus Liberibacter species, those are Ca. Liberibacter asiaticus, Ca. Liberibacter africanus, and Ca. Liberibacter americanus [1]. Historically HLB bacterium was specific organism because a culture system was not uncovered yet. Amplification of 16S rDNA gene is one way to characterize the bacterium [2].
Studies of the comparative gene organization and gene order among related bacteria, can lead to better understanding of the functional significance of gene arrangements among them [3,4]. Such information can either be derived from phylogenetic profiles [5] or from comparative genome analyses [6]. The information may also provide a deep insight into these organisms' evolutionary history and metabolic capabilities [7]. The comparison of 16S ribosomal DNA is a useful tool for deducing phylogenetic and evolutionary relationship among bacteria and other prokaryotes [2]. This research was conducted to uncover the genetic variation of HLB bacteria \textit{L. asiaticus} from some regions in Indonesia, those are from Bali, Bima, Cianjur, and Tulungagung based on 16S rDNA sequences, as well as to compare them with 16S rDNA HLB bacteria strain of Poona (India) and Nelspruit (South Africa).

**MATERIALS AND METHODS**

This research was done by isolating DNA from the leaves of citrus infested by HLB bacteria indicated by chlorotic symptoms from Bali, Bima, Cianjur, and Tulungagung. DNA isolation results were then amplified by PCR technique. The DNA isolation and amplification 16S rDNA fragments of \textit{L. asiaticus} referred to previous methods using OI1 and OI2c primers [8]. The DNA amplification results were sequenced by automatic machines fluorescent DNA sequencer (ABI 377A) at Eijkman Molecular Biology Laboratory. The results of the DNA sequence of these regions were identified using the BLAST program (Basic Local Alignment Search Tool) at NCBI GenBank (National Center for Biotechnology Information) at \url{http://www.ncbi.nlm.nih.gov} and then were analyzed by alignment procedure. The bacterial 16S rRNA of ‘Ca. L. asiaticus’ isolated from Poona and L. africanus’ isolated from Nelspruit gene sequences used for comparisons were obtained from the GenBank database.

**RESULTS AND DISCUSSION**

The research results showed that there were some genetic variations among all of those bacteria tested, as shown in the appendix. Based on the frame of mind that the Poona strain emerged early, it can be expected some of the following phenomenon.

1. There are some unique sequences from Bali, Bima, Cianjur, and Tulungagung compared to Poona and Nelspruit strains sequences. Those sequences are marked with green color, at position of 504-505, 531-532, 777, 778, and 1125.
2. There are some similar sequences from Bali, Bima, Cianjur, and Tulungagung compared to Poona strain but are different compared to Nelspruit strain. Those sequences are marked with orange color, at position of 57-59, 207, and others.

3. There are some different sequences from Bali, Bima, Cianjur, and Tulungagung compared to Poona strain but are similar compared to Nelspruit strain. Those sequences are marked with blue color, at position of 774, 797 and 1078.

4. There are some nucleotide insertions from Bali, Bima, Cianjur, and Tulungagung. Those sequences are marked with yellow color, at position of 342 to 343, 400 to 401, and others.

5. There are some base inversions from Bali, Bima, Cianjur, and Tulungagung compared to Poona strain, indicated by hatching, at the position of 556-557, 566-567, and others.

6. There are some nucleotides deletions marked with a sign (-), at the position of 99, 552, 573, 574, and 731.

7. There are some purine base transitions with other purine base.

8. There are purine bases transversions with pyrimidine bases.

9. There are pyrimidine base transitions with other pyrimidine base.

10. There are transversions of pyrimidines base with purine bases.

Based on the analysis, it can be stated that there are some genetic variation among the 16S rDNA L. asiaticus from several regions in Indonesia, such as nucleotide insertions and deletions, base transversions, transitions, and inversions. It was also found that there were unique 16S rDNA sequences of L. asiaticus from Bali, Bima, Cianjur, and Tulungagung. Those unique sequences showed differences in 16S rDNA sequences compared to the Poona and Nelspruit strains. This phenomenon can be well understood on the frame of mind that the Poona strain emerged more early than the other HLB bacteria. Advanced analysis will be needed related to other sequences to find out more comprehensive information related to HLB bacteria in Indonesia.
Table 1. The differences of *L. asiaticus* 16S rDNA sequences from Bali (Bl), Bima (Bm), Cianjur (Ci), and Tulangagung (Ta) compared to *L. asiaticus* 16S rDNA sequences of Poona (India, In) and *L. africanus* Nelspruit (South Africa, Af) strains. The numbering of nucleotide positions follows the nucleotides numbering of 16S rDNA sequence of *L. asiaticus* from Poona, LIF16SRRNA on NCBI GenBank database.

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**Keterangan:**

- : The nucleotides from Bali, Bima, Cianjur and Tulungagung were different compared to Poona and Nelspruit strains

- : The nucleotides from Bali, Bima, Cianjur and Tulungagung were similar compared to Poona strain but were different compared to Nelspruit strain

- : The nucleotides from Bali, Bima, Cianjur and Tulungagung were similar compared to Nelspruit strain but were different compared to Poona strain
REFERENCES


P-MB18

CLONING *PALL* GENE ENCODING SUCROSE ISOMERASE FROM LOCAL BACTERIAL ISOLATE

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sony@sith.itb.ac.id

Abstract

Sucrose, which is widely used in foods and drinks, has many disadvantages such as causing tooth decay, cariogenic, and not suitable for diabetic patients. On the other hand, Palatinose, which is an isomer of sucrose has different property than sucrose. Hydrolysis of palatinose in small intestine runs very slowly compared to sucrose or maltose. Therefore, it will not increase the blood glucose significantly in diabetic patient. Palatinose is derived from conversion of sucrose by isomaltulose synthase (*Pall*). Furthermore, palatinose cannot be used by microbes that can cause tooth decay. The *pall* gene encoding sucrose isomerase was cloned from bacteria that was isolated from sugarcane waste product by nested PCR and the DNA fragment was cloned successfully into pGEM®-T Easy vector plasmid. The length of DNA fragment confirmed by sequencing is 1211 bp. Sequence analysis of the DNA fragment has 97% similarity to the *pall* gene from *Erwinia rhapontici*. This is the first attempt to clone the *Pall* gene from local isolate in Indonesia.

Keywords: palatinose, *pall* gene, sucrose isomerase.
P-MB19

IDENTIFICATION OF AMYLOLITIC BACTERIA CAUSING SOURNESS ON RAW STARCH SAGO BASED ON 16SrDNA SEQUENCE

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ABSTRACT

Raw starch sago under traditionality processing can be sourness caused organic acid excreted by amylolitic bacteria. There were variation bacteria can growth on sago, therefore have to isolated and selected. Bacteria isolated from raw starch sago under traditionality processing in Jayapura, Papua Provience. The highest organic acid production of isolates was decided to select them. There were three isolate selected and continued to identied based on 16SrDNA sequence. Phylogenetic tree were contructed by Phylip programe with neighbour joining algorithm and Juke-Cantor evolution model. Isolate TG 31 and TG12 were similarity genetic with Bacillus subtilis EU982541. In other hand isolate TG19 was similarity genetic with Bacillus cereus FJ685763.

Key words: Amylolitic bacteria, sourness, and raw starch sago, 16SrDNA Sequence

INTRODUCTION

Traditional extraction of raw starch sago (RSG) in rural Jayapura-Papua Provience uses labour-intensive methods of pith maceration and washing with a considerable volume of water. Following starch extraction, the starch may be consumed fresh, or more commonly moist starch is stored for up to 6 weeks and sometimes longer, allowing fermentation to occur if appropriately stored. The amylolitic bacteria which produce organic acid caused sourness can grow up on RSG under tradionality processing in Jayapura. There were various bacteria can growth on sago, therefore have to isolated and selected. Vairously and amounitly in organic acid production of isolates was decided to select them. Identification
based on 16S rRNA gene sequences of amylolitic bacteria causing sourness on RSG provided a powerful way of uncovering genetic of selected strain.

MATERIALS AND METHODS

Isolation amylolitic bacteria from RSG, it was taken from traditional extraction area in Jayapura. Medium for isolation was used agar nutrient + 0.3% soluble starch and pour plate methods (1), while amylolitic activity tested by 0.2% iodine in 2% potassium iodide (2). Selection isolate depend on organic acid productivity of isolates. Analysis of organic acid productivity was conducted using an Hawlett Packard 5890 series II gas chromatography (GC). Acids were identified by comparison of retention times and quantified by comparison of peak areas with those of identically treated standards (4). Before genomic DNA sequences analyses, isolates were generic assignment determined. Genomic DNA from test isolates was prepared by using a standard extraction method (3). Futhermore, 16S rRNA genes were amplified by PCR using two concerved primers. The Purified PCR products were sequenced by using four concerved primers with an ABI PRISM 310 DNA sequencer. The 16S rDNA data were aligned with corresponding available Bacillus sequences retrieved from the NCBI data base using the CLUSTAL X software.

RESULTS AND DISCUSSION

Isolation were resulted 118 amylolitic bacteria and 121 non amylolitic bacteria isolates

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Fig.1. Productivity organic acids of selected amylolitic bacteria RSG
Fig2. Phylogenetic tree of amylolitic bacteria causing sourness on raw starch sago based on 16S rRNA
REFERENCES


   
http://www.jlinquist.net/generalmicro/dfstarch.html.


## TOPIC 2: ECOLOGY AND CONSERVATION

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P-EC01

TROPICAL EELS (Anguilla spp.) RECRUITING TO PROGO RIVER: IDENTIFICATION AND HATCHING DATES

Agung Budiwarjo¹, Jusup Subagja², Tjut Sugandawaty Djohan², Djumanto³
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ABSTRACT

Progo River is the entrance of eels to the inland waters of Java Island. This research aimed to identify and estimate the hatching dates of glass eel that recruiting in Progo estuarine. Glass eels were collected in a Progo estuarine from Februari 2007 – June 2009 at night during the new moon. Glass eel identification was based on characters previously defined by Ege (1939), Watanabe et al., 2008; Tabeta et al.,1976; and Tabeta dan Ozawa, 1979. Hatching dates were estimated with the back calculation of glass eel ages at recruit. Among 1.082 specimens collected, there are 3 species were identified. About 31,42% of the specimens were Anguilla marmorata, 63,96 % were Anguilla bicolor bicolor, and 4,62% were Anguilla nebulosa nebulosa. All juvenile pigmentation corresponded to the glass eel stage (VA). Otolith readings and sampling dates showed that glass eels are entry Progo River hatched during the low light intensity around the new moon.

INTRODUCTION

Eels (Anguilla spp.) were the catadromous fish (McKinnon 2006). Eels larvae migrated into the rivers, including Progo River (Aoyama et al. 2003; Jellyman & Tsukamoto 2002; Tesch 1977 ; Soetjipta & Sagi 1975). Until now, there has been no scientific information about the species of eels larvae which entered the Progo River. Hatching dates of eels larvae at sea take place only at a certain time. Hatching dates of eel larvae were entered Progo River unknown. This research aimed to identify and estimate the hatching dates of eels larvae that recruiting in Progo estuary. Eels larvae collected in Progo estuary from Februari 2007 – June 2009 at night during the new moon. Eels larvae identified were based on characters previously defined by Ege (1939), Watanabe et al. (2008); Tabeta et al. (1976); and Tabeta & Ozawa (1979). Eels age determinated with whole-otolith method (Vollestad et al. 1988). Hatching dates estimated with the back calculation of eels larvae ages at recruit. Among 1.082 specimens that had been collected, there are 3 species were identified. About 31,42% of the specimens are Anguilla marmorata, 63,96% are Anguilla bicolor bicolor, and 4,62% are Anguilla nebulosa nebulosa. Ages of eels larvae were migrated to Progo River was 58 to 190 days with the age groups 58-68 (N = 153), 84-97 (N =
217), 114-127 (N = 491), 145-155 (N = 195), and 188-190 (N = 26) days. Age groups were equal to the age of 2,3,4,5, and 6 months. The age groups show that eels larvae were entered the Progo River hatch take place only at a certain time. Eels larvae hatched in the sea only at low light intensity. Eels larvae hatched began on 19 to 4 (lunar dates), and most of them hatched on 23 to 28 (lunar dates).

![Figure 1](image)

**Figure 1.** Hatching dates of glass eels were sampled in Progo River in February 2007 - May 2009.

Based on the hatching dates of eels larvae were entered to Progo River, can be estimated duration time of eels spawning. According to Tsukamoto et al. (1992), within 36-39 hours after fertilization, which took place just after spawning, eggs are hatched. On the basis of this, eels spawning that their larvae entered Progo River also take place during very low light intensity. The results are in correspond with Tsukamoto et al. (2003); Ishikawa et al., (2002); and Fricke and Tsukamoto (1998) who explains that the spawning of tropical eels did not take place any time, but only held on certain days during the low light intensity. Based on the age and dates of sampled, eels larvae were entered Progo River hatched during the low light intensity.

**References**


P-EC02

IMPACT OF THE CILIWUNG RIVER ENVIRONMENT ON ITS WATER QUALITY

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Abstract

The Ciliwung River waters from upstream to downstream are heavily polluted by gray water from households, together with discharges from industries, pesticide and fertilizer run-off from agricultural land. The usage of land area in the upstream area of the riverbed have poured copious amount of waste and pollution, which has risen in level, into the river every year. The water quality monitoring of the Ciliwung River was done based on the specific environment alongside the river. Monitoring was done on dry season (2007) and rainy season (2008). Water quality parameters monitored was physical character (temperature, pH, conductivity, TSS and turbidity) and chemical character (DO, NH$_4^+$, NO$_3^-$, Sulfite and Chlor). Nine sampling side, three at upstream (Puncak), three at midstream (Katulampa, Bogor) and the other three at downstream (Manggarai) were monitored during the research. The results show that water quality at Puncak is relatively good. The level of conductivity was high at Manggarai, its level reach up of 40 at the dry season. The level of turbidity and TSS also tend to be higher at Manggarai, level of TSS reach on the range of 150-300 ppm. The concentration of DO at all locations was not significantly difference, but its level at dry season was very low, below 1 ppm. The Concentration of ammonium (NH$_4^+$) at Manggarai at the dry season was the highest (3-5 ppm), its level was above the standard levels that allowed by Government. The concentration of Sulfite and Chlor was also tend to be higher at Manggarai, at dry season those levels become on the range of 17-28 ppm and 20-30 ppm for Sulfite and Chlor respectively. From all of the data could be concluded that the pollution at downstream (Manggarai) was extremely high. The results of this research my help in giving consideration on creating a policy to regulate the environment, especially land use planning to prevent pollution.

Keywords: Ciliwung River, pollution, water quality, conductivity, sulfite, chlor

INTRODUCTION

The Ciliwung River is running along a line from South (Bogor) to North through the city (Jakarta). The upstream is located in Puncak Mountain, runs through the Bogor city and on through Jakarta. The downstream is located at Marina Beach in Jakarta Bay. The length of the river from upstream to downstream is about ± 76 km, with the catchments area of
about 322 km2. The Ciliwung River waters from upstream to downstream are normally polluted by gray water from households, commercial buildings, together with discharges from industries, pesticide and fertilizer run-off from agricultural land, solid waste, and fecal matter from overflowing or leaking septic tanks. The conversion of the land use will change the land function and cause increasingly the quality and quantity of waste entering to the river. In the last two decades, condition of the river basin of Ciliwung upstream has gotten worse due to uncontrolled land use changes. The conversions from agricultural to settlement uses have significant impact on the magnitude of variation of discharge. Especially in lowland rice field areas, due to a wide gap between the economic land rent with settlements and rice field uses, a large amount of highly suitable land for rice field have been converted. The purpose of this study is to assess the water quality differences of the Ciliwung River from the upstream to the downstream and describe the relationship between the land use and water quality in the Ciliwung River basin.

RESEARCH METHODOLOGY

Study site selection strategy: start from relatively clean headwater streams draining forests or rarely populated natural areas (Puncak) and go to downstream rivers that flows through rural, agricultural areas (Katulampa) and ends up in heavily populated urban and/or industrial areas (Manggarai). It is expected that the selected sampling sites represent representative land-use patterns and water pollution gradients in the whole watershed. We selected a total of nine sites, consisting of three relatively clean headwater streams (IB1, IB2, IB3), three rural or moderately polluted river sites (IB4, IB5, IB6), and three most polluted river sites (IB7, IB8, IB9). Monitoring was done on dry season (2007) and rainy season (2008). Water quality parameters monitored was physical character (temperature, pH, conductivity, TSS and turbidity) and chemical character (DO, NH4+, NO3-, Sulfite and Chlor).

RESULT AND DISCUSSION

The climate and landscapes of Ciliwung have significant influence on natural water quality conditions. Factors such as vegetation, geology and hydrology cause surface water quality to vary extensively. As the results of the physical characters monitoring showed that in general, water quality at Puncak is relatively good. On the other hand the level of conductivity was high at Manggarai, which reach up of 40 at the dry season (Fig.1). The level of TSS also tended to be higher at Manggarai, especially at rainy season, with level of TSS reach on the range of 150-300 ppm (Fig. 2).
The concentration of DO at Puncak and Katulampa were not significantly difference, but its level at Manggarai, on dry season was very low, below 1 ppm (Fig. 3). Surprisingly, the concentration of ammonium (NH₄⁺) at Manggarai on the dry season was also the highest (3-5 ppm) (Fig. 3), its level was above the standard levels that allowed by Government. In fact, it usually causes eutrophication problems. Ammonia levels greater than approximately 0.1 mg/L usually indicate polluted waters. The concentration of nitrate at Puncak (IB 1-3) and Katulampa (IB 4-6) were higher than Manggarai. Since over 60% of nitrate enters water usually from agricultural land, indicated that most of the land in those areas use as agricultures. These areas are well known as agropolitan area, that very high agro-chemical applied. The concentration of Sulfate and Chlor was also tend to be higher at Manggarai, at dry season those levels become on the range of 17-28 ppm and 20-30 ppm for Sulfite and Chlor respectively (Fig. 4). Sulfates (SO₄--) can be naturally occurring or the result of municipal or industrial discharges.

From all of the data could be concluded that the pollutions of Ciliwung River are affected by the environment, where the in let water are found. During dry season, especially at downstream (Manggarai), the pollution was even worse due to accumulation from the upstream and its surrounding environments. The results of this research my help in giving
consideration on creating a policy to regulate the environment, especially land use planning to prevent pollution.

ACKNOWLEDGEMENT

This work was supported by APN project “Climate Change on Surface Water Quality in East Asian Watersheds”.

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P-EC03

MOLLUSK DENSITY AT RECLAMATION AREA IN THE GULF OF MANADO, NORTHERN SULAWESI, INDONESIA

Frans Lumuindong¹, Marsoedi², Soemarno³, and Yenny Risjani²
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Abstract
Reclamation project at gulf of Manado was conducted along coastal line. To know the effect of coastal structure changing to the living organisms, especially mollusk, important to know numbers of mollusk species and observed their density to that area. Samples were collected in each station using transect along 100 meter that linear of coastal line in supralitoral, midlitoral, and sublitoral zone, when seawater on the tide condition. Substrates compositions were found at reclamation area only rock. Variances of rocks were grey rock, black rock, and sponge rock. Substrates composition after reclamation was found more homogenous than before. Mollusks that found in 2007 were 30 species and in 2008 were 34 species. This condition showed that during one year, mollusk in that area increased 4 species, such as Collisella striata, Drupella ricina, Littoraria scabra, and Littoraria undulata. Higher density at supralitoral zone in 2007 and 2008 was found at station I 86,7 and 83,1 individual/ m², in midlitoral zone was also found at station I 49,9 and 34,7 individual/ m², and sublitoral zone was found at station III 32,4 and 17,1 individual/ m². Higher density of mollusk in each years 2007 and 2008 at station I because of much Calyptraea extictorium species in supralitoral 391 and 452,4 individual/ m², and midlitoral 271,5 and 283,7 individual/ m². On the other hand, relative composition analysis showed similarly trend with result of density, whereas higher relative composition at supralitoral in 2007 and 2008 was found at station I 60,04% and 39,68%, in midlitoral was also found at station I 27,46% and 32%, and sublitoral was found at station II 45,76% and 46,23%.

Keywords: density, mollusk, reclamation, coastal
P-EC04

REMEDIATION MODEL OF CORAL REEF ECOSYSTEM VIA POLYCULTURE

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Abstract

Less than 1% of the ocean floor is covered by coral. Yet, 25% of the ocean's biodiversity is supported in these areas. Thus, conservationists are concerned when coral disappears, since the biodiversity of the region disappears shortly thereafter. In this research will be discussed how to make viable polyculture system to replace the current monoculture farming of milkfish that would improve water quality sufficiently that coral larvae could begin settling and decolonizing the area. The other goal of this research is doing a harvesting model using a simulation. The topic to be discussed is a fascinating mathematical problem because the population of any species may changed slightly over time where the populations of each species must remain roughly stable relative to the other population to keep the system in equilibrium. Even in the milkfish pens, a constant population of milkfish produces a relatively constant level of excretion. The mathematical modeling to develop a remediation model using an ordinary differential equation system, because the systems have stability and all population growths are directly proportional to each other. In our model polyculture ecosystem there exists one mollusk species and one algae species instead the coral reef. This model based on the food chain at the ecosystem. The result of this model obtained equilibrium among all species and the waters quality level.

Key Words: Mathematical Modeling, Coral Reef, and Polyculture System

INTRODUCTION

Coral reefs are a precious natural resources. They support a large percentage of the population’s resources. Coral reefs are vital to the ecosystem, but their existence is in extreme danger. The reefs can be damaged by the nearby milkfish pens, which are creating excess bacteria and light blocking algae. This excess buries the slow growing coral reefs beneath feet of debris. In ocean, coral reefs are one of a renewable resource that we need to preserve and prevent it from being destroyed. General estimates show approximately 10% of the coral reefs around the world are already destroyed. Coral reefs grow much faster in clear water which provides more sunlight. Corals can get up to 90% of their nutrients from their zooxanthellae symbionts. Whereas, fish is a staple source of protein in the diets of nearly all coastal societies and plays a large role in the world economy. Both fish catching and fish
consumption is predicted to increase drastically over the next 30 years. For example, milkfish (bangus) is an important foodfish and aquaculture commodity.

MATERIALS AND METHODS

Model Assumptions

Formulation of mathematical model of polyculture systems based on the assumptions as follows:

1. Milkfish produced feces/waste and source of feed derived from human.
2. Algae usually grow normally at tropical regions. Algae is a plant that can not move on active so not to compete each other, the source of food for the algae comes from the process of photosynthesis, milkfish feces, CO2, nitrogen, and phosphate.
3. Shellfish (mollusks) consumed feces / fish waste that is not consumed by the algae.
4. There are coral reefs in the system.

Mathematical Model

Based on these assumptions, the mathematical models can be developed that is expected to represent well the dynamics of ecosystems. This model uses a five variable that is Coral population (C), the population of Shellfish / Mollusks (M), population of Milkfish (B), population of Algae (A), and Feces/fish waste (F). Population dynamics of the five variables can be expressed in the following differential equation system:

\[
\begin{align*}
\frac{dB}{dt} &= \mu_B B(1 - \theta F) \left(1 - \frac{B}{K_B}\right) \\
\frac{dM}{dt} &= \mu_M M \left(1 - \frac{M}{K_M + \beta F}\right) \\
\frac{dC}{dt} &= \mu_C C \left(1 - \gamma F\right) \left(1 - \frac{C}{K_C}\right) \\
\frac{dA}{dt} &= \mu_A A \left(1 - \frac{A}{K_A + \sigma F}\right) \\
\frac{dF}{dt} &= \delta B - \alpha MF - \gamma F
\end{align*}
\]
Mathematical Model of Harvesting

\[ dB = \mu_b B (1 - \theta F) \left(1 - \frac{B}{K_b}\right) - pB \]

\[ dM = \mu_m M (1 - \frac{M}{K_m + BF}) - qM \]

\[ dC = \mu_c C (1 - \gamma F) \left(1 - \frac{C}{K_c}\right) - rCA \]

\[ dA = \mu_a A \left(1 - \frac{A}{K_a + \sigma F}\right) \]

\[ dF = \delta B - \alpha MF - \gamma F \]

\[ dB = \mu_b B (1 - \theta F) \left(1 - \frac{B}{K_b}\right) - pB \]

\[ dM = \mu_m M (1 - \frac{M}{K_m + BF}) - qM \]

\[ dC = \mu_c C (1 - \gamma F) \left(1 - \frac{C}{K_c}\right) - rCA \]

\[ dA = \mu_a A \left(1 - \frac{A}{K_a + \sigma F}\right) \]

\[ dF = \delta B - \alpha MF - \gamma F \]

RESULTS AND DISCUSSION

Fig. 1: Model before harvesting

Fig. 2: Harvesting model
If the concentration faeces (waste) of milkfish increases so will also increase the shellfish/mollusk population because of the excess food. Whereas after harvesting of shellfish and milkfish the shellfish populations, fish, algae, and coral always exist in the ecosystem. From the fig.2 with simulation harvesting model occurs the balance between each species in the ecosystem which affect to water quality.

REFERENCES


P-EC05
POTENCY OF LEMNACEAE AS BIOINDICATOR OF Cadmium (Cd) IN FRESHWATER

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Abstract
The purpose of this study was to determine the resistance lemnaceae (Lemna major, Lemna minor and Wollflia) of heavy metals Cd absorption. This research as a mechanism for water treatment systems Bioremediation river waters have been polluted by heavy metals (Cd) with Lemnaceae plants (floating plant) as a living organism to improve the water body from heavy metals to water can be reused as drinking water, agricultural water and industrial water. This research method is to experiment with random block design of two factors namely the concentration factor and the type of plant. Data consist of the lemma resistance levels and heavy metals were analyzed with ANAVA. Cd concentracion was analyzed using AAS. The results showed that treatment of heavy metals (Cd) of Lemnaceae plants, showing that Wollfia arrizha have the ability to absorb heavy metals is very large compared with the two other Lemnaceae plants.

Key words: Lemnaceae, heavy metals Cd, bioindicator

INTRODUCTION
Water is often contaminated by inorganic components, including a variety of dangerous heavy metals and come from various industries. Some heavy metals are widely used in a variety of purposes, therefore, are routinely produced in industrial scale. The use of heavy metals in a variety of daily necessities directly or indirectly can pollute the environment (Fardiaz, 1992). Dangerous heavy metals and often pollute the environment is particularly mercury (Hg), Lead (Pb), arsenic (Ar), Cadmium (Cd), Chromium (Cr), and Nickel (Ni). (Kristanto, 2002). The results of such research in the waters at times Surabaya by Puspitasari (2004), levels of heavy metals lead (Pb) on kale (Ipomoea aquatica) is greater than water. This can disrupt the survival of organisms in the water, and may even kill certain species, but does not endanger other species. Instead there is the possibility that a contaminant can support the development of certain species (SASTRAWIYAYA, 2001).

Lemnaceae which is a water plant (floating float) can also be used for wastewater treatment using a floating water plant system. Lemnaceae have the ability to survive in a bad environment (high heavy metal content, nitrogen content and phosphat high and high salinity). Quickly, lemnaceae plant will be able to absorb nitrogen, phosphor, calcium, sodium, potassium, carbon and chloride from waste water to be used for population growth. (Leng, et al.1995). Lemnaceae research primarily as a plant Lemna minor bioindicator heavy metals
have been made as study of introduction. The result of study showed Lemna minor can reduce levels of heavy metals in waste water containing 2.273 ppm Fe. Lemna minor can reduce levels of heavy metals up to 50% for Fe, (Safiludin, Fida R, Yuliani, 2005).

These plants can grow and develop without control due to environmental factors such as the number of supporting nutrients and sunlight in the stagnant waters. Lemnaceae can grow rapidly, especially in waters that contain nitrogen and high phosphat. Unlike plants in general, can survive on Lemnaceae bad environment, which menginsyaratkan the potential benefits of Lemnaceae. For example Lemnaceae can be tolerant of different pH range (5-9), but the best growth at pH 6.5 to 7.5, Except that Lemnaceae can survive in conditions of high salinity (up to 4000 mg / liter). They also can collect the remains of the metal in industrial waste that can produce water suitable for release into the flow of water or irrigation so that at the same time can provide economic solutions to restore the source of valuable resources that would otherwise be handled will pollute the environment (Leng, et al, 1995).

Based on the above background problems can be formulated as follows "How resistance lemnaceae (Lemna major, Lemna minor and Wolffia) of the absorption of heavy metals Cd?"

METHODS

This research includes experimental studies. Subject of this research is composed of Lemnaceae that Lemna minor, Spirodela polyrhiza (Lemna major) and Wolffia arrhiza

Variables of Research

Variable of manipulation: Provision of heavy metals in different concentrations, the levels of heavy metals Cd of 1.2 and 3 mg / liter. While the response variable content of heavy metals in the Lemnaceae and water.

Design of Research

In the first phase of the research, study design used was random block factorial design, this design is used because in this study had an experiment with two factors. The first factor concentration and the second factor of metals.

Procedures

a. Adaptation of lemnaceae: Planting the crop species Lemna minor, Lemna major, and Wolffia by aquades and every two days later made the replacement of water, plants adapted in 10 days and was analyzed levels of heavy metals contained in the initial plant body.
b. Planting media creation

Plants that provide media containing metals Cd, with appropriate levels of experimental design and planting media placed in the measuring vessels 5 liters. Preparing the planting medium that includes the control without metal content of Cd with the media control akuades. Cd in the plants. One also carried out pH and temperature measurements as supporters.

Data Analysis

Data analysis was carried out research in three ways. The first two-way analysis of Anava will be followed by DMRT test to see the difference in experimental research for the first phase of lemnaceae tolerant of heavy metals. For other data analysis is performed quantitative descriptive.

RESULT

This study uses three types of plant Lemna major Lemnaceae (Spirodela polyrhiza), Lemna minor and Wolffia arrizha. Third Lemnaceae plants showed different resistance to heavy metals Data early levels of heavy metals Cd at the three Lemnaceae plants and water sources in Mojokerto Pacet Lemnaceae plants used came from the campus area Sukolilo ITS Surabaya, from the three plants each, although there are heavy metals below the threshold as in the following table:

Table 1. Result of initial levels of Heavy Metals Pb (mg/l) in Lemnaceae Plants

<table>
<thead>
<tr>
<th>Plants</th>
<th>Heavy Metal</th>
<th>Concentration (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lemna minor</td>
<td>Cadmium (Cd)</td>
<td>0.019</td>
</tr>
<tr>
<td>Lemna major</td>
<td>Cadmium (Cd)</td>
<td>0.0016</td>
</tr>
<tr>
<td>Wolffia arrizha</td>
<td>Cadmium (Cd)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Table 2. Result of initial levels of Heavy Metals Pb (ppm) of water sources Pacet, Mojokerto

For research use water from Pacet, Mojokerto with heavy metals analysis as follows:

<table>
<thead>
<tr>
<th>Material</th>
<th>Heavy metal</th>
<th>Concentration (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water Resources Pacet</td>
<td>Cadmium (Cd)</td>
<td>0.00</td>
</tr>
<tr>
<td>Mojokerto</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Heavy metal content of Cd in the Lemnaceae plants after treatment

The results of variance analysis showed that treatment of heavy metal with various types and concentrations affect the content of heavy metals (Cd) is absorbed by plants Lemnaceae. Similarly, the interaction of the metal type and concentration affect the absorption of heavy metals in plants Lemnaceae. Content of heavy metals contained in the Lemnaceae plants after treatment for 10 days are presented in Table 3 as follows: Table 3. Absorbing ability of the three plants to heavy metals Lemnaceae Pb (mg / l)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentrations (mg/l)</th>
<th>Lemna major</th>
<th>Lemna minor</th>
<th>Wolffia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cd</td>
<td>K₀ (0 mg/l)</td>
<td>0.06ᵃ</td>
<td>0.05ᵇ</td>
<td>0.024ᵃ</td>
</tr>
<tr>
<td></td>
<td>K₁ (1 mg/l)</td>
<td>0.59ᵇ</td>
<td>0.65ᵇ</td>
<td>0.06ᵃ</td>
</tr>
<tr>
<td></td>
<td>K₂ (2 mg/l)</td>
<td>0.61ᵇ</td>
<td>0.59ᵇ</td>
<td>0.19ᵇ</td>
</tr>
<tr>
<td></td>
<td>K₃ (3 mg/l)</td>
<td>0.64ᵇ</td>
<td>0.63ᵇ</td>
<td>0.08ᵇ</td>
</tr>
</tbody>
</table>

Note: Figures followed by the same letter in a column are not significantly different at level 5% test BNT

BNT test results at the 0.05 level in the plant Lemna major, Lemna minor and Wolffia show that there is a real difference to the concentration of 1 mg / l, 2 mg / l and 3 mg / l with the concentration of 0 mg / l.

Content of heavy metals in the medium water treatment plant after the Lemnaceae.

After planting Lemnaceae for ten days, heavy metal content of Cd in the planting medium analyzed by AAS, the results are given in Table 4.
Table 4. Content of heavy metals Cd (ppm) in water media planting after treatment

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentrations of heavy metals in water media (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Konsentrası (mg/l) Lemna major Lemna minor Wolffia</td>
</tr>
<tr>
<td><strong>Heavy</strong></td>
<td></td>
</tr>
<tr>
<td><strong>metal</strong></td>
<td></td>
</tr>
<tr>
<td>Cd</td>
<td></td>
</tr>
<tr>
<td>K₀ (0 mg/l)</td>
<td>0,05 0,04 0,004</td>
</tr>
<tr>
<td>K₁ (1 mg/l)</td>
<td>1,17 1,13 0,002</td>
</tr>
<tr>
<td>K₂ (2 mg/l)</td>
<td>1,19 1,15 0,005</td>
</tr>
<tr>
<td>K₃ (3 mg/l)</td>
<td>1,14 1,14 0,006</td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td>0,89 0,86 0,01</td>
</tr>
</tbody>
</table>

Overall average levels of heavy metals in the media indicate that planting vegetation planting media Wolffia levels of heavy metals showed the smallest compared to the planting medium in Lemna major and minor.

**Percentage decrease in levels of heavy metals Cd on plant water media Lemnaceae plants.**

From Table 5. calculated the percentage reduction of heavy metals in the media measured by planting early assay medium water plant with a metal levels after treatment.

Table 5. Percentage data reduction Pb levels of heavy metals in water media

<table>
<thead>
<tr>
<th>Plant/ Heavy metal</th>
<th>The initial concentration of heavy metals (mg/l)</th>
<th>The concentration of water state final (mg/l)</th>
<th>% decrease in the concentration of heavy metals (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Lemna minor</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cd</td>
<td>1,0</td>
<td>2,0</td>
<td>3,0</td>
</tr>
<tr>
<td>Lemna major</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cd</td>
<td>1,0</td>
<td>2,0</td>
<td>3,0</td>
</tr>
<tr>
<td>Wolffia</td>
<td>Cd</td>
<td>1.0</td>
<td>2.0</td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
</tbody>
</table>

In Lemna major and Lemna minor plants showed that the higher the concentration of the absorption of heavy metals are also more. For Wolffia plants, showing different things. In Cd absorption increased, but in line with the increase concentration of absorption becomes stable. From the results of research on the effects of heavy metals Lemnaceae plants, showed that Wolffia arrizha have the ability to absorb heavy metals is very large compared with the two other Lemnaceae plants. The results showed that the Lemnaceae plants have the capacity to absorb heavy metals Cd in water planting media, although the absorption capacity of each plant is different, from the three plants Lemnaceae, Wolffia has the largest absorption ability compared with the two other plants. Duckweeds grow together, no true leaves and stems, but only has a root and leaf-like layers containing carrier networks nutrient (Marianto, 2001). Duckweeds roots in plants has an important role in the process of absorption of heavy metals, because in addition to having a large capacity to absorb heavy metals compared with other parts, from the root surface is used as a place to live for some microorganisms that play a role in reducing heavy metal. So on the surface roots will occur physical processes, chemical and biological processing of heavy metals. Based on this, the metal-metal around it will easily be absorbed by the roots and into the body Duchweeds (Connell and Miller, 1995) . Plants that live in polluted areas have a mechanism which makes adjustment of pollutants to be switched off and stored in the old network that does not endanger the growth and life. According to Fitter and Hay (2001) suggests four main mechanisms that this is a concern, namely: a) Avoidance (escape) Fenologis, if the stress occurs on seasonal crops, plants can adaptation of life, that grows in the perfect season only; b) exclusion, the plant can recognize the toxic ions and prevent them from not drawn so as not experiencing toxicity; c) Ameliorasi, plants can absorb ions, but to act in such a way as to minimize its influence. Type includes the creation of khelat, dilution, localized or even excretion; d) Tolerance, plants can develop a metabolic system that can function on the potential toxic concentrations, the enzyme molecule and genetic work /With the environment a lot of heavy metals is a regulatory protein in all three crops a gene expression in order to form a binding compounds called fitokhelatin. Fitokhelatin a peptide containing amino acids 2 -8 in the central system and an acid molecule of glutamate and a glycine at the opposite end. Fitokelatin formed in the core then passes through the endoplasmic reticulum (ER), Golgi apparatus, for till Vasikula Sekretori cell surface. This lot contains Fitokelatin functional group SH-, Sz-, RS-clan groups have a function in amino acid which is a compound system builder
fitokelatin. Fitokelatin will form a bond at the end of the sulfide sulfur in systein when met with an iron, cadmium, timbale and other heavy metals, and form complex compounds. So that heavy metals will be carried into the plant tissue (Salisbury and Ross, 1995). In plants cadmium (Cd) is a non-essential elements of which, when entered into the plant, it will be dikelat by a protein that is then stored in the roots and some will be forwarded to the leaves. Cadmium (Cd) in plant tissue will cause damage to epidermal tissue, sponges, and fence network. Damage is characterized by chlorotic and necrosis (Palar, 1994). Wolfia has the largest absorption ability compared with the two other plants, have roots Wolfia not only has a single leaf or two that are interconnected networks of nutrient transport, create non-root Wolfia leaves on the plant has an important role in the process of absorption of heavy metals, thus the metal weight is not attached to the root of it is directly absorbed by the leaves, where the metal will accumulate in the vacuole is not going to do with the process of plant cell physiology.

CONCLUSION

The results of this first year can be concluded that: The results of testing the resistance of plants to lemnacea heavy metals (Cd) showed Wolfia plants more resistant than Lemna minor and Lemna major.

REFERENCES


P-EC06

POPULATION OF CELLULOLITIC FUNGI AND AMMONIFICATION BACTERIA IN AN EX-COAL MINING AREA OF PT BERAU COAL, EAST KALIMANTAN

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Abstract

An ex coal mining area is critical. It possesses physical and chemical soil properties that unfavorable for growth of vegetations. Consequently it has low population of vegetation as well as soil organisms including important soil microorganisms. In the process of ecosystem rehabilitation, soil microorganisms, such as cellulolitic fungi and ammonification bacteria, play important roles because their activity may enhance the process of soil development. This study aimed to assess the population of cellulolitic fungi and ammonification bacteria in an ex-coal mining area of PT Berau Coal in East Kalimantan.

Surface soil (0-5 cm) were sampled from a newly rehabilitated area (no vegetation), rehabilitated area under *Trema cannabina* and from an adjacent remaining forest in Binungan Site, PT Berau Coal in East Kalimantan. Population of cellulolitic fungi and ammonification bacteria were respectively enumerated using plate count and most probable number methods.

Result of the research showed that population of cellulolitic fungi in the newly rehabilitated area, under *T. cannabina* and in the forest soil do not varied widely (1.5 x 10^6 cfu, 12.5 x 10^6 cfu and 4.5 x 10^6 cfu, respectively). In contrast, population of ammonification bacteria was the lowest in the newly rehabilitated area (1.71 x 10^6 cell/g) followed by under *T. cannabina* (3.75 x 10^7 cell/g) and the greatest was in the forest soil (1.1 x 10^9 cell/g).

Keywords: cellulolitic fungi, ammonification bacteria, ex-coal mining area

INTRODUCTION

An ex-coal mining area is critical. Productivity of this area decreased due to inappropriate management through a serial degradation which interacted as physical, chemical and biological processes (Nugroho & Soewandito, 2000). Ex-coal mining area is characterized by bioecophysical changes that include (a) physical and chemical soil properties: no soil horizon particularly organic horizon, no soil structure, soil compaction; (b) low population of flora, fauna and microorganisms; and (c) harsh microclimate due to the opening of canopy (Hidayati *et al.*, 1999). Rehabilitation of degraded ecosystem requires soil development in which microorganisms plays important rules. Vegetation type and litter quality determine activity of microorganisms more importantly than the soil substrate.
(Sourkova et al., 2005). Decomposition of soil litter is conducted by cellulolitic fungi and further release of mineral nitrogen was conducted by ammonification bacteria (Rao, 1978). This study aimed to assess the population of cellulolitic fungi and ammonification bacteria in an ex-coal mining area of PT Berau Coal in East Kalimantan.

MATERIALS AND METHODS

Soil samples were collected from PT. Berau Coal, East Kalimantan at Binungan site. Surface soils (0-5 cm) were taken from a newly rehabilitated area (no vegetation), rehabilitated area under *Trema cannabina* (pioneer vegetation) and from an adjacent remaining forest. Population of cellulolitic fungi and ammonification bacteria were respectively enumerated using plate count method on Czapek cellulose agar (Yeoh et al., 1985) and most probable number method using peptone broth (Seeley & VanDenmark, 1972) in Physiology Laboratory, Department of Silviculture, Faculty of Forestry, Gadjah Mada University.

RESULT AND DISCUSSION

The first aim of rehabilitation process through vegetation succession approach is to restore microclimate. This is achieved by planting pioneer vegetation that has shallow root and produce litter in short period. This condition allows growth of microorganisms that have rules in litter decomposition (Soekotjo, 2004) and therefore the soil development (Sourkova et al., 2005).

Result of this research showed that population of cellulolitic fungi was greater in soil under *T. cannabina* and in the forest soil (12.5 x 10^6 cfu and 4.5 x 10^6 cfu, respectively) than in the newly rehabilitated area (1.5 x 10^6 cfu)(Table 1). Population of ammonification bacteria was the greatest in the forest soil (1.1 x 10^9 cell/g) followed by under *T. cannabina* (3.75 x 10^7 cell/g) and the lowest in the newly rehabilitated area (1.71 x 10^6 cell/g) (Table 2). This is possibly related to the availability of organic matter in the forest soil that serves as substrate for growth.
Table 1. The average amount of cellulolytic fungi in ex coal mining PT. Berau Coal East Kalimantan.

Table 2. The average amount of ammonification bacteria in ex coal mining PT. Berau Coal East Kalimantan.

*T. cannabina* also increased soil organic matter in the rehabilitated area, because it grew fast and produced great amount of litter. It also altered condition (reduced light intensity hitting the soil and temperature) thus becoming favorable for growth of soil microorganisms (Nurjanto et al., 2007). Although the population was much smaller than in the forest soil, it was clear that the growth of *T. cannabina* was able to create condition favorable for the growth of ammonification bacteria. It also supported the best growth of cellulolytic fungi.

**CONCLUSION**

Population of cellulolytic fungi and ammonification bacteria were greater in soil under *T. cannabina* and in the forest soil where availability of organic matter was greater than in the newly rehabilitated area. *T. cannabina* is able to increase soil organic matter and condition favorable for growth of soil microorganisms.
REFERENCES


P-EC07

Study of Heavy Metal Removal from Polluted Waters and Land Area Using Acacia mangium: Bioadsorption and Phytoremediation


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ABSTRACT

Environmental pollution caused by heavy metal bring to serious health problems. This research developed eco-friendly heavy metal removal technique from polluted water and land area through physical, chemical and biological based remediation methods. The main purpose of this project is utilization of non edible plant for heavy metal handling. For polluted water, it was developed a heavy metal bioadsorber agent, which capable to adsorb oil without adsorbing water, utilizing pyrolyzed sawdust. Whereas, for land area, it was developed a phytoremediation agent using heavy metal hyperaccumulator. Both of bioadsorber and phytoremediation agent were using A. mangium. The sawdust (20-40 mesh) pyrolysis process for bioadsorber synthesis was conducted at various temperatures 200, 250 and 300°C with various pyrolysis duration 30, 45, 60 and 75 min. Then the carbon residue was used as bioadsorber for Pb and Cd solution with various treatments including of heavy metal concentraion (Pb: 0, 50, 100, 200 and 400 ppm; Cd: 0, 1, 3, 6 and 9 ppm), the ratio of bioadsorber : solution (1:100, 2:100 and 3:100 w/v) and agitation time (0.5, 1, 3, 5 and 24 h). The AAS result showed that the optimum condition for bioadsorber pyrolysis was at 300°C for 60 min, which was able to absorbp Pb and Cd almost perfectly (>99%) for 1 h agitation time with 1:100 (w/v) ratio of bioadsorber and heavy metal solution. These data were also supported by water soluble content, tannin content and SEM analysis. The phytoremediation was conducted in the greenhouse, where the plant (4 month growth time) was given the heavy metal treatment with various concentration as like as the bioadsorber treatment for 28 days. The AAS result showed that Pb adsorption in A. mangium increased with increasing of Pb concentration presented in the soil and the optimum adsorption for Pb was obtained in Pb 400 ppm, i.e. 77.297 ppm/g plant. Adsorption of Cd in A. mangium reached the optimum condition in Cd 3 ppm, i.e. 2.054 ppm/g plant. The Bioaccumulation Coefficient (BC) in A. mangium for Pb were 0.021-0.038 and Cd were 0.011-0.054. This research can be used as input for the bioremediation and phytoremediation practitioner.

Keywords: Environmental pollution, heavy metal, A. mangium, bioadsorption, phytoremediation
INTRODUCTION

Oil sludge (OS) is a waste product produced in petroleum refinery activities. In general, this OS come from sedimentation of fine particles of fuel. Sediment is accumulated in the bottom of storage tanks or in the distribution fuel pipes. OS also contain heavy metals from the oil refinery activities. Until now there has been no effort to handle waste processing thoroughly in the operation of oil refinery activities. Heavy metal often comes from former area of OS bioremediation. In general, to degrade the oil content (total poly-hydrocarbon) it can be done by bioremediation process, but the heavy metal content of the post bioremediation process are still left behind in the area. Heavy metal can infiltrate into the ground and carried by water and contaminate groundwater.

Removal techniques of heavy metal in polluted waters and soil can be done with combination of physics, chemistry and biology based remediation methods. The combination of chemical and physical processes can be done by using adsorbent material, such as synthetic polymer or natural material. For example, adsorption of heavy metal can be done by sawdust. Sawdust that has been previously heated at 180°C can be selectively adsorbing oil in the sea without adsorbing water. Components of lignin, tannins and other aromatic extracts are also capable of absorbing heavy metals (Roffael & Dix, 1989).

In addition to physical chemical processes, remediation of OS could be done by phytoremediation. Naturally plants have the ability to tolerate the number of heavy metal absorbed into the plant. The selection of appropriate plants as phytoremediation agents play an important role that will determine the success of absorption of heavy metals. The selection of plants should be adapted to environmental conditions where the plants will be grown, the type of heavy metals to be taken, as well as the ease and availability of plants around the location.

Heavy metal contamination has a direct impact to health. Bioadsorption and phytoremediation are alternative methods to overcome the heavy metal contamination. Therefore, the purpose of this study is to reduce environmental pollution due to heavy metal of petroleum waste with environmentally friendly methods by utilizing non-edible plant. The first goal is adsorbing oil and heavy metal in polluted waters with bioadsorber made from plant material. And the second goal is to use hyperaccumulator plant to absorb heavy metal contamination in polluted land area.
MATERIALS AND METHODS

Bioadsorption

This bioadsorption process was conducted at Laboratory of Chemistry Process (RC for Biotechnology-LIPI, Cibenong). A 200 g of A. mangium sawdust (20-40 mesh bark/wood powder, UPT Biomaterial–LIPI, Cibenong) was pyrolyzed using electric furnace (RC for Physics–LIPI, Bandung) at various temperatures 200, 250 and 300°C with various pyrolysis duration 30, 45, 60 and 75 min (Berrueco et al., 2004; Guo, 2004; Grioui et al., 2007; Krzesińska et al., 2007). The carbon residue (bioadsorber) were characterized, i.e. tannin content, water soluble content and pore structure. Then bioadsorber was used for adsorbing Pb (Pb(NO₃)₂, Merck) and Cd (CdSO₄·8H₂O, Merck) solution with various treatments including of heavy metal concentration (Pb: 0, 50, 100, 200 and 400 ppm; Cd: 0, 1, 3, 6 and 9 ppm), the ratio of bioadsorber : solution (1:100, 2:100 and 3:100 w/v) and agitation time (0.5, 1, 3, 5 and 24 h), with shaking speed 130 rpm and was filtered using Whatman No. 1. The filtrate was used to determined the percent adsorption.

Phytoremediation

This phytoremediation process was conducted at the greenhouse (RC for Biotechnology-LIPI). The seed of A. mangium (The Bureau of Java and Madura Forest Plant Seeding, Sumedang) was used as the phytoremediation plant. The plant (4 month growth time, 4 plants for each treatment) was given the heavy metal treatment with various concentration as like as the bioadsorber treatment for 28 days. The whole part of the plant (leaves, stem, root) and the soil samples were dried at 100°C for 36 h, then were grinded using mortar. A 1 g sampel powder was added with 15 mL of HNO₃/HClO₄ (3:1) (v/v)

![Image](image-url)
(Yanqun et al., 2005). Sample was heated using hot plate at 300 W for 1 h, then was cooled and was added with 10 mL of HNO₃ 0.2%. The solution was filtered using Whatman No. 1 and the filtrate was used to determined the percent absorption and bioaccumulation coefficient.

![Picture 2. A. mangium plant was given heavy metal treatment](image)

Characterization

Tannin and water soluble content was determined as the following. A 5 g sample (sawdust or pyrolyzed sawdust) was dried at 105°C for 5 h, then was repeated for 1 h until constant weight. Then the volatile content was determined. Furthermore, sample was added with aquadest (ratio 1:25) and was heated in autoclave with pressure 1 atm at 121°C for 15 min, then was filtered using Whatman No. 1 and was obtained tannin extract. Tannin content was determined qualitatively using UV-Vis spectrophotometer (UV-160 Shimadzu) at 280 nm (Subiyakto and Prasetya, 2003). Whereas, the residue was dried at 105°C for 5 h, then was repeated for 1 h until constant weight and water soluble content was determined.

Pore structure of the sawdust or pyrolyzed sawdust was analyzed qualitatively using SEM (Scanning Electron Microscopy). Whereas, the concentration of heavy metal absorbed by bioadsorber or phytoremediation agent was determined using AAS (Perkin Elmer).

![Picture 3. Extraction of heavy metal from plant and soil samples](image)

RESULTS AND DISCUSSION

Bioadsorption

Pb and Cd heavy metals adsorption were conducted by analyzing the effect of ratio of heavy metal solution and sawdust (v/w) and the effect of agitation time. The data were presented on the Fig. 4 and 5.
Figure 4. Pb adsorption with variation of sawdust amount

Figure 5. Cd adsorption with variation of sawdust amount
The result of analysis showed that Pb and Cd adsorption using carbon residue of *A. mangium* have a high adsorption value. Actually, adsorption value was increased as like as increasing of temperature and duration of pyrolysis, but in several results of analysis showed that unpyrolyzed *A. mangium* sawdust have a high adsorption capability. There are several factors who responsible to adsorption value, i.e. chemical compound of the sawdust, pyrolysis process, surface area and pore size of the sawdust. Based on the process happened, this can be explained by physical and chemical mechanism.

Chemically, sawdust consist of chemical compounds which have potency to adsorb heavy metal through inter molecule binding mechanism. These compounds, such as tannin, have functional group which is able to bind heavy metal strongly. Greater amount of tannin in
sawdust resulted greater chance of heavy metal adsorption. *A. mangium* was one of wood having high tannin compound. Moreover, sawdust having high of tannin content and low of volatile and water soluble content will be able to adsorb heavy metal and oil from waters optimally.

Fig. 8 described that the water content of pyrolyzed sawdust of *A. mangium* were relatively low. It indicated that pyrolysis has been decreased water adsorption capability of *A. mangium* sawdust that contributed to application for heavy metal adsorption in oil waste. Whereas, the water soluble content of *A. mangium* sawdust before and after pyrolysis were relatively similar, so this parameter gave less effect to the adsorption capability of *A. mangium* sawdust. Result of UV-Vis Spectrophotometric showed that on 3 variation of pyrolysis condition, i.e. at 200°C for 30 min, at 200°C for 45 min and at 250°C for 45 min, tannin content (represented by absorbance of tannin extract) was relatively constant. Then generally, tannin content decreased sharply by the increase of temperature and duration of pyrolysis. Based on the above results, it was known that the optimum condition of pyrolysis of *A. mangium* sawdust was at 250°C for 45 min.

![Figure 8](image)

**Figure 8.** Volatile content, water soluble content and absorbance of tannin extract solution from *A. mangium*.

Physically, adsorption of heavy metal involved surface area and pore structure factors. According to Sjöström (1993) pyrolysis process will be able to degradate chemical compounds of wood, i.e. hemicellulose at 200–260°C, cellulose at 240–350°C and lignin at 280-500°C. The chemical compounds from the pyrolysis product consist of small compounds which have large of surface area and a lot of functional groups that able to bind and to adsorb heavy metal. The degradation processes on the chemical compounds of wood gave an
impact to heavy metal adsorption capability of wood. It can be increased as like as increasing quantity of chemical compounds as a product of degradation process. Increasing of pyrolysis temperature and duration will cause a lot of wood chemical compounds were degraded into small molecules. Small molecules having more functional groups which are able to bind heavy metal have higher surface area and smaller pore structure. According to Rahayu (2004), adsorption was physic phenomenon that depended on surface area and pore structure. Small pore structure would cause limited size of adsorbed molecules. Greater surface area enabled the increase of heavy metal adsorption. Pyrolysis had role to enlarge surface area and pore size. So it could be assumed that the higher pyrolysis temperature and the longer pyrolysis duration resulted higher adsorption of heavy metal because of greater surface area and pore size. Pyrolysis caused shrinkage of dimension and enlargement of hollow space compared with initial condition (Anggono, et al.). SEM would describe the variation of particle size and pore size which could help the analysis for achieving optimal condition for heavy metal and oil adsorption. Example of visual appearance of pyrolyzed sawdust can be shown on Fig. 10.

Carbonization on high temperature would damage transversal wall of cell on axial cut so that enlarging surface area (Krzesińska et al., 2007). The higher surface area would increase adsorption and reaction with adsorbent. While trend of weight loss increase toward pyrolysis duration hasn’t been appeared, except at 200°C for 30-60 min.

Fig. 9 described changes of shape and size of A. mangium pore before and after pyrolysis. At 300°C for 75 min pyrolysis treatment, structural change was clearly appeared on the depth of surface lines. The hollows also became greater in consequence of the release of volatile matters from sawdust during pyrolysis.

**Phytoremediation**

Table 1 showed Bioaccumulation Coefficient (BC) that was ratio of heavy metal concentration in the plant to heavy metal concentration in the medium (P. Tanhan et.al., 2007). BC on the Pb absorption by A. mangium was 0.021-0.038 while Cd absorption was 0.011-0.054. This difference possibly caused by variation of metal solution, metal compound
and kind of plant. Metal was translocated from root to shoot with different mobilities for each metals. Cd and Zn were more mobile than Pb and Cu (P. Tanhan et.al., 2007).

<table>
<thead>
<tr>
<th>Metal</th>
<th>C (ppm)</th>
<th>BC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pb</td>
<td>50</td>
<td>0.038</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>0.036</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>0.021</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>0.030</td>
</tr>
<tr>
<td>Cd</td>
<td>1</td>
<td>0.047</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.054</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>0.011</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>0.012</td>
</tr>
</tbody>
</table>

As have been discussed above, generally, heavy metal content absorbed in the plant would increase by the increase of heavy metal concentration in the given solution (P. Tanhan et.al., 2007). It was shown that there was trend of the increase of absorbed heavy metal (ppm/g plant) by the increase of heavy metal concentration in the medium. This phenomenon was appeared in Pb absorption (Fig. 10), while the same phenomenon was not shown in Cd adsorption. Fig. 11 described the absorption of Cd by A. mangium sawdust reached optimum condition by addition of 3 ppm Cd solution.

![Absorbed Pb concentration for each gram of plant weight.](image)
Many studies proved that heavy metal concentration absorbed in the plant was the function of heavy metal concentration in the environment (medium). The amount of absorbed Pb and Cd would be higher by the increase of those metal concentration in the soil. Concentration of absorbed Pb was higher than Cd because Pb had tendency to be precipitated easier by phosphate and sulphate in the root system. It indicated that the absorption of Pb did not require high energy. Pb could be absorbed from environment against concentration gradient and would be deposited in the high amount in the root (P. Tanhan et al., 2007). In conclusion, Cd absorption level of *A. mangium* plant with the age of 4 months was higher than Pb absorption level.

**CONCLUSION**

Carbon residue of *A. mangium* had a high adsorption value of Pb and Cd. Actually, adsorption value was increased as like as increasing of temperature and duration of pyrolysis. There are several factors who responsible to adsorption value, i.e. chemical compound of the sawdust, pyrolysis process, surface area and pore size of the sawdust. Further experiment should be done for obtaining the optimum condition of pyrolysis. The amount of absorbed Pb and Cd in the plant would be higher by the increase of those metal concentration in the soil. *A. mangium* plant with the age of 4 months has already shown the absorption capability for heavy metal. However, it is important to continue studying Indonesian plants to discover hyperaccumulator plants for heavy metal removal.

**ACKNOWLEDGEMENTS**

We greatly thanks to Dr. Tri Muji Ermayanti for the discussion and suggestion, and also PT. Pertamina UP V Balikpapan for collecting the oil sludge samples. This research was funded by the 2008 Insentive Programme of Ministry of Research and Technology.
REFERENCES


P-EC08

INSECTS BIODIVERSITY AT GUNUNG GEDE-PANGRANGO NATIONAL PARK

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Abstract
Insects are the main group of Arthropods. They are the most dominant animals in the terrestrial ecosystems. They play an important role in forest ecosystem, such as decomposers, natural enemies and succession. The study of insect biodiversity was carried out in Gunung Gede-Pangrango National Park, West Java, Indonesia. The objectives of this study were to measure insect composition and abundance using two types of trap. Yellow-pan trap and malaise trap were used to collect insect samples. The result showed here were twelve orders consisted of 99 families and 896 individuals. Diptera and Hymenoptera were the dominant orders. Sciaridae family was the higher number for Diptera, while Ichneumonidae was the most abundant for Hymenoptera.

Keywords: Insect, Yellow-pan trap, Malaise trap, Diptera

INTRODUCTION
Insects are the main group of Arthropods. They are the most dominant animals in the terrestrial ecosystems. They play an important role in forest ecosystem, such as decomposers, natural enemies and succession. Based on their important role therefore, species diversity as well as their its abundance to be important factors in managing forest. Variation in species richness at the landscape scale is useful tool and an important consideration in conservation planning, environmental monitoring and natural resource management (Magurran, 1988). Although species richness is but one component of biological diversity and only one of many criteria conservationists and planners may use when evaluating sites (Vane-Wright et al., 1991). It becomes especially important as the global loss of species by extinction accelerates and the need for species preservation increases. The study of insect biodiversity was carried out in Gunung Gede-Pangrango National Park (GGPNP), West Java, Indonesia. The objectives of this study were to measure insect composition and abundance using two types of trap.

MATERIALS AND METHODS
Insect collections were carried out from June to Oktober 2007. Two types of trappings were used for sampling of forest insects they were yellow-pan trap and malaise
trap. Yellow-pan traps were placed on the forest floor. Each trap was filled with water containing a little detergent and sorbic acid as preservative agent. Malaise trap consisted of a mosquito net like structure fastened on the ground. A plastic bottle containing 70% ethanol was attached at the upper end. The traps were emptied after 1 week and the insects were stored in 70% ethanol. Trapping was done three times. The specimens were sorted in the laboratory and then identified. Traps were set in three plots inside the sampling plot. The sampling plot design used in FHM is called Cluster-Plot Design (Figure 1). The cluster-plot design based on Forest Health Monitoring: Field Methods Guide (Alexander and Bernard, 1997). FHM Cluster-plots in GGPNP was used as the case study.

RESULTS AND DISCUSSION

Twelve orders of insect were found in GGPNP, consisted of 99 families and 896 individuals. The order collected were Hymenoptera, Diptera, Coleoptera, Collembola, Orthoptera, Homoptera, Lepidoptera, Hemiptera, Psocoptera, Neuroptera, Grylloblattaria and Blattaria. The common orders were Hymenoptera and Diptera, having 80% (Figure 1). Among the Hymenoptera, 362 individuals were recorded and the most dominant family was Ichneumonidae, almost 50%. Twenty two families of Hymenoptera were sampled from the site. A total of 327 individuals of Diptera from 31 families were captured and Sciaridae was the dominant family comprising about 20% of the total number. The primary forest, such as GGPNP, with its thick layer of litter, denser canopy and slightly less understorey plant makes microclimate conditions more conducive to Diptera and Hymenoptera, particularly for Ichneumonidae. Diptera play important role in decomposition of leaf litter, fungal feeders and scavengers (Frouz 1999). The ichneumonid in primary forest might reach stable condition, start to colonize the population and no competitive to others (Haneda, 2004).
Figure 1. The number of individuals per order collected using yellow-pan trap and malaise trap in Gunung Gede-Pangrango National Park

REFERENCES


P-EC09

CHARACTERISTIC DISTRIBUTION OF *Pinus merkusii* ON EARLY SUCCESSION AT MERAPI MOUNTAIN NATIONAL PARK, JAVA, INDONESIA

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Abstract

Characteristic unique of Merapi Mountain National Park is a primary succession post eruption where *Pinus merkusii* is one of the species on early succession. The research aimed to determine the characteristic distribution of *P. merkusii* two year after Merapi eruption 2006.

The data collection was done using line plot sampling methods, based on the distance outside eruption borderline (OEB) that divided into 6 plots and labelled as JT₁, JT₂, JT₃, JT₄, JT₅ and JT₆ (1 plot= 100m). Second based on *P. merkusii* stand (PS), 3 plots were JB₁, JB₂ and JB₃ (1 plot=150m). The relation of parameter density and height of *P. merkusii* was calculated using correlation analysis. Responses of the parameter were calculated using analysis variance and continued with DMRT test for significant level.

The results showed that the distance from EOB was significant (p-value=0.014) with average density (individu/m²) JT₁ (0.131), JT₂ (0.169), JT₃ (0.169), JT₄ (0.202), JT₅ (0.179) and JT₆ (0.096) whereas the distance from PS was not significant with *P. merkusii* density. Relation pattern between distance and density was quadratic (R²=0.626 and p-value=0.003) and the relation was undeviating proportion. Trend of height both EOB and PS were similar, the more distant the higher. The distribution performance of *P. merkusii* can be used as a reference in the rehabilitation of Merapi post eruption.

Keywords: distribution, succession, *P. merkusii*

INTRODUCTION

Merapi Mountain National Park (MMNP) was established in 2004 (Ministry of Forestry–Indonesia, 2004). Characteristic unique of MMNP is a primary succession post eruption and *Pinus merkusii* is one of the species on early succession. The objective of this research were to determine the characteristic distribution of *P. merkusii* two year after Merapi eruption 2006.

MATERIALS AND METHODS
The data collection was done using line plot sampling methods, based on the distance outside eruption borderline (OEB) that divided into 6 plots and labelled as JT1, JT2, JT3, JT4, JT5 and JT6 (1 plot= 100m). Second based on P. merkusii stand (PS), 3 plots were JB1, JB2 and JB3 (1 plot=150m). Quadratic plot size was done on every 10 m with size 4 x 4 m. The relation of parameter density and height of P.merkusii was calculated by using correlation analysis. Responses of the parameter were calculated by analysis variance and continued with DMRT test for significant level.

RESULTS AND DISCUSSION

Pinus density and height

The distance from OEB has significant effect with pine density but the distance from pine stand not significant. Based on fit estimation curve showed that pattern of relation between distance and density follow quadratic pattern with $R^2=0.626$ and $p$-value=0.003, whereas density of pine with distance near the OEB to JT4 increase trend and after JT4 decreasing of the density (Figure 1).

![Figure 1. The trend of density based on outside eruption borderline](image)

Based on the variance analysis and DMRT test, long distance from PS has positive respond on height. The height near stand pine is lower than the pine at long distance. According to Dalling and Denslow (1998) the soil seed banks have been historically surrounded by mature forests. The trend of height based on outside eruption borderline has similar with trend with SP. The real interaction clearly at JB1 and JB2 with JT5 dan JT6. The
height at JB₂ decrease and then increasing significantly at JT₆, opposite with it that the height at JB₁ decreasing at JT₆.

**Relation between density and height**

The correlation showed that between density and height are undeviating proportional except at JB₂, JT₂, JB₁, JT₅, JB₁, JT₅, JB₃ and JT₅ (Figure 2)

Figure 2. The relation between density and height at level distance outside eruption borderline and pine stand

The early characteristic of pinus can use reference to design rehabilitation post eruption related with ecological function especially local community surrounding MMNP. Tree species composition, dispersion and diversity biodiversity is essential for human survival and economic well being and for the ecosystem function and stability (Singh, 2002).

**REFERENCES**


P-EC10
LOWLAND FOREST COMPOSITION AND REGENERATION IN SEMPU ISLAND NATURE RESERVE,
EAST JAVA - INDONESIA

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Abstract

A study on forest composition and regeneration was conducted in lowland forest ecosystem of Sempu Island Nature Reserve (SINR), East Java Province – Indonesia, employing parallel line search method. Beside very vast diversity of unique ecosystem types, this small island of 877 hectares of total area has excellent plant species richness in terms of Shannon-Wiener diversity index for all growth phases (tree, pole, sapling and seedling). Overall result shows that lowland forest composition in SINR consists of 128 plant species representing 44 families. In terms of IVI (importance value index), dominant species in tree stands is Pterospermum diversifolium (Sterculiaceae), whilst those of pole, sapling and seedling stages are Polyalthya rumpfii (Annonaceae), Mitrephora polypirena (Annonaceae) and Mallotus floribundus (Euphorbiaceae), respectively. Lowland forest regeneration in SINR indicates a pattern of 'mosaic theory' where dominant species in tree stage are poorly represented as sapling and seedling stages. SINR also contains one of the most important remaining populations of Myristica teijsmannii, an Indonesian endangered plant species, in which natural regeneration occurs and which supports a complete growth phases.

Keywords: lowland forest, composition, regeneration, Sempu Island Nature Reserve

INTRODUCTION

Recent study suggests that Sempu Island Nature Reserve (SINR) lowland forest has valuable function in conservation and protection, and is considered to be an important ecological region in Indonesia [Risna and Narko, 2009]. This no-inhabitant island of 877 ha total area has not only biological richness with various ecosystem types: lowland tropical forest that covers most area of the island, mangrove and coastal forest ecosystems with two freshwater lakes and natural spring [Purwanto et al., 2002], but acts as an important barrier island protects adjacent villages at the Java mainland from high tides such as Tsunami from the Indian Ocean as well [Risna and Narko, 2009]. The forests in each ecozone differ considerably in species composition, age structure, ecological characteristics, economic importance, and other characteristics. In terms of plant diversity and special ecological region conservation, as undisturbed natural areas for general ecological and botanical studies of fundamental significance, the requirements for research and evaluation of biodiversity data
especially forest structure and composition become more essential. An account of the general structure and floristic composition of the forest in this small island is lacking however. The objectives of this study is to present such an account, and to get information on the SINR’s lowland tropical forest as to species diversity and composition, vegetation structure, as well as species important values.

MATERIALS AND METHODS

The study was conducted in SINR lowland forest, Malang District, East Java, in June – August 2007. The island has very hilly topography with various terrains and partly consists of limestone karsts system. The climate is categorized as C-type with annual rainfall of 2,132 mm [Tjandra and Rosni 2009].

Vegetation analysis using a systematic parallel lines search [Cropper 1993] was employed by establishing ten square plots of 20x20 m for tree stage (species with stem diameter at breast height or DBH >20 cm) along 200 m base line transect. Square sub-plots of 10x10 m, 5x5 m and 2x2 m were constructed within each 20x20 m plots using nested plot method for pole, sapling and seedling stage, respectively. Botanical names, individual number and DBH of each species encountered within plots were recorded, as well as forest physiognomy. Shannon-Wiener diversity index (H’) and species abundance [Krebs 1999; Cox 2002] were also calculated.

RESULTS AND DISCUSSION

Forest Stratification and Floristic Composition

Vegetation in SINR lowland forest is in relatively good condition with very high species diversity for all stages observed. Many species with >50 cm DBH were found: Artocarpus elasticus, Ficus spp., Bischovia javanica, Garcinia spp., Myristica teijsmannii, Pterospermum spp., and Sterculia spp. to name some. Additionally, species of Artocarpus elasticus, Myristica teijsmannii, Pterocarpus javanicum and P. diversifolium seemed to characterize the lowland forest in SINR. The layer of stand canopies on the lowland forest has 4 tree strata from A to D. The dominant trees in high come from Artocarpus elasticus occupying the A stratum or emergent layer. A total of 120 plant species encountered in the plot, representing 89 genera and 43 families. This figure excludes herbs, vines and epiphytes. The most important families in terms of species number among trees were
Euphorbiaceae (15 species), Annonaceae (8), Moraceae (8), Meliaceae (7), Sterculiaceae (6), Ebenaceae (5) and Myristicaceae (3).

Species Diversity

Species richness of the study sites varied moderately between four DBH classes, as well as plant species diversity (Table 1). All stages of DBH class diversity shown excellent diversity (H’>4). Sapling stage was found as the highest species richness and diversity compare to other stages observed, resulting a pyramid population structure. The lower number or species and diversity shown by seedling stage indicated some common species in larger DBH were poorly represented as seedlings. This phenomenon is expressed in Aubreville’s account of forest regeneration as the ‘Mosaic Theory’ [Hikmat et al., 2007].

Table 1. Results of species inventory in Sempu Island Nature Reserve lowland forest

<table>
<thead>
<tr>
<th>Variable</th>
<th>Tree</th>
<th>Pole</th>
<th>Sapling</th>
<th>Seedling</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species richness</td>
<td>72</td>
<td>79</td>
<td>97</td>
<td>84</td>
</tr>
<tr>
<td>Shannon-Wiener diversity index, H’</td>
<td>5.07</td>
<td>5.19</td>
<td>5.22</td>
<td>4.98</td>
</tr>
<tr>
<td>Density (individual numbers ha⁻¹)</td>
<td>120</td>
<td>602</td>
<td>5133</td>
<td>13567</td>
</tr>
<tr>
<td>Stand basal area (m²ha⁻¹)</td>
<td>27.85</td>
<td>12.85</td>
<td>51.07</td>
<td>n.a.</td>
</tr>
<tr>
<td>The most abundant species IVI (%)</td>
<td>29.82</td>
<td>28.75</td>
<td>28.68</td>
<td>47.84</td>
</tr>
<tr>
<td>Pterospermum diversifolium</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mitrephora polypyrena</td>
<td>1.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mitrephora polypyrena</td>
<td>0.38</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mallotus floribundus</td>
<td>0.006</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Area of sampled plot (ha)

REFERENCES


P-EC11

PRELIMINARY STUDY OF FOREST VEGETATION, TUKUNG GEDE MOUNTAIN NATURE RESERVE, SERANG REGENCY

Siti Sunarti
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Abstract
Tukung Gede Mountain Nature Reserve covered almost 1.700 ha area. This includes four administrative districts of Serang Regency, i.e. Pabuanan District, Mancak District, Anyer District and Cinangka District.

This research was conducted in the west part of Nature Reserve that is in the Cadas Gantung Mountain and Cadas Pagar. The aim of the study is to inventory the Nature Reserve vegetation. Plot about 0.2 ha has been made in each location, names, tree diameter, tree total height, dbh and its position of every tree with the diameter ≥ 10 cm, and sapling with the diameter ≥ 2 cm are recorded.

The inventory study from two location showed that 125 trees and 407 sapling of 63 species are recorded. The number of trees in Cadas pagar forest (66 trees) is higher than those of Cadas Gantung Forest (59 trees), however the number of sapling (185 trees) is less than those of Cadas Gantung Forest (222 trees). Some species dominated in those area are almost similar. Cadas Pagar Forest dominated by Nangsi (Villebrunea rubescens), Pulus, Purut (Paratocarpus venenosus), Kelapa ciung, Bengang, whereas Cadas Gantung Forest dominated by Nangsi (Villebrunea rubescens), Teureup (Artocarpus elasticus), Jirak, Ki jebug, Dahu (Dracontomelon dao) and Purut (Paratocarpus venenosus).

INTRODUCTION

Tukung Gede Mountain Nature Reserve (TGMNR) covered almost 1.700 ha area. This includes four administrative districts of Serang Regency, i.e. Pabuanan District, Mancak District, Anyer District and Cinangka District. The reasons why study of forest vegetation in TGMNR is necessary to be conducted, because (1). The data and information of vegetation are still limited and (2). The progressive deforestation because forest conversion as coffee and gnemon plantation. The aim of the study is to inventory the Nature Reserve vegetation.

MATERIALS AND METHODS

This research was conducted in the west part of Nature Reserve that is in the Cadas Gantung Mountain (06° 09,437’ S & 105 ° 57,085’ E) and Cadas Pagar (06° 09,529’ S & 105 ° 56,830’ E). Plot about 0.2 ha has been made in each location. Each plot was divided into 10
subplots of 10 x 10 m size. Names, tree diameter, tree total height, dbh and its position of every tree (the diameter ≥ 10 cm), and sapling (the diameter ≥ 2 cm) are recorded. Nomenclature study based on Backer & Bakh. v/d Brink (1963, 1964, 1965), Flora Malesiana, or other publications in Malesia. To analyze the data, diversity and similarity index are employed (Partomihardjo & Rahajoe, 2004).

RESULTS AND DISCUSSION

The inventory study in two location (0.4 ha) showed that 125 trees (42 species) and 407 sapling of 63 species are recorded. So the density for one hectare there are 310 trees. The number of trees in Cadas pagar forest (66 trees) is higher than those of Cadas Gantung Forest (59 trees), however the number of sapling (185 trees) is less than those of Cadas Gantung Forest (222 trees). Some species dominated in those area are almost similar. Cadas Pagar Forest dominated by Nangsi (Villebrunea rubescens), Pulus, Purut (Paratocarpus venenosus), Kelapa ciung, Bengang, whereas Cadas Gantung Forest dominated by Nangsi (Villebrunea rubescens), Teureup (Artocarpus elasticus), Jirak, Kijebug, Dahu (Dracontomelon dao) and Purut (Paratocarpus venenosus). The result study can be concluded that the composision of the trees species in Cadas Gantung (plot I & II) more diverse than Cadas Pagar (plot III & IV) with H' value = 1.21 and 0.98 (Table 1.). The density of the trees species in TGMNP are more rarely than Sukawayana, Janlappa, and Dungus Iwul forest (Table 2.).

Table 1. Index diversity (H') and similarity (e) trees and sapling

<table>
<thead>
<tr>
<th>Plot</th>
<th>Trees H'</th>
<th>Sapling H'</th>
<th>Similarity e</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>0.98</td>
<td>1.07</td>
<td>0.73</td>
<td>0.80</td>
</tr>
<tr>
<td>II</td>
<td>1.21</td>
<td>1.11</td>
<td>0.95</td>
<td>0.8</td>
</tr>
<tr>
<td>III</td>
<td>0.91</td>
<td>0.88</td>
<td>0.82</td>
<td>0.74</td>
</tr>
<tr>
<td>IV</td>
<td>0.79</td>
<td>2.04</td>
<td>0.67</td>
<td>1.43</td>
</tr>
</tbody>
</table>
Table 2. Comparison of individual numbers of trees per ha in some lowland forests of W Java

<table>
<thead>
<tr>
<th>Site Location</th>
<th>Density (trees/ha)</th>
<th>Sources</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sukawayana, W. Java</td>
<td>418</td>
<td>Othman (1975)</td>
</tr>
<tr>
<td>Janlappa, W Java</td>
<td>523</td>
<td>Basharuddin (1975)</td>
</tr>
<tr>
<td>Dungus Iwul, W Java</td>
<td>567</td>
<td>Dengjel (1993)</td>
</tr>
<tr>
<td>Tukung Gede</td>
<td>310</td>
<td>Present study</td>
</tr>
</tbody>
</table>

REFERENCES


P-EC12
BIOTECHNOLOGY AND AGRICULTURE DEVELOPMENT IN INDONESIA
Tantono Subagyo and Fadiila Dewi Rahmawaty
Seeds Division, PT Syngenta Indonesia. E-mail: tantono.subagyo@syngenta.com

Abstract
Biotechnology has been placed as a high priority technology in Indonesia since 1998 in anticipation of the sharp increase in food demand. Several priorities have been stated, among them are plant transformation programs to improve food crops, estate crops and forestry. Transformation programs has been done in various public institutes among them are ICABIOGRAD, Indonesian Institute of Sciences, Bogor Agriculture University etc. Several regulations has been laid out, among them are the ratification of Cartagena Protocol, Government Regulation in Biosafety of Genetic Engineering Products etc. However the application in the field is still far away and Indonesian farmers do not have a chance to use the technology. This paper will summarize a history of biotechnology in Indonesia, especially modern biotechnology, state of the art of the research and its constraints in the development.

INTRODUCTION
As of September 2009, the Earth’s population is estimated by the to be 6.786 billion, and is expected to reach about 9.1 billion by the year 2050. (United States Census Bureau, 2009). In order to nourish this population, FAO stated that world food production have to increase by 70% (AFP, 2009). It is not an easy task because right now there are several constraints already, namely climate change, water scarcity, land scarcity, moreover even now there are competitions for food and biofuel. It is imperative that country have to decide its strategy to increase food production using the most effective and efficient way, increasing production in the same area of land and using less energy. One of the available options is through modern biotechnology using transgenic crops. Planting transgenic crops, yield per ha can be increased, the use of pesticides can be decreased and food quality can be increased as well. The advancement of modern biotechnology in the world was quite rapid, starting from 1996, in 2008 the area of transgenic crops reaching 120 million ha, the number of country planting transgenic crops were increasing rapidly as well, from 6 in 1996, the first year of commercialization, to 18 in 2003 and 25 in 2008. Among the 25 countries there are several developing countries such as China, India, South Africa, The Philippines, Egypt, Burkina Faso, Bolivia, Brazil. Introduced in 2003, transgenic crops adoption in the Philippines increases rapidly, the area became 248000 hectares in 2007, while in 2008 increase 40% and become 350000 hectares. 13.3 million farmers benefitting from transgenic crops among them 12.3 million are small and resource poor from developing countries, 5 million from India and 7.1 million from China. (James, 2009)
The development of modern biotechnology.

Besides the growth in planting area, the application of modern biotechnology is also rapidly developed, the first generation of transgenic crops planted so far are carrying trait resistance to pests, tolerance to herbicide or both. Soon there will be new generation of transgenic crops carrying trait for abiotic stress tolerance such as drought, cold, saline soil, submergence and nitrogen utilization efficiency. Still under development there will be C4 rice, converting rice from C3 to C4 with estimation of 15-20% increase in yield by increasing photosynthetic efficiency. Further development in transgenic crops are increasing food quality such as rice containing provitamin A, rice with high iron content; delayed ripening fruits such as papaya, tomato; and amylase corn to increase efficiency of biofuel production. Moreover, in the pipeline, there are plant derived pharmaceuticals enabling vaccines productions such as for hepatitis B, insulin, rabies etc. derived from plant. In the pipeline there are plants developed for bioremediation to absorb metals such as zinc, lead, cadmium, nickel and boron.

Development of biotechnology in Indonesia.

The development of biotechnology research in Indonesia is not far behind compared with other developing countries, from number transformation events under research, Indonesia come third (24) only less than China (30) and South Africa (28) and more compared to India (21) or Philippines (17). (Atanassov et al, 2004). Several transgenic crops under research and already reach the contained field trial i.e rice resistant to stem borer, drought tolerant sugarcane, potato resistant to disease, low amylase content cassava, sugarcane with high sugar content. Those research are done in the research centers such as ICABIOGRAD, Central Research Institute for Biotechnology Indonesian Institute of Science, Bogor Agriculture University, Bandung Technological Institute, as well as by state company such as PTPN XI a (Herman, 2009). Several private sectors such as Syngenta, Monsanto and Pioneer are also tried to introduce transgenic crops. However, since 1998 there are no transgenic crops released by the governments, it is due to low research budgets (only about 0.1-0.2% from GDP) and the incompleteness system of the regulation.

Indonesia regulates biotechnology since 1997 with The Decree of the Minister of Agriculture No 856 Kpts/Hk.330/9/1997 on the Provision of Biosafety of Genetically Engineered Agricultural Biotechnology Products which was later revised with the Joint Decree of Four Ministers (Minister of Agriculture, Minister of Forestry and Estate Crops, Minister of Health and State Minister for Food and Horticulture No 998.1/Kpts/OT.210/9/99
790.a/Kpts-IX/19991145A/MENKES/SKB/IX/1999 015A/NmenegPHOR/09/1999) on Biosafety and Food Safety of Genetically Engineered Agricultural Products in 1999. On October 19th, 2004, the Republic of Indonesia officially ratify Cartagena Protocol on Biosafety with Law No 21, 2004. Further to implement the Cartagena Protocol Indonesia establish Government Regulation No 21, 2005 for Biosafety of Products resulted from Genetic Engineering. However, although the Government Regulations No 21, 2005 is already regulate about the conduct of biosafety assessment, until now there is no Biosafety Committee being implemented, causing constraints to biotechnology innovations in Indonesia. Low research budgets can be alleviated by research cooperations, but the uncertainty of the regulations have to addressed by the Government.

**Conclusions and recommendations.**

1. In the long run, Indonesia will face a food shortage and have to use all of the available technology to boost food production.
2. Biotechnology is one of the tools which can be use to increase food production.
3. Indonesia already have a strong research in biotechnology, however lack in implementation due to low budget and lack of regulatory implementations.
4. To benefit from biotechnology, Government of Indonesia have to allocate enough research funds and implement the existing regulation with the efficient implementation system.

**REFERENCE**


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P-EC13

BIOREMEDIATION OF WATER CONTAMINATED LAPINDO MUD VOLCANO BY USING AQUATIC PLANT IN SITU

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*).Department of Biology, Faculty of Mathematic and Science, Universitas Negeri Surabaya

ABSTRACT

That research aims was to investigate the aquatic plant in situ efficiency as well as bioremediator heavy metal (Cd and Pb) in waters contaminated of lapindo mud volcano. Experiment design used was Completely Randomized Design (CRD) with bioremediator Typha latifolia and Eichhonia crassipes Mart(Solms) as an experiment object. Cultivation of bioremediator for 2 weeks. Parameter measured include heavy metals content in water and plants. AAS (Atomic Absorption Spectrophotometer method was used to determine heavy metal content. The capacity of bioabsorption and bioaccumulation data were analyzed descriptively and compared with basic standard of water quality. The results of revealed that the efficiency of bioabsorption and bioaccumulation aquatic plants in situ about Cd up to 41- 67 % and Pb up to 14,29%, so can used as well as heavy metals bioremediators on waters contaminated lapindo mud volcano.

Key worth: Bioabsortion, bioaccumulation, heavy metal, aquatic plant in situ, lapindo mud volcano.

INTRODUCTION

One effect of lapindo mud is the decrease environmental carrying capacity for cultivation, and aquatic ecosystem contamination by heavy metals, especially cadmium (Cd) and Lead (Pb). This condition has implications for the life of farmers and the sustainability of ecosystems and safety (biosafety) fishery products on the east coast of Sidoarjo, so the threatened entry blacklist in the commodity trading arena of international fisheries (Suryadarma, 2007), because it does not meet the standards of Safety Assurance of Imported Foods.

Heavy metal pollutants that are most commonly found in waters (Cahaya, 2003) and used as indicators of water pollution. Heavy metals have a high mass density (5 gr/cm3) so that small concentrations are toxic, harmful and even cause death. Cadmium and lead is a natural component of soil that can not be degraded. Cd and Pb into the human body through food, drinking water, or air. Cadmium (Cd) is dangerous because it causes food poisoning and disease "itai-itai". The symptoms marked by lack of abnormalities of bone and some organs of the body becomes dead. Chronic poisoning by Cd is damage to the respiratory system, blood circulation, smell, reproductive glands, kidneys, heart and bone fragility. Lead
(Pb) has a high toxicity of power because it can damage brain development in children, obstruction of red blood cells, and anemia. Pb can be accumulated directly from water and sediments by aquatic organisms.

In the field of fisheries has been implemented eco-labeling related to sustainable environmental management. Aquatic ecosystem contamination issues can be a cause for developed countries to reject Indonesian fishery products into the world market, for reasons not to apply eco-labeling or eco-friendly. Thus, rehabilitation of polluted aquatic ecosystem by heavy metals lapindo mudflow absolutely must be done in order to re-utilized for the cultivation of agriculture or fisheries that their products meet food safety standards (foodsafety).Bioremediation is the restoration of contaminated environmental conditions using the process or products of living organisms. Several species of aquatic plants (hidrophyta) has the ability to absorb heavy metals and accumulation in the organ and known as hyperakumulator. Observations indicate, in the lapindo mud gripped the region in several species hydrophyta resistant lapindo mud stress, namely Typha latifolia, Eichhornia crassipes, Cyperus iria, Lemna major, and Pistia stratiotes. Plant species in situ is suspected the heavy metal hyperaccumulator that can be used as a heavy metal bioremediator.

This study aims to (1) knowing bioabsorption and bioaccumulation capacity plants in situ Water hyacinth (Eichhornia crassipes) and Cattail (Typha latifolia) of Cd and Pb, (2) found plant species in situ as an efficient bioremediator used to rehabilitate water from the stress of heavy metals Cd and Pb, (3) produce prototype model aquatic ecosystem Bioremediation of heavy metals was seized with economical, environmentally friendly, and applicable. Results of this study can be used for (1). Lapindo mudflow manage ecologically to be a small negative impact possible, (2) restore the aquatic ecosystem carrying capacity in the areas lapindo mudflow affected to recover productivity, (3) reduce pressure on marine and coastal ecosystems due to the impact of lapindo hot mud, (4) support the achievement of fishery products biosafety in the Sidoarjo area.

MATERIALS AND METHODS

Research carried out experimentally in the Green house and laboratory Ecology, Department of Biology, Faculty of Mathematic and Science, State University of Surabaya (UNESA). Consists of 3 treatment bioremediator biomass. As the test bioremediator is water hyacinth (Eichhornia crassipes Mart (Solms) biomass 200 g, 400 g, and 600 grams, and a cattail (Typha latifolia) biomass 300 g, 600 g, and 900 gr. Each treatment is repeated 3 times. Bioremediator was cultivation for 2 weeks. Experimental units arranged according to Completely Randomized Design (CRD). Parameters measured include content of Cd and Pb
in water sediments, water hyacinth leaves, stems, and roots of *Typha latifolia*. Analysis of content of heavy metals (Cd and Pb) conducted at Central Laboratory of Health Laboratory Surabaya (BBLKS), using the method of Atomic Absorption Spectrophotometer (AAS) (λ = 283.3 nm). Data were analyzed by qualitatively descriptive statistics and compared with water Quality Standards (Litbang Perikanan Deptan, 1987; and PP.No.82 Thn. 2001).

RESULTS AND DISCUSSION

1. Heavy metal content of Cd and Pb in waters contaminated Lapindo mud

Lapindo muddy water before bioremediation containing cadmium (Cd) and lead (Pb) far exceeds the threshold of water quality standards (Litbang Perikanan Deptan, 1987; and PP.No 82/2001), which is indispensable bioremediation.

<table>
<thead>
<tr>
<th>Heavy metals</th>
<th>Content of Heavy metals (ppm) before bioremediation</th>
<th>Standard of quality (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>Eichhornia crassipes</em></td>
<td><em>Typha latifolia</em></td>
</tr>
<tr>
<td>Cd</td>
<td>0.038</td>
<td>0.024</td>
</tr>
<tr>
<td>Pb</td>
<td>0.323</td>
<td>0.217</td>
</tr>
</tbody>
</table>

2. Capacity Bioabsorption Water hyacinth (*Eichhornia crassipes*) against Cd and Pb Lapindo mud.

After bioremediation using water hyacinth (*Eichhornia crassipes*) Lapindo muddy water content decreased Cd 0.010-0.019 ppm, and Pb 0.012-0.039 ppm. decrease in Pb levels of 5.88 to 12.07%, while the Cd up to 26.32 to 50%. as in Table 2.

<table>
<thead>
<tr>
<th>Heavy metals</th>
<th>Content of Heavy metals (ppm)</th>
<th>After bioremediation</th>
<th>Bioabsorption Capacity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>before bioremediation</td>
<td>Biomass of <em>Eichhornia crassipes</em> (gram)</td>
<td>Replication</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1. Heavy metal content (Cd and Pb) Lapindo mud water before bioremediation

Table 2. Heavy metal content of Cd and Pb in the mud After bioremediation Using *E. crassipes*
Water hyacinth (*Eichhornia crassipes*) is capable of absorbing heavy metals in water and sediments because it has roots branching smooth, the surface roots are used by microorganisms as a place of growth (Neis, 1993), which functions as a biological filtration system that can absorb nutrients, minerals and heavy metals including cadmium and lead. Bioabsorption of metals in water by water hyacinth is influenced by several factors, including composition and levels of metals, biomass, and the residence time (cultivation).

![Image](image.png)

**Figure 1. Histogram Content of Cd Lapindo mud After bioremediation using *E. crassipes***

Bioabsorption Cd and Pb by the water hyacinth lasted through rhizofiltrasi, the process of absorption of contaminants by the roots of substance. Heavy metals in the water (in the form of essential heavy metals), after contact with the roots will be absorbed into the roots. The mechanism of metal absorption in water hyacinth metal accumulation by the transport ligands into the roots will then be entered into the xylem in a row from the organs root, stem, until the leaf cells (via plasmalema, cytoplasm, and tonoplast) into the vacuole (vacuole transport in metal complexes react with terminal acceptor ligand) which eventually will form the metal complex acceptors. The next transport off and acceptor ligand metal complexes accumulate in the vacuole. This explains why the levels of Cd and Pb in water hyacinth petiole much higher than the mud, because in this organ more vacuole. Heavy metal Bioabsorption influenced by water hyacinth biomass. Greater water hyacinth to absorb Cd, because the roots are widespread and floating in the water. The ability of water hyacinth
to absorb heavy metals depends on the age, metal absorption was higher in younger age than old age (Widiyanto and Suselo, 1977).

Water hyacinth bioabsorption capacity of Cd reaches 26.32 to 50.00%, and Pb 03.71 - 12.07%. Capacity bioabsorption influenced by biomass, the largest bioabsorption in biomass Cd 200 grams (50.00%), whereas Pb in biomass 600 grams (12.07%). Thus water hyacinth absorb more efficient in Cd than Pb. Water hyacinth bioabsorption capacity of Cd and Pb can be seen in the picture below.

![Figure 2. Capacity Bioabsorption Water hyacinth (Eichhornia crassipes) of Cd and Pb lapindo mud water](image)

### 3. Capacity Bioaccumulation *Eichhornia crassipes* against Cd and Pb Aquatic

Table 3. Content of Cd and Pb in water hyacinth (*Eichhornia crassipes*) After bioremediation

<table>
<thead>
<tr>
<th>Heavy metals</th>
<th>Content of heavy metals (ppm)</th>
<th>Bioaccumulation Capacity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before bioremediation</td>
<td>In <em>Eichhornia crassipes</em> (after bioremediation)</td>
</tr>
<tr>
<td></td>
<td>Biomass <em>E.crassipes</em> (gram)</td>
<td>Replication</td>
</tr>
<tr>
<td>Cd</td>
<td>0.038</td>
<td>200</td>
</tr>
<tr>
<td></td>
<td></td>
<td>400</td>
</tr>
<tr>
<td></td>
<td></td>
<td>600</td>
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<tr>
<td>Pb</td>
<td>0.323</td>
<td>200</td>
</tr>
<tr>
<td></td>
<td></td>
<td>400</td>
</tr>
<tr>
<td></td>
<td></td>
<td>600</td>
</tr>
</tbody>
</table>

*E.crassipes* are able to accumulate Cd and Pb, it is shown content of Pb from 1.628 to 1.804 ppm or 504.02 to 558.51% higher than the water sediment and 0.371-0.425 ppm Cd or 915.79 to 1118.42%.
Figure 3. Content of Cd and Pb in the leaf stalk water hyacinth (Eichhornia crassipes) after bioremediation.

The largest accumulation of Pb (1.804 ppm or 558.51%) on *E.crassipes* biomass 600 g, and Cd (0.425 ppm or 1181.42%) in 200 g biomass.

![Content of Cd and Pb in the leaf stalk water hyacinth](image)

Figure 4. Capacity Bioaccumulation water hyacinth (*Eichhornia crassipes*) of Cd and Pb lapindo mud

Water hyacinth is a Cd and Pb hiperakumulator efficient. Bioaccumulation Pb reached 504.02 to 558.51%, and Cd from 915.79 to 1118.42%, so the potential use asbiofilter lapindo mud.

### 4. Capacity Bioabsorption Cattail (*Typha latifolia*) against Cd and Pb aquatic.

<table>
<thead>
<tr>
<th>Heavy metals</th>
<th>Content of heavy metals (ppm)</th>
<th>Bioabsorption Capacity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before bioremediation</td>
<td>After bioremediation</td>
</tr>
<tr>
<td></td>
<td>Biomass <em>Typha latifolia</em> (gram)</td>
<td>Replication</td>
</tr>
<tr>
<td>Cd</td>
<td>0.024</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
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<td>Pb</td>
<td>0.217</td>
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<td></td>
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</tr>
</tbody>
</table>

Table 4. Content of Cd and Pb in the mud after bioremediation Using cattail (*Typha latifolia*)
Typha latifolia efficiency in absorbing Cd reaches 12.50 to 41.67%, and Pb 07.83 - 14.29%. Efficiency is influenced by the biomass, capacity bioabsbtion largest biomass occurred in 900 grams (41.67%), such as the following figure.

![Histogram Content of Cd and Pb](image)

**Figure 5. Histogram Content of Cd and Pb Lapindo mud water after bioremediation using *Typha latifolia*.

Typha latifolia efficiently absorb and accumulate Cd and Pb dissolved in the water. Absorption and accumulation of heavy metals by plants is divided into 3 mutually continuous process, ie heavy metal absorption by roots, translocation from roots to other plant parts, and localization of heavy metals on the part of certain cells that do not inhibit the metabolism of these plants (Prayitno and Prayitno, 2008).

Metal ions are absorbed by root cells and transported through the carrier network (phloem and xylem) and the metal bound by phytochelatin (PCs), a peptide that is rich in amino acids and contains a cysteine sulfur with the general structure (γ-Glu-Cys)n Gly. Metal ions Cd and Pb will chelated by S atoms from cysteine in translocated and phytochelatin then stored in the stem and leaf tissue. Several heavy metal ions such as Pb2+, Cd2+, Cu2+ and Zn2+ can induce the synthesis phytochelatin in plants, if plants fail to synthesize phytochelatin hampered the growth or death (Brooks, 1998). phytochelatin be synthesized from the transfer of γ-Glu-Cys at the catalyst trypeptide glutamate transpeptidase (phytochelatin synthase). Concentrations of heavy metals in the cytosol in control transpeptidase enzyme activity. If there is no heavy metal ions, the enzyme will be inactive. The enzyme becomes active by the metals Pb, Cd, Cu, Hg and metal ions other. A reduction in the cytoplasm of ions of heavy metals-free resulting in lower enzyme activity (Salisbury and Ross, 1995).

Heavy metal absorption by plants of water going through the roots, and buried in a particular species in the canopy. According to Muhayati (2000) the greatest absorption of heavy metals in the water plant is at the root and leaves. This event is largely a passive process, although there are some who are involved in cell metabolism. The factors that affect bioabsbtion heavy metals by plants of water is the water pH, temperature, and light intensity.
5. Capacity Bioaccumulation cattail (*Typha latifolia*) against Cd and Pb Aquatic

The results showed levels of Cd and Pb in the roots of *Typha latifolia* well above the water levels in the mud. Distribution of Pb in the roots of water plants associated with translocation of water and salt from the root to the top of the plant (Artantiwi, 1995 in Puspitasari, 2004). This is evidenced by the accumulation of Pb in the epidermal cells, and endodermis root metaxylem which is the current line of salt and water translocation. In the trunk Pb accumulation occurred in metaxylem and protoxylem vessels which are vessels that channel network solute inorganic salts from the root to the top of the plant. Whereas in the leaf, palysade parenchymal and metaxylem can accumulate Pb. Water and mineral salts not only by bone and expansion of leaves, but also by the mesophyll cells (Fahn, 1995).

Table 5. Content of Cd and Pb in roots of the cattail (*Typha latifolia*) after bioremediation

<table>
<thead>
<tr>
<th>Heavy metals</th>
<th>Before bioremediation</th>
<th>After bioremediation</th>
<th>Bioaccumulation Capacity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Replication (ppm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Biomass <em>Typha latifolia</em> (gram)</td>
<td>Replication (ppm)</td>
<td>Average</td>
</tr>
<tr>
<td>Cd</td>
<td>0,024</td>
<td>300</td>
<td>0,298</td>
</tr>
<tr>
<td></td>
<td></td>
<td>600</td>
<td>0,307</td>
</tr>
<tr>
<td></td>
<td></td>
<td>900</td>
<td>0,298</td>
</tr>
<tr>
<td>Pb</td>
<td>0,217</td>
<td>300</td>
<td>1,287</td>
</tr>
<tr>
<td></td>
<td></td>
<td>600</td>
<td>1,324</td>
</tr>
<tr>
<td></td>
<td></td>
<td>900</td>
<td>1,294</td>
</tr>
</tbody>
</table>

*Typha latifolia* is bioaccumulator Cd and Pb. Content of Cd in the roots of *Typha latifolia* reached 0.290 to 0.315 ppm or 1216.67 to 1284.72% higher than the water sediment. Pb reaches 1,265-1,296 ppm or 582.95 to 609.98%. The largest accumulation of Pb (1.324...
ppm or 609.98%) in 600 g of biomass, while the Cd (0.308 ppm or 1284.72%) in 900 g of biomass, such as the following figure.

Figure 7. Content of Cd and Pb in the roots of Typha latifolia after bioremediation.

Efficiency of Cd and Pb bioaccumulation very high because of Typha latifolia able to bind heavy metals in the tissue. Typha latifolia mechanism in accumulating heavy metals that are not harmful to their growth: (1) save a lot of water to dilute the heavy metals and reduce toxicity. (2) to form a compound chelat that will phytochelatin bind heavy metals and heavy metals by atomic chelated S peptye derived from cysteine. (3) heavy metals that are bonded to phytochelatin will enter into the root cells by active transport, will be transported by the network phloem and xylem to goto the other body. (4) heavy metals Cd and Pb which have entered into the body of Typha latifolia will drop by excreted older leaves, thereby reducing their concentration.

Figure 8. Capacity Bioaccumulation of Typha latifolia Cd and Pb lapindo mud

Heavy metals in the water, usually bound by another compound that will form the molecule. It could be a bond of salt (organic or inorganic salts). Salt is absorbed by the roots in the form of ions and can move through the cortex in apoplas, simplas or both. If the past is apoplas, the ions will diffuse through the walls of cortical cells without entering the protoplasm. While moving on simplas is breaking the continuity of endodermis occurs because of the suberin bands of a watertight, so water and dissolved substances can not pass from one side of the endodermis to the other side except the diffusion through the endodermis and protoplas cells through plasmodesmata by the movement of the plasma. Cells that are considered bad in the Stele have a low capacity to hold ions, which tend to
leak into the xylem (wood vessels) (Fahn, 1995). Important components as the storage of metal ions is the vacuole, where the metal ions are bound by phytochelatin. Unlike the salt is absorbed by the roots, the salt is not absorbed by the roots to eventually find their way into and onto the surface of the leaf, which when accumulated in high concentrations of the salt can be stored as a precipitate when water evaporates dillution (Loveless, 1987).

6. Physical and chemistry parameters of lapindo mud water After Bioremediation

Changes in physical and chemistry factors lapindo muddy water using *Typha latifolia* after bioremediation as shown in the following table.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Before bioremediation</th>
<th>After bioremediation</th>
<th>Quality standard</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><em>Eichhornia crassipes</em> (gr)</td>
<td><em>Typha latifolia</em> (gr)</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>400</td>
<td>600</td>
</tr>
<tr>
<td>Salinity (o/oo)</td>
<td>20</td>
<td>6</td>
<td>9</td>
</tr>
<tr>
<td>Suhu (oC)</td>
<td>30,33</td>
<td>29,6</td>
<td>28,0</td>
</tr>
<tr>
<td>pH</td>
<td>7,43</td>
<td>7,5</td>
<td>7,1</td>
</tr>
<tr>
<td>DO (ppm)</td>
<td>1,31</td>
<td>1,88</td>
<td>2,41</td>
</tr>
<tr>
<td>CO2 (ppm)</td>
<td>24,7</td>
<td>23,3</td>
<td>23,7</td>
</tr>
</tbody>
</table>

Bioremediator water hyacinth and *Typha latifolia* to reduce water salinity up to 26%, 1-2 °C temperature, pH and CO₂. While DO has increased, though not meet quality standards.

From this research can be concluded that (1). water hyacinth (*Eichhornia crassipes*) and the cattail (*Typha latifolia*) is a plant *in situ* with the ability hyperakumulator heavy metals Cd and Pb lapindo mud, making it potentially useful as a heavy metal bioremediator lapindo mud. (2). Bioabsorbtion and bioaccumulation Cd and Pb influenced by the bioremediator biomass. Bioabsorbtion capacity water hyacinth (*Eichhornia crassipes*) on Cd reaches 50.00% and Pb 12.07%, whereas Pb bioaccumulation ability to reach 504.02 to 558.51%, and Cd from 915.79 to 1118.42%. (3). *Typha latifolia* bioabsorbtion efficiency of Cd reached 41.67% and Pb 14.29%, while the ability of Cd bioaccumulation reach 1216.67 to 1284.72% and Pb reached 582.95 to 609.98%.
REFERENCES


P-EC14

BIODIVERSITY OF AQUATIC ORGANISM AND HEAVY METAL CONTENT IN WATERS AND AQUATIC ORGANISM AS THE IMPACT OF LAPIndo MUD VOLCANO

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ABSTRACT

The aims of this research was to investigate the change in community structure of aquatic organism (plankton, benthos, fish, and aquatic insects) and the content heavy metals in waters ecosystems and aquatic organism as the impact of lapindo mud volcano. This research was done through field observation and laboratory analytical. The sample was taken from 6 stations in Porong, District of Sidoarjo. AAS (Atomic Absorption Spectrophotometer) method was used to determine heavy metal content. The biodiversity data of aquatic organism and heavy metal content were analyzed descriptively and compared with basic standards of waters quality. The results revealed that the lapindo mud volcano caused biodiversity degradation and increasing content of heavy metals in waters and aquatic organisms. Content of heavy metals in waters contaminated of lapindo mud volcano and aquatic organisms (Cd>0.001 and Pb>0.005 ppm) have been exceeded the concentration standard so that necessary bioremediation.

Key words: lapindo mud volcano, biodiversity, aquatic organisms, heavy metals, aquatic ecosystem.

INTRODUCTION

Mud flow that overflowed since May 29, 2006 from a gas exploration wells PT. Lapindo Brantas in Porong, District of Sidoarjo has led to ecological impacts. With volumes 160,000 m/day, mud on the surface temperature 80°-100°C, and the content of heavy metals cause soil and water contaminated by salt water mud and heavy metals, so that the carrying capacity for the cultivation of agricultural / fishery declined. Lapindo mud composition of a fluid mixture of salt water (30%) and solids (70%) in the form of coarse sand (sand rather subtle), fine sand, Silt (clay) containing hydrocarbons, steam and gas (H₂S). The results of X-ray analysis shows the mineral mud consists of pyrite, albit, caolinite, paragonit, and halite, comes from rock that has undergone changes in hydrothermal. Water mud dominated by elements Sodium (Na), Magnesium (Mg) and Calcium (K), (> 8 ppm) and Chloride (Cl) (1.8 ppm) (Timnas PSLS, 2007), which is brine. High salinity causes changes to freshwater ecosystems become brackish. As a result most of
the plants and aquatic organisms in the mud contaminated sites die (Mawardi, 2006). Lapindo mud containing values of BOD, COD, oils and fats is very high, and contain toxic heavy metals (Mawardi, 2006), the mud disposal to the surrounding aquatic environment is continuously causing deposited heavy metals in sediments and aquatic organisms (Palar, 1973). Heavy metals can accumulate in the body of water organisms, the phytoplankton and zooplankton because the average absorbed by the organisms in the water is faster than the process of excretion (Soule, 1988).

Heavy metal is a metal group density greater than 5 g / cm (Palar, 2004). In water, the heavy metals found in the soluble form complexes with organic and inorganic compounds and does not dissolve in the form of particles that form colloids and compounds adsorbed on suspended particles (Razak, 1980). Heavy metals can not be degraded in nature and tend to accumulate in the food chain through a process biomagnification (Darmono, 1995). Most of the heavy metals are essential for aquatic organisms for growth and development, among others in the formation and enzymatic haemocyanin (Darmono, 1995). But if that comes into the body of excess amounts, will turn into toxic to the body (Palar, 2004).

Porong topography and surrounding areas is the watery swamp area throughout the year. These regions included in the lowland areas of East Java, North, ground level is almost the same as the sea level with an average elevation difference 1 - 1.5 m (Anon, 2006). With these characteristics, spraying mud easily distributed to the surrounding area, including the area of rice fields and ponds.

Cadmium (Cd) is one type of hazardous heavy metals that affect humans in the long term and can accumulate in the liver and kidneys. At low concentrations of the interference effect in the lungs, emphysema and renal disease, chronic turbular. According to Suhendrayatna (2001) Cadmium is more easily accumulated by plants (including phytoplankton) compared with other heavy metal ions. This heavy metal joining of lead and mercury as the big three heavy metal that has the highest danger level on human health. According to the FAO/WHO, consumption per week for men tolerance is 400-500 ug per person or 7 ug per kilogram of body weight (Suhendrayatna, 2001). Cd toxicity can cause pain, heat in the chest, lung disease and acute bone damage can cause death.

Lead (Pb) is a heavy metal that is toxic, the main source of lead derived from alkyl groups lead components. Lead showed toxic to the nervous system, hemetologic, hemetotoxic and affect the kidneys work. Weekly consumption of these elements recommended by the WHO tolerance for adults is 50 ug/kg body weight and for infants or children 25 ug/kg body weight. Mobility of lead in soil and plants tend to be slow
(Suhendrayatna, 2001), so if the metal is contained in the phytoplankton, it will contaminate other aquatic organisms, including fish and shrimp.

Changes in waters environmental Porong territorial that had to be fresh to be salty from Lapindo mudflow and heavy metal contamination can alter the community structure of aquatic organisms, mainly of plankton as a producer in the water. So that parameter changes that occur waters will cause changes in aquatic organism communities in this area. It needs to be examined for content and distribution of heavy metals (Cd and Pb) in aquatic ecosystems contaminated mud lapindo. So it can be studied possible impact on aquatic organisms, especially biosafety fishery commodities (fish, shrimp, crab, etc.) so that its security is guaranteed. Of this research will be known (1). content and distribution of heavy metals in polluted waters lapindo mud, (2). diversity of aquatic organisms, (3). physical-chemical factors of water (temperature, salinity, pH, dissolved oxygen (DO), CO$_2$ levels).

MATERIALS AND METHODS

Research conducted in the affected area of lapindo mud Porong Sidoarjo, research areas include aquatic ecosystem in the mud shelters around the dike, rivers, ponds and Porong river. Data from 6 stations purposively determined, namely (1). Waters in the village of Kedung Bendo, (2). Waters in the village of Reno Kenongo, (3). Waters in the Village of Pejarakan,(4). Channel water to the ponds area, (5). Ponds (Village Permisan), and (6). Porong River estuary.

Research parameters include the content and distribution of heavy metals (Cd and Pb), the biodiversity of plankton, macrobenthos, aquatic insects, and fish, as well as physical-chemical factors of waters (salinity, temperature, pH, dissolved oxygen (DO), CO$_2$). Analysis of heavy metal content conducted in Balai Besar Laboratorium Kesehatan Surabaya, using Atomic Absorption Spectrophotometer method. Identification of plankton species, macrobenthos, aquatic insects, and fish in the Ecology Laboratory, Department of Biology Faculty of Mathematic and Science UNESA Surabaya. Data content of heavy metals (Cd and Pb) and water quality descriptively qualitatively analyzed and compared with quality standards (according Litbang Perikanan Deptan, 1987; and PP.No.82 Thn. 2001, East Java Governor Decree No.45 of 2002). Biodiversity of organism and diversity index were analyzed quantitatively using descriptive statistics. Calculation of diversity index using the Shannon-Weaner formula (Odum,1993). Abundance index is calculated using the formula of abundance (Soegianto, 1994 in Habibi, 1996)
RESULTS AND DISCUSSION

1. Content of Heavy Metals (Cd and Pb) in polluted waters lapindo Mud

Lapindo mud has caused waters ecosystem on the east coast Sidoarjo polluted by heavy metals (Cd and Pb), as in table 1 below:

Table 1. Content and distribution of heavy metals (Cd and Pb) in aquatic ecosystems contaminated lapindo mud

<table>
<thead>
<tr>
<th>Heavy metals</th>
<th>Station 1</th>
<th>Station 2</th>
<th>Station 3</th>
<th>Station 4</th>
<th>Station 5</th>
<th>Station 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cd</td>
<td>0.004</td>
<td>0.031</td>
<td>0.011</td>
<td>0.000</td>
<td>0.001</td>
<td>0.046</td>
</tr>
<tr>
<td>Pb</td>
<td>0.018</td>
<td>0.024</td>
<td>0.012</td>
<td>0.030</td>
<td>0.022</td>
<td>0.097</td>
</tr>
</tbody>
</table>


Cd content in the waters around the pool of mud reservoir have exceeded the quality standards (Cd> 0001 ppm). This is due to the stations 1, 2, and 3 there are many wild bursts. Cd and Pb levels highest in Porong River estuary because it is a place of lapindo mud sediment that are drawn to the sea through Porong river.

![Content and distribution of Heavy Metals (Cd and Pb) at each station](image)

Figure 1. Content and distribution of Heavy Metals (Cd and Pb) at each station

Lead is toxic and accumulate in body tissues through the food chain, then this suggests a potential danger through fishery products from this region. According to Fogg & Thake (1987) Cd and Pb are non-essential elements that can be accumulated on the phytoplankton and then can enter the food chain. If contained in a water pond, will eventually enter and accumulate in the body of cultivated organisms (Milkyfish, Shrimp, Crab) or harvested by a fisherman (shellfish, Kupang).

2. Heavy metal Content Porong River Water

Content of heavy metals (Cd and Pb) of water Porong river have exceeded the standard river water quality, because Lapindo mud containing lead and cadmium exceeded the threshold, is applied to the sea through Porong river. This fact is demonstrated by the high content of heavy metals in stations II and III that have been in the lapindo mud.
3. Plankton Biodiversity

In the waters contaminated lapindo mud can be identified 16 species of plankton, consisting of 13 genera of phytoplankton and 3 genera zooplankton. Distribution and diversity index of plankton at each station shown in the following Table.

Table 3. Plankton diversity, density and diversity index in the polluted waters Lapindo Mud

<table>
<thead>
<tr>
<th>No.</th>
<th>Plankton</th>
<th>Station 1</th>
<th>Station 2</th>
<th>Station 3</th>
<th>Total (ni)</th>
<th>Diversity index</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Gleocystis</em></td>
<td>7</td>
<td>9</td>
<td>4</td>
<td>5</td>
<td>13</td>
</tr>
<tr>
<td>2</td>
<td><em>Ankyra</em></td>
<td>8</td>
<td>9</td>
<td>3</td>
<td>10</td>
<td>13</td>
</tr>
<tr>
<td>3</td>
<td><em>Bracteacoccus</em></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td><em>Sphaerocystis</em></td>
<td>0</td>
<td>9</td>
<td>0</td>
<td>14</td>
<td>11</td>
</tr>
<tr>
<td>5</td>
<td><em>Eutetramorus</em></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>6</td>
<td><em>Golenkinia</em></td>
<td>6</td>
<td>9</td>
<td>0</td>
<td>13</td>
<td>14</td>
</tr>
<tr>
<td>7</td>
<td><em>Spirotaenia</em></td>
<td>8</td>
<td>12</td>
<td>25</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>8</td>
<td><em>Terpsione</em></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>9</td>
<td><em>Peronia</em></td>
<td>17</td>
<td>6</td>
<td>0</td>
<td>9</td>
<td>16</td>
</tr>
<tr>
<td>10</td>
<td><em>Cocconeis</em></td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>11</td>
<td><em>Gyrosigma</em></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>12</td>
<td><em>Amphipleura</em></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>13</td>
<td><em>Chromulina</em></td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>14</td>
<td><em>Pompholyx</em></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

Figure 2. Content and distribution of Heavy Metals (Cd and Pb) Porong River Water.

Table 2. Heavy metal content (Cd and Pb) Porong River Water

<table>
<thead>
<tr>
<th>No</th>
<th>Heavy metals</th>
<th>Level of heavy metals (ppm)</th>
<th>Standard quality</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Station I</td>
<td>Station II</td>
</tr>
<tr>
<td>1</td>
<td>Cadmium (Cd)</td>
<td>0.013</td>
<td>0.078</td>
</tr>
<tr>
<td>2</td>
<td>Plumbum (Pb)</td>
<td>0.018</td>
<td>0.072</td>
</tr>
</tbody>
</table>

740
15. *Brachionus*  1  0  0  1  1  3  6  0.04
16. *Halicyclops*  1  0  4  0  1  2  8  0.08

Total individu  48  54  39  53  78  121  393  2.01


At all stations was dominated by *Gleocystis, Ankyra, Sphaerocystis, Golenkinia, Spirotaenia, and Peronia*. While the genus *Bracteacoccus, Gyrosigma, Amphilpleura* and *Pompholyx* found in small amounts. Based on the Shannon-Weaner index diversity (Odum, 1993) the diversity of plankton in the waters affected by Lapindo mud varies with the value 2.01. The highest index (0.29) is occupied by the genus *Gleocystis* totaling 65 individuals, while the lowest number of genus *Bracteacoccus, Gyrosigma, Pompholyx* with *Amphilpleura* (0.01). *Spirotaenia* dominant in the station 2 and 3 because of this genus are more resistant to higher salinity conditions. *Golenkinia* dominate in the station 5, this genus is a member of a group of green algae that are primary producers in the waters (Prescott, 1970). There are only three genus of zooplankton with a total of 15 individuals are observed in waters contaminated mud lapindo, namely *Pompholyx* and *Brachionus*, which is a member of the group of *Rotifera* and *Halicyclops* from the group of *Crustacea*.

4. Macrobenthos Biodiversity

Lapindo mud drainage into the sea through of Porong River has an impact benthos community structure changes, ie the degradation of biodiversity macrobenthos Porong River, as seen in the following Table.

Table 4. Type, Density, and Distribution Macrobentos Porong River After Lapindo Mud Disposal

<table>
<thead>
<tr>
<th>No.</th>
<th>Species</th>
<th>Stasiun I</th>
<th>Stasiun II</th>
<th>Stasiun III</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td><em>Bulimus tentaculata</em></td>
<td>12</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2.</td>
<td><em>Goniobasis virginica</em></td>
<td>12</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3.</td>
<td><em>Ligumia latissima</em></td>
<td>16</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4.</td>
<td><em>Lymnae auricularia</em></td>
<td>12</td>
<td>-</td>
<td>18</td>
</tr>
<tr>
<td>5.</td>
<td><em>Parathelpus maculata</em></td>
<td>-</td>
<td>-</td>
<td>30</td>
</tr>
<tr>
<td>6.</td>
<td><em>Pleurocera acuta</em></td>
<td>124</td>
<td>28</td>
<td>58</td>
</tr>
<tr>
<td>7.</td>
<td><em>Psidium singleyi</em></td>
<td>14</td>
<td>12</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>190</td>
<td>40</td>
<td>106</td>
</tr>
</tbody>
</table>
Biodiversity and density macrobentos highest in the station I, because it is not flowing through the lapindo mud so lowest pollution levels. Location of stations I, 4 km in the upper spillway outlet (where the lapindo mud disposal to Porong river), so that no contaminated mud. Station II right spillway outlets, which at this point that receive input lapindo mud in very high volume. Physically, at stations II and III occurred extraordinary sedimentation. Water depth ranged only from 0.3 to 0.5 m, high turbidity level so that the level of transparency reaches 0 m. Distribution and types of Porong river macrobentos are as follows:

![Figure 3. Biodiversity and density of Macrobenitos Porong River Post mudflow Disposal](image)

Macrobenitos found at all stations are *Pleurocera acuta* and *P. singleyi*. *P. acuta* found in high density in the three stations, because this species can live in polluted habitats (low dissolved oxygen levels, BOD and high CO2). Therefore a *P. acuta* bioindikator polluted aquatic ecosystem. According Wilhm (1975) in Hidayati (1995) benthos in general can not move quickly and aquatic habitat. Addition of pollutants into the waters will affect the abundance, composition and diversity macrobentos level. Macrobenitos diversity index is as follows:

<table>
<thead>
<tr>
<th>No</th>
<th>Species</th>
<th>Diversity Index of Macrobenitos</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Station I</td>
</tr>
<tr>
<td>1</td>
<td><em>Bulimus tentaculata</em></td>
<td>0,174</td>
</tr>
<tr>
<td>2</td>
<td><em>Goniobasis virginica</em></td>
<td>0,174</td>
</tr>
<tr>
<td>3</td>
<td><em>Ligumia latissima</em></td>
<td>0,208</td>
</tr>
<tr>
<td>4</td>
<td><em>Lymnea auriculalia</em></td>
<td>0,174</td>
</tr>
<tr>
<td>5</td>
<td><em>Parathelpusa maculata</em></td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td><em>Pleurocera acuta</em></td>
<td>0,278</td>
</tr>
<tr>
<td>7</td>
<td><em>Psidium singleyi</em></td>
<td>0,192</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>1,202</td>
</tr>
</tbody>
</table>
Macrobentos diversity index of the highest in station I. and dominated by *P.acuta*, conditions on the station I including contaminated (Sastrawijaya, 1991). At station II is dominated by *Psidium singleyi* (0.361), and the station III is dominated *Parathelpusa maculata* (0.357), both classified as heavily polluted.

<table>
<thead>
<tr>
<th>Table 7. Abundance of Macrobentos in the Porong River</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameter</td>
</tr>
<tr>
<td>Total species</td>
</tr>
<tr>
<td>Abundance</td>
</tr>
</tbody>
</table>

This is in accordance with the contaminated status is (Station I), and heavy polluted (Stations II and III) (Sastrawijaya, 1991). Content of heavy metals (Cd and Pb) in macrobentos found in Porong river, as in the following Table:

<table>
<thead>
<tr>
<th>Table 8. Heavy metal levels (Cd and Pb) in Porong River Macrobenthos</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
</tbody>
</table>

Accumulation of heavy metals both Cd and Pb in the body macrobentos exceeds the threshold quality standards so dangerous for consumption.

The high content of heavy metals in the water due macrobentos Porong river contain high heavy metal too. Heavy metals into the body through absorption macrobentos then distributed to all body tissues. Certain metal contacts of epithelial cells would affect the route of ion movement intercelluler and transcelluler, so that heavy metal ions can enter through the mucosa or ligands bound to proteins in the mucosa. Thus the flow of water containing heavy metal can enter the body crustacea and distributed to the tissue. In the tissue heavy metal is then accumulated. Factors affecting degree of heavy metal accumulation among
others, the concentration of heavy metals in water, while animals are alive in polluted habitats, body size, and the rate of consumption. Metals which are then accumulated in certain organs. Heavy metal concentrations in the tissue respectively from high to low is the gill> labial> kidney> intestine> liver> feet (Hemeral et al, 1986a in Darmono, 2006).

5. Aquatic Insect Biodiversity

The influx of lapindo mud into Porong River has caused decreased diversity and density of aquatic insects Porong river. The main cause is the increased salinity of water and turbidity, the apparent difference between the station I was not affected by the mud with stations II and III are affected mud.

<table>
<thead>
<tr>
<th>No</th>
<th>Type of Aquatic insect</th>
<th>Station I</th>
<th>Station II</th>
<th>Station III</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Agrion virgo</td>
<td>3</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>Berosus</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>Gomphus vulgarissimus</td>
<td>2</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>Halipus fulvus</td>
<td>19</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>Gerris najus</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>Leuctra hippocus</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>7</td>
<td>Micronecta poweri</td>
<td>7</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>Platambus maculates</td>
<td>18</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>9</td>
<td>Hydrometra stagnorum</td>
<td>7</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>Eristalis tenax</td>
<td>0</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>65</td>
<td>4</td>
<td>9</td>
</tr>
</tbody>
</table>

Aquatic insects are animals that are sensitive to dissolved oxygen content and water transparency. The diversity of aquatic insects Porong river declined sharply multiplied due to the disposal of lapindo mud. This is evidenced by the high species diversity and density in the station I which is not affected by lapindo mud, and the low diversity and density at station II and III. That is supported by the data physically and chemistry water is qualified. Water quality parameters at the station I have exceeded the standard, especially for the parameters DO, CO₂, BOD, temperature, brightness. The calculated abundance and diversity index of aquatic insects at Porong river, as in the following Table:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Station I</th>
<th>Station II</th>
<th>Station III</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
6. Fish Biodiversity

Various species of fish found in the waters of the lapindo mud gripped can be seen below:

<table>
<thead>
<tr>
<th>No.</th>
<th>Species</th>
<th>Local name</th>
<th>Abundance (%)</th>
<th>Content of Heavy metals (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Oreochromis mossambicus</em></td>
<td>Mujair</td>
<td>48,70</td>
<td>Cd: 0,617, Pb: 1,362</td>
</tr>
<tr>
<td>2</td>
<td><em>Mystus gulio</em></td>
<td>Keting</td>
<td>18,18</td>
<td>Cd: 0,413, Pb: 0,845</td>
</tr>
<tr>
<td>3</td>
<td><em>Poecilia reticulata</em></td>
<td>Kepala timah</td>
<td>8,45</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td><em>Trichogaster trachopterus</em></td>
<td>Sepat</td>
<td>5,84</td>
<td>Cd: 0,354, Pb: 0,894</td>
</tr>
<tr>
<td>5</td>
<td><em>Oreochromis niloticus</em></td>
<td>Nila</td>
<td>5,19</td>
<td>Cd: 0,412, Pb: 0,997</td>
</tr>
<tr>
<td>6</td>
<td><em>Anabas testudineus</em></td>
<td>Betok</td>
<td>5,19</td>
<td>Cd: 0,335, Pb: 0,962</td>
</tr>
<tr>
<td>7</td>
<td><em>Placostomus sp.</em></td>
<td>Sapu-sapu</td>
<td>3,90</td>
<td>Cd: 0,469, Pb: 1,008</td>
</tr>
<tr>
<td>8</td>
<td><em>Barbodes schwanefeldi</em></td>
<td>Bader</td>
<td>2,60</td>
<td>Cd: 0,271, Pb: 0,622</td>
</tr>
<tr>
<td>9</td>
<td><em>Channa striata</em></td>
<td>Gabus</td>
<td>1,95</td>
<td>Cd: 0,380, Pb: 0,824</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>9</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

From 9 species of fish, 7 of which are common fish consumed by the public. Even fish is a favorite surrounding communities. However, with Cd and Pb content well above the threshold of quality standards, then the fish are dangerous to consume.

7. Physical-chemical factors of water

Qualification measurements waters are affected by the lapindo mud as the following:

<table>
<thead>
<tr>
<th>No.</th>
<th>Parameters</th>
<th>Station</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Salinity (%)</td>
<td></td>
<td>6</td>
<td>20</td>
<td>9</td>
<td>0,67</td>
<td>0</td>
<td>23</td>
</tr>
<tr>
<td>2.</td>
<td>Temperature of water (°C)</td>
<td></td>
<td>29,67</td>
<td>31</td>
<td>30,33</td>
<td>28</td>
<td>28,67</td>
<td>29</td>
</tr>
<tr>
<td>3.</td>
<td>pH</td>
<td></td>
<td>7,5</td>
<td>6,7</td>
<td>6,2</td>
<td>6,67</td>
<td>7,13</td>
<td>7,43</td>
</tr>
<tr>
<td>4.</td>
<td>DO (ppm)</td>
<td></td>
<td>1,20</td>
<td>1,15</td>
<td>1,31</td>
<td>1,88</td>
<td>2,41</td>
<td>2,58</td>
</tr>
<tr>
<td>5.</td>
<td>CO₂ (ppm)</td>
<td></td>
<td>23,33</td>
<td>24,67</td>
<td>23,67</td>
<td>24,67</td>
<td>19,33</td>
<td>18,67</td>
</tr>
</tbody>
</table>
Lapindo mud has caused the change from freshwater to brackish. Salinity at all stations is very varied, ranging from 0-23 ‰. Based on the content of heavy metals (Cd and Pb), the quality of water in Porong river have exceeded water river quality standards.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Station I</th>
<th>Station II</th>
<th>Station III</th>
<th>Standard of Quality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salinity</td>
<td>o/oo</td>
<td>0,06</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Temperature</td>
<td>°C</td>
<td>27,6</td>
<td>32</td>
<td>30</td>
</tr>
<tr>
<td>Transparency</td>
<td>m</td>
<td>3</td>
<td>0,2</td>
<td>0,3</td>
</tr>
<tr>
<td>Current of flow</td>
<td>m/dt</td>
<td>0,152</td>
<td>0,242</td>
<td>0,203</td>
</tr>
<tr>
<td>Total Dissolved Solid</td>
<td>gr/l</td>
<td>0,107</td>
<td>0,179</td>
<td>0,109</td>
</tr>
<tr>
<td>Wide</td>
<td>m</td>
<td>176</td>
<td>182</td>
<td>200</td>
</tr>
<tr>
<td>Deeps</td>
<td>m</td>
<td>4,6</td>
<td>0,3</td>
<td>0,5</td>
</tr>
<tr>
<td>pH</td>
<td></td>
<td>7,6</td>
<td>8,4</td>
<td>8,3</td>
</tr>
<tr>
<td>DO</td>
<td>ppm</td>
<td>1,32</td>
<td>0,00</td>
<td>0,36</td>
</tr>
<tr>
<td>BOD</td>
<td>ppm</td>
<td>7,92</td>
<td>10,98</td>
<td>13,05</td>
</tr>
<tr>
<td>CO2</td>
<td>ppm</td>
<td>12,90</td>
<td>24,67</td>
<td>28,15</td>
</tr>
</tbody>
</table>

Salinity, clarity, pH, DO, BOD, and CO2 air Porong river has exceeded the threshold of quality standards. Based on river water quality parameters, Porong river classified as contaminated weight.

Based on the research results can be concluded (1). Levels of heavy metals (Cd and Pb) in waters of contaminated lapindo mud has exceeded the threshold quality standards and spread into ponds and marine ecosystems that potentially enter the food chain system. (2). Type of plankton found in waters polluted by Lapindo mudflow as much as 5 phylum consisting of 16 genera, dominated Gleocystis, diversity index 0.29. (3). Lapindo mud disposal to Porong river has led to decreased biodiversity organism. (4). Content of heavy metals (Cd and Pb) in water and body macrobentos living in Porong river has exceeded quality standards. (5). Quality of waters contaminated lapindo mud with heavy to moderate based on the measured DO and the heavy metal content (Cd and Pb). (6). Based on the macrobentho diversity index and the parameters physical-chemical quality of water (DO, BOD, CO2, pH, salinity, clarity, and suspended solids) waters Porong river are classified as contaminated - weight. Recommended before the lapindo mud poured into the sea need the remedies to be free of heavy metals.
REFERENCES

Palar, Heryando. 2004. Pollution and Heavy Metal Toxicology. Jakarta: Rineka reserved.
P-EC15

STUDY OF PURINE DEGRADATION IN AQUEOUS SOLUTION BY LACTOBACILLUS FERMENTUM

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Abstract

In this study the degradation of extracellular purines by the bacterium \textit{Lactobacillus fermentum} was examined with aqueous purine solutions. \textit{Lactobacillus fermentum} was able to decompose free purine bases. The nitrogen-containing products of the degradation were ammonia. Purine uptake was the main control of purine decomposition. In the cases of adenine, guanine, xanthine and hypoxanthine further control was exerted by induction. Furthermore, the uptake of the purines caused differences in the duration and temporal development of the substrate degradation. It was also responsible for the inhibitory effects of the purines on the decomposition of one another when the substrates were used in mixtures. Also, fermentation parameters like biomass and purine concentration, pH, and temperature influenced the purine usage of \textit{Lactobacillus fermentum}.

Keywords: Degradation, Purine, \textit{Lactobacillus fermentum}, Xanthine

INTRODUCTION

Today many foodstuffs contain substances that might be harmful to the human organism. Purines belong to this group because their degradation product, uric acid, causes hyperuricemia and gout. Hyperuricemia is a disease, which results from the overproduction and/or underexcretion of uric acid and is greatly influenced by a high dietary intake of purine. It is usually an asymptomatic condition, which is hypothesized to play a role in cardiovascular disease and hypertension. Some hyperuricemic individuals develop gout, a common disease with a worldwide distribution and are mainly caused by deposition of monosodium urate crystal in joints and other tissues as a result of extracellular urate supersaturation. Although most uric acid is derived from the metabolism of endogenous purine, eating foods rich in purines contributes to the total pool of uric acid. Usually this disease is treated with a diet low in purines and/or the use of medicaments. The patients often reject this treatment because it is contrary to their nutritional habits. A solution to this problem might be the availability of dietetic food low in purines.
Microorganisms could be used for diminishing the purine content of food. However, examinations concerning this particular subject have not been carried out yet. The publications at hand deal with the biological background of microbial purine degradation, especially with studies on enzymes. Therefore, the ability of various microorganisms for decomposing purines was studied at our laboratory. These studies showed that the bacterium *Lactobacillus fermentum* could degrade purines. Purine is used for tested is adenosine.

**MATERIALS AND METHODS**

*Lactobacillus fermentum* (collection of Lab. of Microbiology, Dept. of Biology, Faculty of Natural Sciences and Mathematics, University of Brawijaya) were used and medium MRS Broth (deMan Rogosa Sharpe) for growing that bacterial. Growth of biomass. *Lactobacillus fermentum* was grown on agar plates containing medium MRS broth at 30°C for 2 days. The biomass was suspended in 0.9% NaCl. The solution had an OD$_{560}$ of 1.0 AU. Fermentation conditions. The culture volume amounted to 150 ml, the volume of the inoculum to 1.5 ml. The cultures were incubated at 30°C on a rotary shaker. Preparation of bacterial suspensions. Before analysis, the biomass was separated from the medium by centrifugation. The optimalisation of microbial purine degradation was done in various pH (2-10), temperature (30-45°C), amount of bacteria ($4\times10^5$-$47\times10^6$ cells) and time of incubation (0-24 h). The concentration of adenosine was determined by spectrophotometry at a wavelength of 260 nm and TLC-Densitometry at a wavelength of 254 nm. The ammonia content of the medium was determined with Conway Microdifuison, and measured at a wavelength of 420 nm.

**RESULTS AND DISCUSSION**

The studies of purine degradation showed that *Lactobacillus fermentum* was able to decompose adenosine. Excretion of end products occured at the same time as adenosine usage, there are adenine and ammonia (Fig. 1 and Fig. 2).

![Fig. 1: Adenosine degradation; Densitometry (A), TLC (B), and graph of adenosine and adenine (C)](image)

![Fig. 2: Excretion of ammonia](image)
Fig. 3: Adenosine degradation; pH (A), temperature (B), mount ol (C), and time (D)

The optimalisation of microbial purine degradation was done in various pH (2-10), and optimal pH for microbial adenosine degradation is pH 6, and the optimal temperature of microbial purine degradation 37°C. The optimal amount of bacterial could degrade adenosine until 30% are 9.425x10^6 cells, and time of incubation give the optimal of adenosine degradation are 24 h.

REFERENCES


P-EC16

THE MINIMAL VALVES NUMBER IN THE IDENTIFICATION AND ENUMERATION OF DIATOMS STEP FOR WATER QUALITY ASSESSMENT

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Abstract
Global concern on the environmental issue had been focused on the sustainability of the availability, management and conservation freshwater ecosystem. Research on limnology, therefore, must be conducted as a basic national policy. It was not doubtly about the potential use of diatom as bioindicator. However, there is no Indonesian protocol Standard Methods of Diatom Analysis. Even in the developed countries, the protocol differs, particularly on the number of valves identified, that might be not suitable if implemented in Indonesia. Diatom analysis consist of 3 steps, sediment digestion, preparation, and identification-enumertation. This research was conducted to set up the minimal valves number in the identification and enumeration of diatom for limnological analysis.

Sediment samples had been taken from 3 sites of Rawa Pening Lake using hand auger. The core sediment sample is cutting every 0.5 cm. The enumeration the number of diatom species had been done on the valves number of 100, 00, 300, 400, 500, and 60. Semi quantitative analysis had done by graph and statistical test had done by ANOVA to set up the minimal number of valves that have to be identified.

In the digestion step, research as hyrax or naphrax. Identification-enumertation step had to use 1000 magnification. Based on this research, the number of diatom species on the 100 and 200 valves number tent to increase, and remain stable on the 300 to 600 valves. Therefore, the minimal number valves of 300 had to be reach for the water quality assessment.

Key words: diatom analysis, digestion, preparation, identification- enumeration

INTRODUCTION

Global concern on the environmental issue had been focused on the sustainability of the availability, management and conservation freshwater ecosystem. Research on limnology, therefore, must be conducted as a basic national policy. It was not doubtly about the potential use of diatom as bioindicator. However, there is no Indonesian Protocol Standard Methods of Diatom Analysis. Even in the developed countries, the protocol differs, particularly on the number of valves identified, that might be not suitable if implemented in Indonesia. Diatom analysis consist of 3 steps, sediment digestion, preparation, and identification-enumertation. This research was conducted in order to set up the minimal valves number in the identification and enumeration of diatom for limnological analysis.
MATERIALS AND METHODS

Sediment samples had been taken from 2 sites of Rawa Pening Lake using hand auger i.e. Dangkel and Asinan sites which had 29 cm and 63 cm core sediments, respectively. The core sediment sample is cutting every 0.5 cm.

In the digestion step, diatoms separates from sediment, by adding the 10% of chloride acid, simmer for 2 hours, tooped up with distilled water and allow to cool for overnight, then decant the acid and washed 3 times. Each time of washing allow to settle for minimal 2 hours. This step repeats with 10% hydrogen peroxide. In the preparation step, mountant used had to has refraction index of 1.7, such as hyrax or naphrax. Identification-enumeration step had to use 1000 magnification (Gell, 2008). The enumeration the number of diatom species had been done on the valves number of 100, 00, 300, 400, 500, and 600. Semi quantitative analysis had done by graph and statistical test had done by Analysis of Varian to set up the minimal number of valves that have to be identified. Regression analysis had performed for Dangkel site.

RESULTS AND DISCUSSION

Based on this reserach, the number of diatom species on the 100 and 200 valves number tent to increase, and remain stable on the 300 to 600 valves almost all samples of Dangkel and Asinan sites. Figure 1 illustrated the tend of increase in the 2 cm core sediment layer. Round (1993) identify and count diatoms until found minimally 100 valves for dominant species. In this research, when diatom counting on 300 valves or more, usually, the dominant species will reach at number of 100 valves. DALES (2004), European protocol Diatom Enumeration, assured when one species dominates 1/3 out of all species, the number of valves for dominant species have to reach 200 minimally. The prsent of new species on 300 valves or more, usually by rare or contaminant species.
Figure 1. Trend of increasing number of species for 100 and 200 valves, and remains stable on 300 or more valves. X-axis: counting valves of 100, 200, 300, 400, 500 and 600, Y-axis: number of species.

Analysis of Variance indicates that there is a significant difference the number of species on the difference number of valves counting. Further analysis of Least Square Design, indicates that the number of species on 100 valves counting, significantly differ with 300 valves or more. Meanwhile, there is no significant differ on the number of diatom species found at 100 valves and 200 valves counting. Therefore, the minimal number valves of 300 had to be reach on the diatom identification and enumeration for the water quality assessment, although Pienitz et al. (2006) tend to use total of 500 valves diatom count for paleolimnological analysis.

REFERENCES


P-EC17

COCOA AGROFORESTRY SYSTEMS AT THE EDGE OF LORE LINDU NATIONAL PARK, CENTRAL SULAWESI

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Abstract
The field studies were conducted in three types of cacao agroforestry systems at the northeastern edge of Lore Lindu National Park. Field study sites covering in natural forest (NF) and in three cacao agroforestry systems that is cacao which was planted under remaining forest cover (CF1), under planted trees (CF2), and between shade trees Gliricidia sepium (CP). Soil macro-organic matter fractionated by size and density fractionation in silica suspension (LUDOX solution) with a density of 1.13 and 1.37 g cm-3. The nitrogen and organic carbon of each fraction of soil macro-organic matter were determined.

Fractionation of soil macro-organic matter resulted three fractions with the order of total fractions as followed intermediate fraction (MF) > heavy fraction (HF) > light fraction/active fraction (LF). The percentage of LF in the natural forest was lower than those of in the cacao agroforestry systems. The carbon organic and nitrogen content in the LF in the natural forest was higher than those of cacao agroforestry systems. Among cacao agroforestry system, the organic carbon and nitrogen content in the LF was the highest in the CF2.

Keywords: cacao agroforestry, soil macro-organic matter, lore lindu national park, LUDOX solution.

INTRODUCTION
Changes in soil management practices influence the amount, quality and turnover of soil organic matter (SOM) (Glaser et al. 2000). Soil organic matter is a key of nutrients for plant growth essential for the maintenance of soil structure, and contributes to the ability of soil to retain nutrients and water (Liu et al. 2003). It is generally accepted that SOM contains fractions with rapid turnover rate and fractions with a slower turnover rate (Cambardella and Elliot 1992). The fractions with a rapid turnover (active fractions) are assumed to play a dominant role in soil nutrient dynamics (Janzen et al. 1992). The “active” fraction (light fraction) has been found to be more sensitive to differences in management and inputs of residues than total SOM (Janzen et al. 1992). Size-density fractions have been shown to be more useful to characterize land use changes compared with whole SOM (Hairiah et al. 1995). Fractionation by size and density was shown to be more powerful approach for separating soil macro-organic matter (SmOM) fractions than fractionation based on density alone (Magid et al. 2002). From a density perspective, SmOM can divided into: (1) light fraction (LF), which consist of mineral-free organic matter composed of partly-decomposed
plant debris and less stable, it has a relatively high C/N ratio, low ash concentration, a specific density considerably lower than that of soil minerals; and (2) intermediate fraction (MF) of partly humified material, and (3) heavy fraction (HF), composed of organic matter adsorbed or deposited by microorganisms on aggregate surfaces and sequestered within organo mineral aggregates, more stable, it has a narrow C/N ratio, a slower turnover rate and higher specific density than the light fraction due to its intimate association with soil minerals (Meijboom et al. 1995, Gregorich et al. 1996). Light and intermediate fractions of the SmOM (> 150 μm) respond much faster to changes in C input than smaller size fractions. This shows that the light and intermediate SmOM fractions can be used as early indicators of effects of soil management on changes in SOM (Hassink et al. 1997). The aims of this research are that to determine the amount SmOM fractions in various land use types, from natural forest to cacao agroforestry systems, and their carbon and nitrogen content.

MATERIAL AND METHODS

Study sites

The field studies were conducted in three different types of cacao agroforestry systems at the northeastern margin of Lore Lindu National Park (LLNP), which is located in Central Sulawesi, Indonesia ca. 75 km southeast of Palu. The whole site is located at an elevation of 800 m to 1100 m in Toro village, Kulawi district, Central Sulawesi, Indonesia.

The three different cacao agroforestry systems selected in the margin zone of LLNP were (1) Cacao agroforestry under a remaining forest cover (CF1), (2) Cacao agroforestry under local shade trees (CF2), (3) Cacao agroforestry without forest cover, but with planted shade trees, Glyricidia sepium (CP).

Size and density fractionation of SmOM (Hassink 1995, Meijboom et al. 1995).

Twenty-five soil samples were collected from each subplot in each plot down to 10-15 cm depth. Soil samples were collected four times in a year. Air dried soil samples (500 g, < 2 mm) were washed on two sieves (top sieve, mesh size 250 μm; bottom sieve, mesh size, 150 μm). The mineral fraction was discarded by decantation, and after combining the organic fraction from both sieves it was fractionated in silica suspensions (LUDOX solution) with a density of 1.13 and 1.37 g cm⁻³. The organic fraction recovered on both sieves (diameter > 150 μm) were referred to as soil macro-organic matter (SmOM). The SmOM was separated into three fractions: a light fraction (LF) (density < 1.13 g cm⁻³), an intermediate fraction (MF) (density between 1.13-1.37 g cm⁻³), and a heavy fraction (HF) (density > 1.37 g cm⁻³). Each
fraction was dried into a constant dry weight at 60°C. Nitrogen and carbon of each fraction of SmOM were analyzed by Kjeldahl and Walkley and Black method respectively.

RESULTS AND DISCUSSION

Using size and density fractionation of SmOM in this study, size of SmOM between 150 to 250 μm and density between 1.13 to 1.37 g cm⁻³ was chosen. Table 1 showed the amount of fractions of SmOM (LF-, MF-, and HF-SmOM), was significantly different between natural forest (NF) and cacao agroforestry systems (CF1, CF2, and CP). Intermediate SmOM (MF-SmOM) was the highest proportion of SmOM, except in the cacao agroforestry system CF2. Light fraction of SmOM (LF-SmOM) was the highest in NF. Among cacao agroforestry systems, the CF2 was the highest LF-SmOM. The natural forest had the highest total SmOM (6.1 g kg⁻¹ DW soil), while CF2 (3.9 g kg⁻¹ DW soil) was the highest among cacao agroforestry systems. Quality of SmOM could be determined by nitrogen, and organic carbon content. Quality of SmOM fractions (organic carbon and nitrogen) was significantly different (p < 0.05) between natural forest (NF) and cacao agroforestry systems (CF1, CF2, and CP). Organic carbon and nitrogen content in fractions of SmOM decrease with the changing of land use from natural forest into cacao agroforestry systems. The natural forest has highest organic carbon and nitrogen content.

Table 1. The dry weight of soil macro-organic matter fractions and their fractions (light fractions - LF, intermediate fractions - MF, heavy fractions – HF) in various land use types.

<table>
<thead>
<tr>
<th></th>
<th>NF</th>
<th>CF1</th>
<th>CF2</th>
<th>CP</th>
</tr>
</thead>
<tbody>
<tr>
<td>LF</td>
<td>1.4 ± 0.2a</td>
<td>0.8 ± 0.1a</td>
<td>1.0 ± 0.2a</td>
<td>0.7 ± 0.1a</td>
</tr>
<tr>
<td>MF</td>
<td>2.8 ± 0.4a</td>
<td>1.3 ± 0.2ab</td>
<td>1.2 ± 0.1ab</td>
<td>1.3 ± 0.2ab</td>
</tr>
<tr>
<td>HF</td>
<td>1.9 ± 0.2a</td>
<td>1.0 ± 0.3a</td>
<td>1.7 ± 0.2b</td>
<td>0.7 ± 0.1a</td>
</tr>
<tr>
<td>Total</td>
<td>6.1b</td>
<td>3.1b</td>
<td>3.9b</td>
<td>2.7b</td>
</tr>
</tbody>
</table>

Organic C and N content of fraction of SmOM (mg kg⁻¹ DW soil)

<p>| | |</p>
<table>
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</tr>
</thead>
<tbody>
<tr>
<td>C in LF</td>
<td>428.9 ± 10.7b</td>
</tr>
<tr>
<td>C in HF</td>
<td>353.3 ± 2.1b</td>
</tr>
<tr>
<td>N in LF</td>
<td>17.7 ± 0.5b</td>
</tr>
<tr>
<td>N in HF</td>
<td>22.9 ± 0.1c</td>
</tr>
</tbody>
</table>

Numbers followed by different superscript(s) indicate significantly different. Values are mean ± SE. Significance of Tukey-test given at p < 0.05.

The land-use type in Toro village affected the amount and quality of SmOM, since the different land use types in this study had different tree density and diversity (Ramadhanil 2006). Therefore, land use types contributed to different amount and quality of litterfall, hence; the total of SmOM fractions were significantly different (Table 1). The total of SmOM fractions under NF was the highest due to the highest litterfall (data was not shown). In this
work, the fractions of SmOM apparently were affected by land-use type. The order of the dry weight of total SmOM fractions was MF > HF > LF. This result was different from the reported by Barrios et al. (1996) and Hassink (1995). Barrios et al. (1996) observed the order of SmOM fractions was HF > LF > MF after 4-yr-old maize legume systems on Alfisol in Kenya, but Hassink (1995) resulted the order HF > MF > LF on sandy arable soils in the Netherlands that received various organic input for 25 yr. In both situations their finding reflected condition in annual crops, which is different from the tropical forest and cacao agroforestry system studied here. The amount of LF-SmOM in the soil at any time is small due to its rapid turnover (Paul and Juma 1981). The dry weight of LF-SmOM and MF-SmOM decreased as changing land use from natural forest into cacao agroforestry systems in this study. The HF-SmOM was not affected by land-use change. This is consistent with finding of Hopkins et al. (1993) that SmOM tend toward chemical homogeneity as it decreases in size and increases in density through decomposition. Quality of SmOM fractions in this study was represented by nitrogen and organic carbon contents. Hassink (1995) reported that the quality and amount of the residues input had the strongest effect on the nitrogen and organic carbon content of the SmOM particularly LF-SmOM. Land use change from natural forest into cacao agroforestry systems decreased organic carbon and nitrogen in SmOM fractions. The nitrogen and organic carbon content in the LF-SmOM fractions indicate that the LF-SmOM consists of mineral free, partly decomposed plant debris. The HF-SmOM fraction includes the organomineral complexes SOM (Hassink 1995) with much lower carbon content (Golchin et al. 1995).

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P-EC18

BIODIVERSITY OF MEDICINAL POTENTIAL PLANTS AT DIPTEROCARPACEAE BUFFERING FOREST OF BUKIT BARISAN SELATAN NATIONAL PARKS KRUI, WEST LAMPUNG

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Abstract
Bukit Barisan Selatan National Parks (TNBBS) is one of tropical rain forest conservation areas that has high plant diversity, but it is still exploited and converted into plantation, human settlement and other sifting cultivation. Field data collection of medicinal plants was done by direct observation and interview with the figure or tribe-head and community who used medicinal plant in Krui. The result of this study revealed 78 species and 46 families, those are Piperaceae (8 spp.), Euphorbiaceae (6 spp.), Fabaceae and Zingibeeaceae (5 spp.), Arecaceae (4 spp.), Myrtaceae (3 spp.), Malvaceae, Rubiaceae, Oxalidaceae, Simarubaceae, Verbenaceae (2 spp.) and other families (1 spp.) have high potential as Medicinal plants. There are five species categorized as endangered species such as areh jelateh (Ficus deltoidea), bidara (Eurycoma longifolia), kelawi (Alstonia scholaris), tahi angin (Usnea spp.) and maluag (Shorea javanica). Ethnobotany and potential utilization of medicinal plant diversity will be discussed in this paper.

Key words: Medicinal plants, Biodiversity, West Lampung

INTRODUCTION
Tropical forests are recognized as communities which is rich in plant biodiversity. Tropical forests, particularly tropical rain forests, are also considered as one of part of the world which still remain wildlife, which raise human amazement and astonishment. In relation to that and for human welfare, tropical forests are place for germplasm biodiversity, especially world medicinal plants currently and for the future. Considering the importance of environmental issues and Indonesian biodiversity conservation, the individual or communities awareness should be raised, especially economical performers which carry out in industrial sector, to understand the importance of biodiversity conservation for future generation. The conservation is not only for plants, animals, microorganisms, and ecosystem specialty, but also for culture and traditional knowledge of each tribe in Indonesia. Traditionally, Indonesian people have long been used biodiversity of natural resources, particularly plants existing naturally at their surroundings as medicine, beauty treatment, health and for ritual activities. Various traditional medicine mixtures were created and given to their progenies from one
generation to other generation, eventhough the heritage is less documented. Due to the importance of medicinal plants for human life, and anxiety on the extinction of community knowledge on medicinal plants and in line with damage of forest areas which are continuously exploited, a study was conducted at communities in TNBBS surroundings areas, that are Krui, Pahmongan Village, Sukangra, and Gunung Komala Village, Pesisir Tengah Krui District, and Kubu Perahu Village, istrict of Balik Bukit, Lampung Barat.

MATERIALS AND METHODS

In this study, plant specimen sampling at field was carried out by using exploratory method (1), that are method which is performed by exploring each location. All plants species found in the location have potential as medicinal plants. The plants were then collected and given number. Information on potential plant utilazaton was obtained by two methods, that are: 1) Primary data (interview), and 2) Secondary data (literatures). The plants having high potential use were collected and its herbarium materials were taken for identification.

RESULTS AND DISCUSSION

Study conducted at Village of Pesisir Tengah Krui and Kubu Perahu has successfully collected more than 78 plant species and 46 family which has potential as medicinal plants. The plants species were from Piperaceae (8 species), Euphorbiaceae (6 species), Fabaceae and Zingiberaceae (5 species), Arecaceae (4 species), Myrtaceae (3 species), Malvaceae, Rubiaceae, Rubiaceae, Oxalidaceae (2 species). Of 78 plant species utilized as medicinal materials, there were 5 species which is categorized as endangered plants, that are ara jelateh (Ficus deltoidea), kelawi (Alstonia scholaris), pasak bumi (Eurycoma longifolia), maluang (Shorea javanica), and tahi angin (Usnea sp.). The result of study also recorded that 24 types of diseases suffered by communities, namely jaundice, fever, body heat suffer, malaria, kidney stone, post parturition, diabetes mellitus, hypertension, and gastric disorders.

Plant species that are categorized as endangered medicinal plants are as follows:

1) Kelawi (Alstonia scholaris) which used as medicinal plant for combat malaria fever. The stem bark is peeled, and the bark is cut and boiled with 3 glasses of water into 1 glass, sieved and drunk twice a day in the morning and afternoon. In the Philippines, a decoction of the bark is used as a febrifuge and tonic, as an emmenagogue, anticholeric and vulnerary. The latex is applied to ulcers and for rheumatic pains (2).

The use of the plants as medicinal plants was quite high without efforts in its cultivation. Therefore, the plants are categorized become rare in nature (3).

2) Ara jelateh (Ficus deltoidea) is used as postparturition medicine for keeping women in good health and fresh after giving birth. Based on direct interview with
communites, 'areh jelateh' is drunk as aprodisiac for women. In related to the application of 'tabat barito' product formula, based on activity and inhibit zone from bioassay test, extract produced is potential to be developed as base extract for antiseptic products (4). This plant is classified as one of 41 endangered plant species and it should be conserved (5,6).

3). Pasak bumi (Eurycoma longifolia). The root is used for curing malaria and as tonic. The roots are cut and scraped, and boiled with water, then drunk. E. longifolia was found in buffering zone of Dipterocarpaceae Krui primary forests, its population has been difficult to be found. This was caused by highly use of the plants continuously as medicinal plants. The increasing use of pasak bumi causes the population of the plants in wild nature become less, furthermore forest areas as natural habitat of the plants was damage by illegal logging. Conservation activity such as natural reserve or national parks as habitat for this plant is highly required. Therefore, pasak bumi is categorized as one of Indonesian endangered plant species (1).

4). Tahi angin (Usnea sp), is used as mixture of postparturition herbs, drug materials for lever, and mixture of various traditional medicines. Tahi angin is boiled with 3 glasses of water become 1 glass, and drunk every morning and afternoon. Nowadays extract of Usnea has been traded in form of ointment, processed by factory and packaged in tin tube (7). Use of Usnea as medicine is quite potential, so the population in nature is being extinction and its cultivation is still difficult (9)

5). Maluang (Shorea javanica), its resin sap is used as medicine for scabies, by refining and pouring it to the part of disease. In Krui its resin sap is source of foreign exchange for Indonesia because the sell price of sap follows dollar rates in market, unfortunately maluang tree stands should be cultivated and conserved as heritages from our ancestors. Based on Mogeja, et al (2001), Shorea javanica is classified as one of endangered plant species in Indonesia.

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## Topic 3: Systematic And Evolution

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<td>P-SE02</td>
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<td>Hypomycetes Fungi In Gunung Tukung Gede Nature Reserve, Serang Regency, Banten Province</td>
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<td>Preliminary Study Orchids of Mount Tukung Gede Nature Reserve Serang-Banten</td>
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<td>Taxonomy Study on The Diversity of Palmae in Wilis Mountainous Area, east Java</td>
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P-SE01

PRELIMINARY STUDY OF PTERIDOPHYTE IN WILIS MOUNTAIN AREA, EAST JAVA

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Mountain Wilis as one of many mountainous area in East Java surrounded by within four cities; Madiun, Ponorogo, Tulung Agung and Kediri. Mountain Wilis area is 26,634 ha. so this place is promising for inventory works on its flora especially for the diversity of Pteridophyte. Even though many researchers such Junghuhn (1838), Goot, Backer & Berger (1912-1914), Dorgelo (1923) and Doelitzcsch (1935) and van Steenis (1967) have explored that area, however the diversity of florahas not been reported scientifically. Especially for pteridophyte based on Backer and Posthumus (1939) reported that about 515 species of Pteridophyte present in Java and almost 66.2% (341 species) present in East Java.

The exploration work was carried out recently in Mountain Wilis area especially at Ngebel Lake (742 m asl.), Sigogor Nature Reserve (1,212 m asl.), Ngesep Forest (1,124 m. asl.), Manyutan Forest (1,361 m. asl.) and Bunder Mountain (1,165 m. asl.). Sixty five species of Pteridophyte been collected which have 50 species of terrestrial Pteridophyte and 15 species of epiphyte Pteridophyte. The diversity of Pteridophyte and its potential use provided.

Keywords: Diversity, Pteridophyte, Wilis Mountain, Exploration.
HETEROPHYLLY IN Murraya exotica L. (RUTACEAE)  
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Email: inggit_pa@yahoo.com. \textsuperscript{2}Faculty of Biology Gadjah Mada University. \textsuperscript{3}Centre for Plants and the Environment, University of Western Sydney, Australia

Heterophyly, the existence of different leaf types and shapes on the same plant was observed in Murraya exotica populations from different location in Java, Indonesia. In each population, three types of compound leaves (paripinnate, imparipinnate and intermediate) and three types of leaflet shape (oblong, rounded and obcordate) were observed. This heterophyllous condition was found on both juvenile and adult plants. The variation in leaf type and leaflet shape may be due to the environmental conditions prevailing at the time of leaf production directing leaf development or due developmental noise caused to random interactions between genes and the environment.  

**Keyword:** Heterophyly, compound leaves, leaflet shape, Murraya exotica

**Introduction**  
The genus Murraya extends from India, through Sri Lanka, Southeast Asia, Taiwan, the Phillipines, Borneo, Indonesia Indo-China, Thailand, New Guinea, New Caledonia and northern Australia (Swingle & Reece 1967; Parrotta 2001). Within this genus is the mock orange or orange jasmine, known as kemuning in Indonesia, which is commonly grown as an ornamental or hedge due to its glossy foliage and clusters of fragrant flowers. Among the morphological characters used in the classification of Murraya is leaf shape with leaves typically being imparipinnate compound with 5 to 7 leaflets. However, observations in the field have shown the occurrence of heterophyly (Sculthrope, 1967) with variation occurring in leaflet shape and compound leaf type. The objective of this study was to detail the morphological variation associated with heterophyly to aid the correct identification of forms of Murraya.

**Materials and Methods**  
The materials observed were adult plants and seedlings of M. exotica. A total of 500 three-year-old adult plants and 500 one-year-old seedlings planted at UGM, BBG and PBG were examined for the type of compound leaf and shape of the leaflets (conducted from October, 2004 to August, 2006). The seeds from the three-year-old plants was sourced from UGM, BBG and PBG and the one-year-old plants were propagated from seed from the three-year-old plants. Five randomly-selected individuals were selected from each of these sets of plants and assessed to determine: (i) the position of paripinnate and intermediate compound leaves and (ii) the number and the position of obcordate leaflets on the main stem (one-year-old plants) or the main stem and branches (three-year-old plants). Both immature, recently
formed leaves that were still bright green and mature leaves that had turned dark green were assessed separately. From December, 2006 to December, 2008, plants grown at PBG, UGM and BBG were reassessed; at this time the plants were five-years-old. In addition, five-year-old plants at Purworejo were also assessed; the seed for these plants came from Florida, Brazil and China. Data were analysed by ANOVA using Statistica (Version 7, StatSoft, Inc.). Data were first tested for normality and homogeneity of variances using Levine’s test. Data sets that were heteroscedastic were subjected to square root transformations before analysis.

Results

A. Type of compound leaves

Three types of compound leaves can be found in *M. exotica*: imparipinnate, paripinnate and intermediate. However, most leaves were imparipinnate. In the imparipinnate and paripinnate compound leaves, there were two types of position of the leaflets i.e., alternate and opposite. There were no differences in the numbers of paripinnate or intermediate leaves on the one-year-old plants grown at the three sites nor between the young and mature leaves. Among the three-year-old plants grown at BBG, PBG and UGM, there were no significant differences among sites in terms of the number of paripinnate or intermediate leaves, so the data from the different sites were combined. There was a trend for the stem and older branches to have more immature leaves that were paripinnate (Table 1). However the mature paripinnate leaves were randomly distributed on the different part of the plant as were both immature and old mature leaves.

<table>
<thead>
<tr>
<th>Paripinnate</th>
<th>Intermed</th>
<th>Immature</th>
<th>Mature</th>
<th>Immature</th>
<th>Mature</th>
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</thead>
<tbody>
<tr>
<td>Stem</td>
<td>2.13 a</td>
<td>3.73 ab</td>
<td>1.67 a</td>
<td>3.33 b</td>
<td></td>
</tr>
<tr>
<td>Branch 1</td>
<td>2.07 a</td>
<td>3.73 ab</td>
<td>1.60 a</td>
<td>4.33 a</td>
<td></td>
</tr>
<tr>
<td>Branch 2</td>
<td>1.40 b</td>
<td>3.33 ab</td>
<td>2.07 a</td>
<td>4.13 ab</td>
<td></td>
</tr>
<tr>
<td>Branch 3</td>
<td>1.47 b</td>
<td>3.00 b</td>
<td>1.80 a</td>
<td>3.80 ab</td>
<td></td>
</tr>
<tr>
<td>Branch 4</td>
<td>1.40 b</td>
<td>4.07 a</td>
<td>2.13 a</td>
<td>3.47 b</td>
<td></td>
</tr>
</tbody>
</table>

Table 1. Average number of immature and mature paripinnate and intermediate leaves on the main stem and four side branches of 3-year-old *Murraya exotica* plants. The averages are combined data from plants grown at Bogor and Purwodadi Botanical Gardens and at Universitas Gadjah Mada. Means within a leaf type followed by the same letter are not significantly different according to Fisher’s LSD test at *P* = 0.05.

When the plants were reassessed after two years, there were no statistically significant differences among the numbers of immature and mature leaves of the two types on the stem nor the different branches produced by the plants. Therefore, the data from the stem and all branches on each plant were combined. This analysis revealed small but statistically significant differences in the number of mature paripinnate leaves per plant among the sites and in the numbers of immature intermediate leaves between the sites (Table 2). There were no differences among sites in the numbers of immature paripinnate leaves, nor mature intermediate leaves.
<table>
<thead>
<tr>
<th>Site</th>
<th>Immature</th>
<th>Mature</th>
<th>Immature</th>
<th>Mature</th>
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<tr>
<td>Purworejo</td>
<td>1.40 a</td>
<td>4.40 c</td>
<td>1.17 b</td>
<td>5.27 a</td>
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<tr>
<td>Purwodadi Botanical Gardens</td>
<td>1.30 a</td>
<td>5.17 ab</td>
<td>1.23 b</td>
<td>5.17 a</td>
</tr>
<tr>
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<td>1.80 a</td>
<td>4.87 bc</td>
<td>1.40 b</td>
<td>5.67 a</td>
</tr>
<tr>
<td>Universitas Gadjah Mada</td>
<td>1.70 a</td>
<td>5.73 a</td>
<td>2.07 a</td>
<td>5.70 a</td>
</tr>
</tbody>
</table>

Table 2. Average number of immature and mature paripinnate and intermediate leaves on five-year-old *Murraya exotica* plants grown at different locations. The averages are combined data from the stem and all branches on the plants. Means within a column followed by the same letter are not significantly different according to Fisher's LSD test at P= 0.05.

B. Leaflet shape

The leaflet shape of *M. exotica* is generally elliptical to oblong; however, rounded and obturate leaflets were occasionally found. The number and position the obturate leaflets varied among individuals. In one compound leaf, all leaflets may be obturate, but there were also occurrences where only one, two or three obturate leaflets were produced. The occurrence of obturate leaflets was found in all populations observed; however, there were no differences in the number of obturate leaves at any site at any time of measurement. The one-year-old plants at PBG, UGM and BBG had between 5.6-5.8 obturate leaves per plant. The three-year-old plants at these sites had between 3.8-5.8 obturate leaves per plant and, when these plants were assessed two year later, they had between 4.0-7.8 obturate leaves per plant. The plants grown at Purworejo had ~3.2 obturate leaves per plant.

Discussion

Swingle & Reece (1967) described *M. exotica* as producing unpaired-pinnate or occasionally pinnately 3-foliolate leaves with alternate, cuneate-ovate or almost obliquely rhomboid, blunt or bluntish acuminate leaflets. Stone (1985) described the genus as possessing alternate, imparipinnate leaves and described the leaves of *M. exotica* as having 3-7 leaflets that are alternate or the lowest pair opposite mostly obvate to subelliptic, obtuse to bluntly acuminate with the apex minutely notched. This study is the first to report obturate leaflets and the occurrence of paripinnate and intermediate leaves in either *M. exotica*. Variation in leaf shape within a plant has been well documented and has been assigned to a number of different causes. There can be differences between immature and mature leaves, a phenomenon termed heteroblasty (Kersetter and Poethig 1998). However, many plants have the ability to develop leaves of different shape in response to environmental conditions such as nutrient availability (Sussex and Cutter 1960) and light (Njoku 1956) and the evolutionary significance of this has been reviewed by Bradshaw (1965). In addition, phenotypic variability may be due to random interactions between genes and the environment (Mather 1953) and this has been termed developmental noise by Waddington (1957). The cause of the variation in leaf type and leaflet shape and whether these features have a genetic component has yet to be determined in *M. exotica*. The variation in leaf type
or leaflet shape observed in this study may be due to prevailing environmental conditions
directing leaf development, thus a particular stress such as drought or high temperatures
may lead to a particular leaf morphology. However, as there appears to be little difference
between populations of M. exotica nor between different parts of individual plants in the
number of paripinnate or intermediate leaves produced nor in the number of cordate
leaflets; therefore, it may be that leaf development in M. exotica is particularly susceptible to
developmental noise.

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P-SE03
HYPHOMYCETES FUNGI IN GUNUNG TUKUNG GEDE NATURE RESERVE, SERANG-BANTEN

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ABSTRACT

Hyphomycetes diversity research was carried out in Gunung Tukung Gede Nature Reserve. Most of these fungi grew as saprobic and a few of them as parasitic. Samples were collected from dead leaves, twigs and culms from various plants in Gunung Tukung Gede forest. Some of them are new record for Indonesia since we knew that there is still lack of Hyphomycetes research in Indonesia. As many as 12 genera were found, e.g. Acrodictis, Corynespora, Coronospora, Endophragmia, Exosporium, Helicoma, Melanographium, Periconia, Sporidesmium, Stachylium, Verticillium, and Zygosporium.

Keyword: Fungi, Hyphomycetes, diversity, Gunung Tukung Gede Nature Reserve

INTRODUCTION

Gunung Tukung Gede Nature Reserve is located in western part of Java and is located beside Rawa Danau Nature Reserve. This nature reserve was named based on a name of a hill inside the area i.e. Tukung Gede. Located between 6°14'-6°20'S and 105°52'-105°57' E, this nature reserve covers 1700 ha. This area is mostly sloping between 30-60° and hilly. This area's climate is tropic humid with annual rainfall ca. 2151 m and daily rainfall ca. 116,64 days. Dry season is in June until August and wet season is in September until May. And average of temperature minimum is 19° Celsius and maximum is 25° Celsius.

Hyphomycetes is anamorphic fungi which grow mostly on dead part of plants or some of them are parasite on living plants. They play role as decaying agent in the nature. Since we knew that there was no data for fungal collection from this area (Dephut 1982), this research was conducted as a preliminary study for Hyphomycetes research in this nature reserve.
Material and Method

The fungi were collected from dead part of plants mostly from class Monocotyledoneae. A total of 40 samples were collected from 3 sampling sites: Cadas Gantung, Cadas Pagar and Pasir Menteng forest. Samples were placed into zip-lock bag with an addition of moistened tissue paper. Morphological characteristics of fungi were observed using dissecting and light microscope. They are mounted with lactophenol. Herbarium specimens were deposited in Herbarium Bogoriense, Cibinong. This material was collected in July as a survey for the representative of dry season.

Result

Fourteen species of Hyphomycetes were identified. The most common species are Stachylidium bicolor and Melanographium citri. The other species are presented in table 1. Of all species, only one species is known as parasite. Coronospora dendrocalami was known as parasite on bamboo leaf of Stachylidium iraten in Indonesia (Dewi, 2005). Rattan (Calamus sp.) is a substrate which has the highest frequency inhabited by fungi, followed by bamboos. Acrodyctis appendiculata, Endophragmia sp. and Helicoma sp. are new record for Indonesia. And Stachylidium pallidum is another finding after the type specimen.

Table 1. Hyphomycetes species from Gunung Tukung Gede Nature Reserve

<table>
<thead>
<tr>
<th>No</th>
<th>Species</th>
<th>Substrat</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Acrodyctis appendiculata</td>
<td>Gigantochloa apus</td>
</tr>
<tr>
<td>2</td>
<td>Coronospora dendrocalami</td>
<td>kasrap</td>
</tr>
<tr>
<td>3</td>
<td>Corynespora sp.</td>
<td>Jirak, kasrap</td>
</tr>
<tr>
<td>4</td>
<td>Endophragmia sp.</td>
<td>kasrap</td>
</tr>
<tr>
<td>5</td>
<td>Exosporium sp.</td>
<td>Calamus sp.</td>
</tr>
<tr>
<td>6</td>
<td>Helicoma sp.</td>
<td>Calamus sp.</td>
</tr>
<tr>
<td>7</td>
<td>Melanographium sp.</td>
<td>Calamus sp.</td>
</tr>
<tr>
<td>8</td>
<td>Melanographium citri</td>
<td>Daun sayar</td>
</tr>
<tr>
<td>9</td>
<td>Periconia sp.</td>
<td>Gigantochloa apus, kasrap</td>
</tr>
<tr>
<td>10</td>
<td>Sporidesmium cf. verrucisporum</td>
<td>Calamus sp.</td>
</tr>
<tr>
<td>11</td>
<td>Stachylidium bicolor</td>
<td>Calamus sp.</td>
</tr>
<tr>
<td>12</td>
<td>Stachylidium pallidum</td>
<td>Calamus sp., bangban,</td>
</tr>
<tr>
<td>13</td>
<td>Verticillium sp.</td>
<td>Donax sp.</td>
</tr>
<tr>
<td>14</td>
<td>Zygosporium oscheoides</td>
<td>sayar</td>
</tr>
</tbody>
</table>
References


P-SE04
PRELIMINARY STUDY ORCHIDS OF MOUNT TUKUNG GEDE NATURE RESERVE
SERANG – BANTEN

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Mount Tukung Gede Nature Reserve located at Serang region, Banten province, West Java,
and covers a forest area about 1700 ha. The study was conducted in the forest areas near Cikedung
village, Mancak district, Serang. No information about the orchid of this Nature Reserve exists.
BKSDA website, mentioned only Phalaenopsis sp. from this area. Based on the field observation,
seven orchids species of Mount Tukung Gede were recorded. One species is recognized as a rare
orchid plant, there is Erythorchis altissima. Phaius amboinensis is also recorded in this area which
was reported that is recorded in Java only in East Java previously.

Keywords: Orchids, mount Tukung Gede Nature Reserve.
P-SE05

TAXONOMICAL RESEARCH OF GUAVA (Psidium guajava L.) VARIETIES
COLLECTION OF B2P2TO2T (MED.PLANT & TRAD.MED. R&D OFFICE) BASED ON
THIN LAYER CHROMATOGRAPHY (TLC) PROFILES

Dyah Subositi¹, Tri Widayat and Rohmat Mujahid
Medicinal Plant and Traditional Medicine Research and Development Office, NIHRO, MOH

Guava (Psidium guajava L.) is native to northern South America, although it is mainly used for
food, the guava plant can be utilized medicinally especially its leaves. Medicinal Plant and Traditional
Medicine Research and Development Office (B2P2TO2T) has 5 varieties of guava: dark purple,
stripped leaf, bangkok, red and small which is different in morphology. The aim of this study was to
make database of guava varieties based on Thin Layer Chromatography profiles and phenetic
relationship among varieties. Leaves of 5 guava varieties and strawberry guava (Psidium cattleianum)
as an outgroup were collected from B2P2TO2T. Identification of compound was conducted using TLC
Densitometry and visualized using TLC scanner 4 to determine compound profiles in 254, 366 and
540 nm of wavelength. There were 84 TLC profiles and used as binary data (0 or 1). Data were
calculated for the coefficient of using Jaccard similarity coefficient method. The relationship was
determined using UPGMA clustering method and presented as dendogram. Guava varieties were
divided into three clusters on the coefficient of association 80%: cluster I consist of bangkok, red and
dark purple varieties, and cluster II consist of small and stripped leaf, also strawberry guava in cluster
III as an out group. The closest relationship was found between bangkok and red varieties on the
coefficient association 85,1%.

Keywords: Guava (Psidium guajava L.), Thin Layer Chromatography (TLC) profile, TLC Densitometry

INTRODUCTION

Guava (Psidium guajava L.) is native to northern South America, although it is mainly
used for food, the guava plant can be utilized medicinally especially its leaves¹. Medicinal
Plant and Traditional Medicine Research and Development Office (B2P2TO2T) has 5
varieties of guava: bangkok, red, dark purple, small and stripped leaf which are different in
morphology. The taxonomic distribution of individual secondary compounds varies
considerably. Chemical similarities are frequently used to infer relationships among plant
taxa in so-called chemosystematics study²,³. The objective of this study were to make
database of guava varieties based on Thin Layer Chromatography (TLC) profiles and
similarity relationship among varieties.
MATERIALS AND METHODS

Leaves of 5 guava varieties and strawberry guava (Psidium cattleianum) as an out group were collected from B2P2TO2T medicinal plant field collection. Methanol extract of samples were obtained maceration method for 24 hours. Leaf extracts of guava varieties were analyzed by Thin Layer Chromatography using 3 solvents system {diethyl ether; chloroform:methanol (1:1); hexane:ethyl acetate (17:3)} at 3 replications. Spots (Retention factor/Rf) detection was conducted using TLC Densitometry and visualized using TLC Scanner 4 (CAMAG) under UV-254 nm, 366 nm and 540 nm. Presence/absence of Rf value was taken as a character state and used as binary data (0 or 1). Data were calculated for the coefficient of association using Jaccard similarity coefficient method. The relationship was determined using UPGMA clustering method and presented as dendogram.

RESULTS AND DISCUSSION

There were 84 TLC profiles found in guava varieties collection of B2P2TO2T and the similarity relationship is described by dendogram below:

![Dendogram of guava varieties collection of B2P2TOT based on TLC profiles (%)](image)

Dendogram above shows that guava varieties were divided into three clusters on the coefficient of association 80%: cluster I consist of bangkok, red and purple varieties, and cluster II consist of small and stripped leaf varieties, also strawberry guava in cluster III as an out group. The closest relationship was found between bangkok and red varieties on the coefficient association 85,1%. Morphological variation of guava varieties followed by chemical profile variations. Few studies done this far have consistently demonstrated that closely related plant species can have highly divergent chemical profiles. The distribution of chemical variation within and among plant species is directly relevant for the evolutionary relationships between plants and their associated herbivores and pathogens also environment. Three solvents were used in TLC because to separated compounds that have wide range polarity from different classes of secondary metabolites. Eighty four profiles
were found in this research because the used instrument so sensitive and visualized under 3 UV-wavelength that the peak/Rf could appear more and some different Rf could represent the same compound. Based on the result TLC profiles could be used to differentiate intraspecific varieties and to strengthen the morphological characters. Secondary metabolite has some chemical characteristics, which implies that specific extraction method, solvent and analysis method should be developed to study detail for further research on chemosystematics especially guava varieties.

REFERENCES
P-SE06

THE VARIATIONS OF POLLEN MORPHOLOGICAL CHARACTERS OF THE
APOCYNACEAE ON MALANG

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Malang Stated of University

ABSTRACT

Pollen has been very useful in determinating pattern of species relationships in plants. The taxonomic characters provided by pollen grains include pollen wall structure, polarity, symmetry, shape and grain size.

The study is aimed to know features pollen grains involving the shape of grains, aperture type, surface sculpturing type of pollen grains of the Apocynaceae. The pollens grains of the Apocynaceae was collected at Malang on 2004 January to July.

The result of the reseach show that: (1) The species of Apocynaceae on Malang are Allamanda, Cerbera, Tabernaemontana, Catharanthus, Thvetia, Nerium, Adenium and Plumeria; (2) Shapes of grains showing polar view of the Apocynaceae are circular, triangular with convex obtuse and quadrangular with convex obtuse; (3) Shapes of grains showing equatorial view of the Apocynaceae are circular, elliptic acuminate obtuse, elliptic acuminate acute, rhombic truncate obtuse, rhombic acute obtuse, and rectangular convex obtuse; (4) The grains had a long variation 35-80 μm on polar view and 25-80 μm on equatorial view; (5) Apertura types of the Apocynaceae are trizonocolpore, tetrazonocolporate, trizonoporate, tetrazonoporate and sincopitate; (5) The colpate grains are acuminate and obtuse, its longs are 25-70 μm, the shape pori are circular and elliptic, its longs are 5-15 μm; The surface sculpturing type of pollen grains of the Apocynaceae are regulate, reticulate and verrucate.

INTRODUCTION

Pollen is useful for species determination in plants. Its provided the taxonomic character include the wall structure of pollen, polarity, symmetry, shape and its size.

OBJECTIVES

To know the type of pollen (shape, aperture type, surface) in Apocynaceae in Malang for determitating its species.

METHODS

The Apocynaceae pollens grains was collected from January to July IN 2004. Methods for preparation of pollen grains were acetolysis methods.
RESULT AND DISCUSSION

The result of the research show that: (1) the genus of Apocynaceae in Malang involves Allamanda, Cerbera, Tabernaemontana, Catharanthus, Thevetia, Nerium, Adenium and Plumeria; (2) the shapes of grains base on polar view are circular, triangular with convex obtuse and quadrangular with convex obtuse; (3) the shapes of grains base on equatorial view are circular, elliptic acuminate obtuse, elliptic acuminate acute, rhombic truncate obtuse, rhombic acute obtuse, and rectangular convex obtuse; (4) the variation of length 35-80 μm (base on polar view) and 25-80 μm (base on equatorial view); (5) the apertura types are trizonocolpore, tetazonocolporate, trizonoporate, tetazonoporate and sincopplate; (6) the type colpate grains are acuminate and obtuse, the length is 25-70 μm, the porous shape circular and elliptic, and its wide is 5-15 μm; (7) the surface sculpturing type of pollen grains of the Apocynaceae are regulate, reticulate and verrucate.

CONCLUSIONS

Apocynaceae in Malang comprises 8 genus, there are Allamanda, Cerbera, Tabernaemontana, Catharanthus, Thevetia, Nerium, Adenium and Plumeria. The type of pollen (shape, aperture type, surface) its genus vary variation.

REFERENCES

P-SE07
ETHNOBOTANY OF RAW VEGETABLES (TESPONG, JOTANG, JONGE, REUNDEU AND PICUNG) OF SUNDAANESE COMMUNITY AT CIHAMBALI VILLAGE, LEBAK REGENCY, BANTEN.

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"Lalap" (raw vegetables) is known as a part of the daily life of Sundanese community and its never been separated from this culture since long time ago. Raw vegetables usually consist of young leaves, young fruits or fresh seed. This diet is a part of WHO programme for health “back to nature” that rich in fibrous, mineral and vitamin. Besides as food, "lalap" some times also used as traditional medicines. The research was conducted on June30th "Lalap" that been consumed by Sundanese community in Cihambali village, Lebak regency, Banten consist of tespong (Oenanthe javanica), jotang (Spilanthes acmella), jonge (Emelia sonchifolia), reundeu (Staurogyne elongata), and picung (Pangium edule). Conservation and ethnobotany aspects of each species has been discussed within paper.

Keywords: Conservation, Ethnobotany, "Lalap", Sundanese community, Banten

INTRODUCTION

In Indonesia, food and food habits differ from one place to the others. Therefore, there is no typical Indonesian dishes, though nowadays “rijsttafel” is labelled as an Indonesian trade mark. It consists of rice with several main dishes such as chicken, meat, or fish and vegetables. One dish which is in common throughout the islands is chili sauce to which shrimp paste is added (1). Leafy vegetables characterize the Sundanese diet. A great deal of leafy vegetables, either raw, half cooked or cooked, are offered during the meals together with chili sauce. Those varieties of vegetables offered as such are referred to as “lalab”. In culture and daily life of Sundanese, lalab could not be separated since previous years. Lalab has special meanings in rural life, but nowadays it being part of modern life of urban societies. Lalab which consists of young leaves, young fruits, or fresh plant seed has been part of WHO “back to nature” program or high fiber, mineral and vitamin foods for health and vitality. Lalab or vegetables are highly fiber food, therefore consuming lalab and fresh vegetables will give considerably benefit for health and body vitality and for skin beauty, especially women face skin. Indigenous vegetables has also several characteristics, that are well adapted at relatively various environmental conditions, being alternative sources of protein, vitamin, mineral, and fiber which is cheap, and traditionally has been one
of cropping pattern components, especially in utilization of backyard and relatively resistant to environmental stress.

MATERIALS & METHODS

The study was carried out on June 30 until July 17, 2009 in Cihambali Village which is directly adjacent to TNGHS areas. Data were collected by interviewing the communities based on questionnaires. Data collected were on knowledge of local communities in utilization of food crops which is daily consumed. Plant sampling was taken one day before end-time study because leaves are quick to decay. The leaves were then analyzed for its nutrition and mineral content.

RESULTS AND DISCUSSION

Result of study at Cibeber District, Banten revealed that there were more than 105 plant species utilized as foodstuffs, in form of leaves, roots, fruits or seeds. Of 105 species of food crops, the most and frequent food crops used and consumed by Sundanese and has not been cultivated were tespong, jotang, jonge, rendeu & picung. Each species was described as follows

1. Tespong (*Oenanthe javanica* DC.)

Uses *Oenanthe javanica* used as a leafy vegetable or condiment in East and South-East Asia. The plant smells strongly of carrot leaves when bruised, and its leaves and young stems are used raw as well as cooked briefly. It is a delicious substitute for celery. The seed and other plant parts are used medicinally (2).

Conservation Tespong (*Oenanthe javanica*) is one of the most popular traditional vegetables in South-East Asia. It is a good alternative for celery, with the additional advantages that it is easier to grow and can be maintained around the year without replanting. In location studied, tespong was still as wild plant and has not been cultivated.

2. Jotang (*Spilanthes acmella* (L.) Mure.)

Uses In Sundanese restaurants in West Java, young Spilanthes leaves are usually served raw with other fresh vegetables together with a chilli sauce. The most important application is the use of the flowerheads as a local anaesthetic, often applied to ease toothache (3).

Conservation Spilanthes greens will remain of minor importance as vegetables. Population of jotang in location studied was still abundant, therefore jotang has not been cultivated yet at their backyard or garden by local Sundanese at the location studied.

3. Jonge (*Emilia sonchifolia* (L.) DC.)
Uses The young, non-flowering plants are eaten raw or steamed as a side dish with rice. The older leaves or plants are cooked. Emilia is a slightly bitter tasting green. The plant has many medicinal applications. It is administered internally against fever, coughs and diarrhoea, as well as externally as a poultice for sores and swellings, drops for dim eyes and sore ears (4). Its leaves are used as diuretic, for curing scurbut, cough, and fever, its root for curing diarrhoeae (5)

Conservation *E. sonchifolia* is a much relished leafy vegetable. Developments in the horticultural sector have diminished the importance of gathered products like emilia. The natural abundance of it’s has not led, and probably will not lead in the near future, to efforts to promote its cultivation.

4. Reunedeu (*Staurogyne elongata* (Bl.) Ktze.)

Uses The roots and leaves are used as a diuretic. Young leaves are eaten raw as a vegetable (6). All parts of the plants are ground, the pressing water is drunk or youn leaves were boiled as diuretic Its flowers were squeezed, the water was rubbed to the tongue to clean the whitish tongue (7).

Conservation Population of rendeu at location studied was quite abundant and found in rice field, bushes, and secondary forests. Cultivation of reundeu has not been performed yet by local communities.

5. Picung (*Pangium edule*)

Uses Most of the medicinal and poisonous applications of *P. edule* are based on the presence of hydrocyanic acid in all parts of the plant, ranging from seeds, fruits, leaves, bark or roots. Fresh leaves, leaf sap, pounded leaves or ground fresh seeds are externally applied as an antiseptic and disinfectant to cleanse ulcerations, infested wounds, and to treat scurf. (8). Local communities at location studied use water from the roots for eye drops of post parturition mothers.

Conservation Due to its benefit, Sundanese at Cihambali village has cultivated picung at their backyard or garden.

REFERENCES


P-SE08
TAXONOMY STUDY ON THE DIVERSITY OF PALMAE IN WILIS MOUNTAINOUS AREA, EAST JAVA

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Willis Mountain which lies at the South of East Java consists of enormous diversity of plants. Unfortunately up to now there is no scientific record on the plants diversity of this area. Recently, a group of researchers from Herbarium Bogoriense has made exploration work in order to inventory the flora of Mountain Wilis and its surrounding area. As a preliminary result of taxonomy study can be reported especially the diversity of Palmae in that area. So far, six species of palms, tree palms as well as rattans or climbing palms, are found in Wilis Mountain this including one proposed new species of Daemonorops sp. nov. This six species are Caryota maxima, Pinanga javana, Calamus spp. (3 species) and Daemonorops sp. nov. This proposed new species has erect inflorescence, where in Java so far represented by Daemonorops melanochaetes only. As reported by local people the young shoot of this proposed new species can be eaten because it has sweet taste. The taxonomy study of the Palmae together with identification key and its illustration will be presented.

Keywords: taxonomy, diversity, Palmae, Wilis Mountain, new species.

INTRODUCTION

Wilis mountain (2,169 m) is located about 36 km from South East of Madiun – Ponorogo, East Java. This area has characteristic of tropical rain forest. Floristic study including taxonomy in Wilis Mountain is highly needed especially because there is no complete floristic book of this area had been published. Steenis (1950) reported some botanist explored Wilis Mountain such as Junghuhn (in 1838), Goot, Backer and Berger (in1912 – 1914), Dorgelo (in 1923), and RF Doelitzsch (in 1935). The last floristic exploration had been made by Steenis in 1967 at Dorowati Mountain, one of Wilis Mountainous area and he reported the flora of Wilis Mountainous is very diverse eventhough he never publish floristic study result of Wilis Mountain taxonomically (Steenis, 1972). Recently many problems such as illegal logging, deforestation and wild hunting tremendously happen in this area (Anonym, 2009). Before the forest is damaged because of those irresponsible human activities, study on the flora especially diversity of palms in Wilis Mountain and its surrounding area is needed in order to reveal the new record or even new species for Java Island. The aim of the expedition is to explore and document the flora of the mountain (especially palms) using conventional botanical and herbarium methods. Results of the
expedition will include new plant discoveries, strengthened collaborative links between institutions and joint research outputs, such as papers describing new species.

Recent field work have made at the West of Wilis Mountain in Gunung Sigogor Nature Reserve, Gunung Picis Nature Reserve and several places maintains by Perhutani KPH Wilis Barat, in the Toyomarto village, Ngebel District, Ponorogo. Many Pinanga javana (Piji) have found associated with Pandan (Pandanus kurzii). Eventhough palms exploration had made by Beccari (1884-1886; 1885; 1902; 1908; 1911; 1913; 1918), however palms study never been scientifically published. Palms are difficult to collect and are often neglected by general collectors. Praptosuwiro et al (2003) reported the newest information regarded to palms flora of Wilis Mountain eventhough only for one species, Pinanga javana. So far, for other genera of tree palms and climbing palms in Java refers to several publication of Beccari (1884-1886; 1885; 1902; 1908; 1911; 1913; 1918) and Backer et al (1968).

MATERIALS AND METHODS

Standard fieldwork methods involving a collaborative team from Herbarium Bogoriense were implemented. Standard plant specimen collection techniques were employed. This involves the collection of representative vegetative and reproductive parts (Dransfield 1986). The specimens were kept in the national herbarium of Indonesia - Herbarium Bogoriense - where they will be processed and distributed according to existing LIPI protocols. Standard GPS equipment/methods were used to acquire collection locality data. Photographs were taken using standard digital photography equipment.

RESULTS AND DISCUSSION

A preliminary result of palms taxonomy study can be reported. Six species of palms, tree palms as well as rattans, are found in Wilis Mountain this including one proposed new species of Daemonorops sp. nov. This six species are Caryota maxima, Caryota mitis, Pinanga javana, Pinanga coronata, Calamus sp. and Daemonorops sp. nov. (fig. 1). This proposed new species has erect main bractea or prophyl which enclosed all the infructescence, where in Java based on Backer et al (1968) represented by Daemonorops melanochaetes only. This species include in the section Daemonorops (formerly known as section Cymbospatha – because the main bractea has cymbo-shape). As reported by local people the young shoot of this proposed new species can be eaten because it has sweet taste.
Calamus sp. (unidentified) found in this area has large cane diameter (3 cm in diameter) almost similar with Calamus manan which has been used for furniture by many local people in Kalimantan. Unfortunately, in this area this Calamus sp. eventhough potentially used but local people never use it except for stick when they go to the field. The fruit is sweet and edible so in the future it can be consumed as pickled fruit. The taxonomy status of this species needs further study. Two species of Pinanga represented by P. coronata and P. javana differentiated by clustering habit and solitary habit respectively. Another information concerning uses of palms in this area is stem usage of Caryota maxima or locally known as Liwung as water pipe by local people. They claimed this stem can be used for up to ten years. Caryota maxima has solitary stem whereas Caryota mitis has clustered stem. Local people use this species as ornamental plant.

REFERENCES


P-SE09

DIversity of Nymphalids Butterfly at Mount Slamet, Central Java
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There are over 600 species of butterflies found on Java and Bali, nearly 40% of which are endemics at sub species level, however until now there is no available data or publication showing recent butterfly records. From about 629 butterfly species on Java and Bali, the higher species richness is found among Nymphalids which consist of 226 species. Based on the feeding preference, this family is divided into two groups i.e.: flower visiting butterfly and frugivorous butterfly. Mount Slamet is a higher mountain at Central Java with almost all part of the mountain covered by forest under Perhutani management, suggested as center of butterfly diversity. The aims of the research was to investigate the diversity of Nymphalids butterfly at different forest types. The research was conducted from June to August 2009 with simple random sampling at two forest types (Plantation Forest and Natural Forest) using line transect (300 m long and 10 m width). Butterfly was captured with hand netting six times during sampling periods. The result showed that at Mount Slamet totally found 30 species of Nymphalids butterfly with different species abundance. Based on the species abundance, there are 5 species categorized as rare species (found < 10 individual during sampling periods), these species are: Centhosia hypsea munjava, Cyrestis lutea, Symbrenthia anna, Stibochiona coresia, and Vagrant egista. One of these species is endemics Java (Cyrestis lutea). Nymphalids butterfly species which categorized as most abundance species at Mount Slamet are: Vanessa cardui, Euthalia monina, Tanaecia trigeta, Junonia hedonia, and Neptis nisea those species were found more than 50 individual during sampling period. From the 30 species of Nymphalids butterfly, only one species not found at plantation forest during sampling periods (Vagrant egista). Based on the clarification above, this research can be concluded that forest at Mount Slamet still support Nymphalids diversity.

Keywords: Mount Slamet, Nymphalids butterfly, diversity, rare species, endemics species.
P-SE10

Premilinary Study : Diversity of the Asteraceae (Compositae)
In The West Wilis Mountain Area, East Java

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ABSTRACT

Premilinary studies on the Diversity on the Asteraceae (Compositae) growing throught in the Wilis mountain area was carried out. The study area included six districts of The Wilis mountain area; Cagar Alam Cigogor, Mayutan Mountain, Picis Mountain, Ngebel Lake area, Selorejo Waterfall area, and Protected forest of Perum Perhutani KPH Lawu DS. A total of 14 species under13 genera of the Asteraceae (Compositae) were collected and identified. One species is very dominant in all of area. Key to species is provided.

Key words: Diversity, Asteraceae, Wilis Mountain. Identification key

Introduction

The Wilis Mountain Area is one of forest area in East Java. It is located between Tulungagung and Kediri about 36 km of south-east Madiun. The West Wilis Mountain area is located Ponorogo around + 2 hours from Madiun. This area is combinanted between proctected forest and production forest. The main shade vegetation of this area are lowland tropical rainforest (700-1000 m altitude) and lower montane forest (up to 1000 m altitude). The West Wilis mountain area have six district; Cagar Alam Cigogor, Mayutan Mountain, Picis Mountain, Ngebel Lake area, Selorejo Waterfall area, and Protected forest of Perum Perhutani KPH Lawu DS. In this area can be found Quercus sp (Pasang), Juglan regia (Morosowo), Litsea sp, etc. The understorey plant dominated by Asteraceae (Compositae) family.

The Asteraceae (Compositae) family is the second largest family of flowering plants in term of number of species. It is known as the aster, daisy or sunflower family. This family containing about 1,317 genera and nearly 21,000 species. The family is divided into 13 tribes and the major tribes are Helianthaceae, Asterae, Senecionae, Eupatorieae, Cynareae, and Lactuaceae. The members of Asteraceae have been used for wide array of medicinal purposes, as herbs and in herbal tea, beverages, poultry feed, marigold oil, and medicine plants, etc.
Materials and Methods

The exploration was conducted on 28 June – 7 July 2009, in The West Mountain Area; Cagar Alam Cigogor, Mayutan Mountain, Picis Mountain, Ngebela Lake Area, Selorejo Waterfall area, and Protected forest of Perum Perhutani KPH Lawu DS. The exploration, in each location used “Exploring Method” following methods by Van Valgooy (1987) and Bridson & Forman (1992). The data and information about the plants in the field such as plant species name, local name, abiotic environment, altitude and number of species were recorded in the field book.

Results & Discussion

Study of species diversity on the Asteraceae (Compositae) growing throughout in the West Wilis mountain area was carried out. A total of 14 species under 13 genera of the family Asteraceae were collected and identified. Of total number of species 9 were cultivated and 5 were wild. The Most of cultivated species had been introduced in Java already much more than a century ago and present widely distributed over.

They are species found in The West Wilis mountain area on the six districts were as follow: Ageratum conyzoides Linn, Bidens biternata Merrill & Sherff, Blumea balsamifera DC, Chromolaena odorata (L) R.M King & H. Robinson, Elephantopus scaber Linn, Erechtites valerianaefolia DC, Eupatorium odoratum Vahl, Eupatorium triplinerve Vahl, Gynura pseudo-china Benth, Porophyllum ruderale Cass, Sonchus arvensis Linn, Spilanthes iabadicensis A.H. Moore, Spilanthes paniculata Wall, and Tithonia diversifolia A. Gray. Eupatorium triplinerve is one dominant species in all of area. Almost of this area can be founded it. Unfortunately, only one individual has blooming flower. All of Asteraceae species can be found in lowland forest and lower montane forest area.

Table. The Asteraceae Collection from The West Wilis Mountain Area

<table>
<thead>
<tr>
<th>Name</th>
<th>Tribe</th>
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<tr>
<td>Ageratum conyzoides Linn</td>
<td>Eupatoriae</td>
<td>Cultivated</td>
</tr>
<tr>
<td>Bidens biternata Merrill &amp; Sherff</td>
<td>Heliantheae</td>
<td></td>
</tr>
<tr>
<td>Blumea balsamifera DC</td>
<td>Inuleae</td>
<td>Cultivated</td>
</tr>
<tr>
<td>Chromolaena odorata (L) R.M King &amp; H. Robinson</td>
<td></td>
<td></td>
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<tr>
<td>Elephantopus scaber Linn</td>
<td>Vernoniae</td>
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<td>Erechtites valerianaefolia DC</td>
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<tr>
<td>Eupatorium odoratum Vahl</td>
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</tr>
<tr>
<td>Eupatorium triplinerve Vahl</td>
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<td>Cultivated</td>
</tr>
<tr>
<td>Gynura pseudo-china Benth</td>
<td>Senecioneae</td>
<td>Cultivated</td>
</tr>
<tr>
<td>Porophyllum ruderale Cass</td>
<td>Tagoeeae</td>
<td>Cultivated</td>
</tr>
<tr>
<td>Sonchus arvensis Linn</td>
<td>Lactuceae</td>
<td></td>
</tr>
<tr>
<td>Spilanthes iabadicensis A.H. Moore</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spilanthes paniculata Wall</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tithonia diversifolia A. Gray</td>
<td>Heliantheae</td>
<td>Cultivated</td>
</tr>
</tbody>
</table>
Key to Species: Adapted from ¹.
The local people have uses the members of Asteraceae as livestock feed, medicinal plants, and ornamental plant. The main uses of Asteraceae species is as livestock feed because most of local people has animal livestock like cow and goat. They took of some the members of Asteraceae every day from The Wilis mountain area forest.

References

P-SE11

THE GECKOS OF DAERAH ISTIMEWA YOGYAKARTA

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Family of Gekkonidae mostly known as Cicak and Tokay Gecko (Tokek). Most of the geckos are cosmopolitan reptiles. They were wide spread in Daerah Istimewa Yogyakarta region. Some species were have similar appearance and difficult to recognize. They also sometimes have similar body shape between male and female. The geckos diversity from Daerah Istimewa Yogyakarta were poorly studied. A herpetological survey was conducted at this area from July to November 2008. This research aimed to give a complete data about geckos diversity from this region. Specimens were studied using purposive sampling in 47 different areas. Seven species of geckos were identified. They were Gekko gecko, Hemidactylus frenatus, Cosymbotus platyrurus, Gehyra mutilata, Cyrtodactylus marmoratus, Ptychozoon kuhlii and Hemiphylodactylus sp.

Keywords: Gekkonidae, Reptile, Diversity, Daerah Istimewa Yogyakarta.

INTRODUCTION

Family of Gekkonidae is mostly known as Cheechak (Cicak) and Tokay Gecko (Tokek). Most of geckos are cosmopolitan reptiles. They are wide spread in Daerah Istimewa Yogyakarta region. Some species have similar appearance and difficult to recognize. They also sometimes have similar body shape between male and female. The gecko diversity from Daerah Istimewa Yogyakarta (DIY) was still poorly studied. This research aimed to give a complete data of gecko diversity from this region.

As a part of Java regions, DIY province is cover from southern part of Mount Merapi (Montane and hilly zones) to the South Sea (Coastal zones). It was divided into five regency viz. Yogyakarta, Bantul, Gunungkidul, Kulonprogo and Sleman which have specific topography for each region. They provide suitable habitat for reptiles to survive but there were no records available about geckos of DIY. We present the recent result of gecko survey of Daerah Istimewa Yogyakarta province as contribution to our understanding of the reptile species of Java.

MATERIALS AND METHODS

Survey was conducted from July until November 2008 in Daerah Istimewa Yogyakarta Province. Data was collected randomly in five regencies: Yogyakarta, Bantul, Gunungkidul, Kulonprogo and Sleman. Purposive sampling was used in several habitats such as plantation, forest, human housing, etc. Species which have encountered during
surveys were captured manually from its habitat using either hand or tools. Individuals that were not captured but visually identified were noted. For identification purposes and records representative, individuals of each species were preserved. Specimens were killed by 70% alcohol injection to the brain. Specimens were then labeled, preserved in 70% alcohol and stored in specimen jars. Identification guides used in this research were de Rooij (1915), Kurniati (2003), Das (2004), Krysko (2005), and Eprilurahman (2007).

RESULTS AND DISCUSSION

Seven species of geckos were identified. They were *Gekko gecko*, *Hemidactylus frenatus*, *Cosymbolotus platyurus*, *Gehyra mutilata*, *Cyrtodactylus marmoratus*, *Ptychozoon kuhlii* and *Hemiphylodactylus* sp. *Gekko gecko* was the biggest gecko among other. It has big head and rough skin. Other geckos have smaller size and smooth skin. Identification could be done by examine the morphology viz. head, limbs, body, tail, scales and colorations. Special features were found in each species. *Gekko gecko* has always big and rough granule in it skin; *Ptychozoon kuhlii* has a large skin fold in the lateral of body to glide; *Hemidactylus frenatus* has rounded tail with several spines; *Gehyra mutilata* has only four toes in the hind limbs; *Cosymbolotus platyurus* has frilly flat tail; *Cyrtodactylus marmoratus* has marbled coloration skin and the finger shows the shape like a bow; and *Hemiphylodactylus* sp. has a slender body with prehensile tail.

<table>
<thead>
<tr>
<th>Species</th>
<th>Common name</th>
<th>Sleman</th>
<th>Bantul</th>
<th>Yogjakarta</th>
<th>Kulonprogo</th>
<th>Garungkidul</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Gekko gecko</em></td>
<td>Tokay Gecko</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
</tr>
<tr>
<td><em>Ptychozoon kuhlii</em></td>
<td>Kuhl’s Gliding Gecko</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>√</td>
<td>-</td>
</tr>
<tr>
<td><em>Hemidactylus frenatus</em></td>
<td>House Gecko</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
</tr>
<tr>
<td><em>Cosymbolotus platyurus</em></td>
<td>Frilly House Gecko</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>-</td>
</tr>
<tr>
<td><em>Cyrtodactylus marmoratus</em></td>
<td>Marbled Bow-Fingered Gecko</td>
<td>√</td>
<td>-</td>
<td>-</td>
<td>√</td>
<td>-</td>
</tr>
<tr>
<td><em>Gehyra mutilata</em></td>
<td>Stump-Toed Gecko</td>
<td>√</td>
<td>-</td>
<td>√</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Hemiphylodactylus</em> sp.</td>
<td>Worm Gecko</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td></td>
<td>6</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>3</td>
</tr>
</tbody>
</table>

Based on Table 1., it can be seen that the distribution of geckos are almost spread evenly in every regency. That could be happened because of human activities. Human make a variety of habitats especially for geckos. One of them, the common *Hemidactylus frenatus*, is such a good adaptable species that wide spread around the world. Other species that also wide spread are *Cosymbolotus platyurus* and *Gekko gecko*. Those three species could be
easily found around human housing area. Other two species, *Cyrtodactylus marmoratus* and *Gehyra mutilata*, were recorded from two regencies. *Cyrtodactylus marmoratus* was sighted from Sleman and Kulonprogo; *Gehyra mutilata* was sighted from Yogyakarta and Sleman. Meanwhile, *Ptychozoon kuhli* and *Hemiphyllodactylus* sp. remain inhabited in hilly area with less human activity.

Generally, we can conclude that DIY is suitable both natural and man made habitats for geckos and contribute seven species to our understanding of the reptiles of Java.

REFERENCES


P-SE12
THE REPTILES SPECIES IN DAERAH ISTIMEWA YOGYAKARTA PROVINCE, INDONESIA

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1) Herpetology Study Club Faculty of Biology Gadjah Mada University, 2) Animal Taxonomy
Department Faculty of Biology Gadjah Mada University

This research was aimed to figure out the diversity of Reptiles species in Daerah Istimewa
Yogyakarta Province. The research was done since November 2007 to August 2009, using a variety
of methods to sample the Reptile species. A total of 63 species of reptiles were obtained during the
survey. Results include five families of lizard, seven families of snake and two families of turtle. On all
of identified species, only two are endemic to java and two species are new record. Unidentified and
possibly undescribed species of unusual specimen referred to genus Cyrtodactylus and still being
examined. Vegetation types, habitat and elevation variability are dominant ecological factors to affect
species richness and distribution of reptiles in Daerah Istimewa Yogyakarta Province. From this study
can conclude that Daerah Istimewa Yogyakarta Province have a high diversity of reptiles species.

Keywords: Reptiles, species richness, Daerah Istimewa Yogyakarta Province

INTRODUCTION

Daerah Istimewa Yogyakarta (D.I.Y.) is one of province that present in Java. This
province consist of five regency that is Kulonprogo, Sleman, Bantul, Gunungkidul and
Yogyakarta. This province has total area ± 318,580.00 km² or less than 0,5% Indonesian
land area. D.I.Y. province has high potential biodiversity, one of them is reptiles species
richness. But the research about reptile diversity in this province still rare. So that, potential
of reptile diversity that live in D.I.Y. province has not been revealed. Infact, reptiles is kind of
fauna which is easily threatened by the environment change and human activities. The aim
of this research is to revealed the reptiles diversity in D.I.Y., also give information about
reptile diversity and their distribution in D.I.Y. province. As a first effort to protect and
conservate reptiles diversity in D.I.Y. province.

MATERIALS AND METHODS

The research was done since November 2007 to August 2009, using visual
encounter survey, pupossive sampling, glue trap and census methods (Hamidy et.al, 2007;
Riyanto, 2008) to collect the reptile species. The major research area are located in five
surveys site, there are Kulonprogo, Sleman, Bantul, Gunungkidul and Yogyakarta regency.
Voucher specimens were collected whenever possible. In identifications, taxonomy of the
Lacertilia were based Roij (1915) and Manthey et.al. (1997), expecially for Sphenomorphus
puncticentralis was based on Iskandar (1994); for Ophidia were based on Roij (1917) and Iskandar & Colijn (2001), especially for Xenochrophis melanzotus was based on Vogel et al. (2006); for turtle based on Iskandar (2000) and Roij (1915).

RESULTS AND DISCUSSION

A total of 64 species of reptiles were obtained during the survey. Results included five families of lizard, seven families of snake and two families of turtle. A detailed species is presented in table 1. On all of identified species, Sphenomorphus puncticentralis, Xenochrophis melanzotus and Calamaria schlegeli cuvieri are endemic to Java; and Lepidodactylus lugubris, Cryptoblepharbus baliensis and Cryptoblepharbus cursor are new record to Java. Unidentified and possibly undescribed species of unusual specimen referred to genus Cyrtodactylus and still being examined.

Lepidodactylus lugubris was found in disturbed terrestrial habitat in South Slope of Gunung Merapi National Park (see figure 1). It was captured by Nur Istiqomah in a small three near the residence. The specimen has SVL 3,21. A species of Lepidodactylus lugubris characterized by the digits dilated, free or with a rudiment of web; below with transverse lamellae, divided by a median groove, the distal joint short and inner digit claws.

Cyrtodactylus sp. was found in disturbed terrestrial habitat in Condongcatur (see figure 1). It was first captured by Tony F. Qumiawan. The specimen has SVL 3,12-5,05. The key character this specimen from another is a tailed form, chin shield and ventral scale. A species of Cyrtodactylus sp. have a unique defence behaviour. It will do unknown reflect as defence from predators.

Lepidocheles olivacea, Eretmocheles imbricate and Chelonia mydas are listed in Appendix 1 and Red List as endangered specied (Iskandar, 2000). They has a few nesting area in this world (Lutz and Musick, 1997). Samas and Trisik Beach at Yogyakarta is one of nesting area of them. But nowadays very difficult to see Lepidocheles olivacea, Eretmocheles imbricate and Chelonia mydas at Samas and Trisik Beach. Because sea turtle hunting and mass murdering are general in our neighborhood and their ecosystem its selves.

Based on species presence/absence (see table 1), the Sleman showed the highest species richness (51) and followed by Kulonprogo (48 species), Bantul (45 species), Gunungkidul (31 species) and Yogyakarta capital (26 species). This result indicated that Sleman, Kulonprogo and Bantul regency have a good condition and still have a lot of natural habitat for reptile’s live than in Yogyakarta and Gunungkidul regency.
Table 1. Reptiles species list recorded at five major surveys site in Daerah Istimewa Yogyakarta Province

<table>
<thead>
<tr>
<th>No</th>
<th>Species</th>
<th>Java</th>
<th>Daerah istimewa Yogyakarta Province</th>
</tr>
</thead>
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<tr>
<td></td>
<td></td>
<td>Kulonprogo</td>
<td>Sleman</td>
</tr>
<tr>
<td>1</td>
<td>Lepidodactylus lugubris</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Cyrtodactylus marmoratus</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Cyrtodactylus sp</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>Gehyra mutilata</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Hemydactylus frenatus</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Hemyphylodactylus typus</td>
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<td>-</td>
</tr>
<tr>
<td>7</td>
<td>Cosymbotus platyurus</td>
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<td>+</td>
</tr>
<tr>
<td>8</td>
<td>Ptycozoon kuhli</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>Gekko gecko</td>
<td>+</td>
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<tr>
<td>10</td>
<td>Draco volans volans</td>
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<tr>
<td>11</td>
<td>Bronchocela cristatella</td>
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<td>Bronchocela jubata</td>
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<td>Gonocephalus chamaeleontinus</td>
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<td>14</td>
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<td>Takydromus sexlineatus</td>
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<td>Eutropis multifasciata</td>
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<td>Eutropis rugifera</td>
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<td>19</td>
<td>Sphenomorphus puncticentralis</td>
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<td>Sphenomorphus sanctus</td>
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<td>21</td>
<td>Lygosoma bowringii</td>
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<td>Lygosoma quadrupes</td>
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<td>Cryptoblepharus cursor</td>
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<td>Cryptoblepharus baliensis</td>
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<td>Varanus salvator</td>
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<td>Dendrelaphis pictus</td>
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<td>Xenochrophis melanzotus</td>
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<td>Xenochrophis vittatus</td>
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<td>Pareas carinatus</td>
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<td>Oligodon bitorquatus</td>
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<td>Bungarus candidus</td>
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<td>61</td>
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<td>Chelonia mydas</td>
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<tr>
<td>63</td>
<td>Eremochelys imbricata</td>
<td>+</td>
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</tr>
<tr>
<td>64</td>
<td>Lepidochelys olivacea</td>
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</table>

**Figures**

Figure 1. Picture Cyrtodactylus sp. (left) and Lepidodactylus lugubris (right) specimen

**REFERENCES**


P-SE13

SPECIES DIVERSITY OF EUPHORBIACEAE IN GUNUNG TUKUNG GEDE NATURE RESERVE, SERANG-BANTEN AND NEW RECORDS FOR JAVA: A PRELIMINARY STUDY

Tutie Djarwaningsih
“Herbarium Bogoriense”, Botany Division, Research Centre for Biology Indonesian Institute of Sciences, Cibinong Science Center

Gunung Tukung Gede Nature Reserve (GTGNR) is located in the Serang District, Province of Banten, and geographically is situated between 6° 14’ – 6° 20’ S and 105° 52’ – 105° 57’ E. In the past Banten was part of West Java province, therefore the GTGNR is still recognized as representative of biodiversity in West Java. The information on the species diversity of Euphorbiaceae in GTGNR has not been reported yet. According to Backer & Bakhuizen van den Brink (1968), totally 60 genera of Euphorbiaceae occur in Java, of which 58 genera were found in West and Central Java, and 10 genera were only found in West Java. The exploration methods followed van Balgooy (1987), used in the permanent plots in the Cadas Gantung, Cadas Pagar and its surrounding area (western part of West GTGNR). Morphological characters of materials were examined based on new collections and specimens which deposit in Herbarium Bogoriense – LIPI. The aim of this study is to explore the species diversity of Euphorbiaceae, especially the economically potential species. This will be the basic understanding for further research on physiology, phytochemistry, phytofarmacology, agronomy etc. Preliminary result of the investigation of Euphorbiaceae in GTGNR has shown that 21 genera occur in GTGNR, and it represents approximately 33,33 % of Euphorbiaceae genera in Java. The number of Euphorbiaceae species in GTGNR are 32, and it is more than 50 % of Euphorbiaceae in West Java. The common species encountered during the field work are Mallotus spp., Antidesma spp. and Bactaera spp. Alchornea rugosa, Aporosa lucida, Botryophora geniculata, Mallotus mollissimus, M. moluccanus and Wetria insignis are new records for the Flora of Java. Key to the genera, habitat and distribution of all species in throughout Java are presented in the paper.

Keywords: Euphorbiaceae, Gunung Tukung Gede Nature Reserve (GTGNR), species diversity, new record.

J. INTRODUCTION

Gunung Tukung Gede Nature Reserve (GTGNR) is located in the Serang District, Province of Banten, and geographically is situated between 6° 14’ – 6° 20’ S and 105° 52’ – 105° 57’ E. In the past, Banten was part of West Java province, therefore the GTGNR is still recognized as representative of biodiversity in West Java. The condition of forest in GTGNR divided to natural forestry, planted forest, and area for cultivation.
According to Backer & Bakhuizen van den Brink (1968), totally 60 genera of 
*Euphorbiaceae* occur in Java, of which 58 genera were found in West and Central Java, and 
10 genera were only found in West Java. The information on the species diversity of 
*Euphorbiaceae* in GTGNR has not been reported yet. The aim of this study is to explore the 
species diversity of *Euphorbiaceae*, especially the economically potential species. This will 
be the basic understanding for further research on physiology, phytochemistry, 
phytofarmacology, agronomy etc.

**MATERIALS AND METHODS**

The exploration methods followed van Balgooy (1987), used in the permanent plots 
in the Cadas Gantung (at 160 m dpl. and 250 m dpl.) is situated between 06° 09,437' LS and 
105 ° 57,085' BT, Cadas Pagar (at 130 m dpl. and 190 m dpl.) between 06° 09,529' LS and 
105 ° 56,830' BT, and its surrounding area. Morphological characters of materials were 
examined based on new collections, and specimens which deposit in “Herbarium 
Bogoriense”, Cibinong Science Center – LIPI. The nomen clature of plant based on naming 
system of Flora of Java (Backer & Bakhuizen van den Brink 1968), Flora Malesiana and 
journals which content of revision of *Euphorbiaceae* in the Malesian region.

**RESULTS AND DISCUSSION**

Java has 60 genera of *Euphorbiaceae* and comprises 210 species. The number of 
*Euphorbiaceae* species in West Java are 59 (Backer & Bakhuizen van den Brink, 1968). 
Preliminary result of the investigation of *Euphorbiaceae* in GTGNR has shown that 21 
genera occur in GTGNR, and it represents approximately 33,33 % of *Euphorbiaceae* genera 
in Java. The number of *Euphorbiaceae* species in GTGNR are 32, and it is more than 50 % 
of *Euphorbiaceae* in West Java. Distribution of *Euphorbiaceae* species in throughout Java 
and in West GTGNR, and new record for Java presented in table below (Table 1).

Table 1. Distribution of *Euphorbiaceae* species in throughout Java and in West GTGNR, and new record for Java 
(*, **, ***,***, ****, *****)

<table>
<thead>
<tr>
<th>No.</th>
<th>Species</th>
<th>Distribution in throughout Java and in GTGNR</th>
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</thead>
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<tr>
<td>1.</td>
<td>Alchornea rugosa Muell. Arg.*</td>
<td>In GTGNR at about 120 m alt.</td>
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<td>2.</td>
<td>Antidesma bunius (L.) Spreng.</td>
<td>Distribution in W.C.E.-Java, Madura at 5 – 1300 m. In GTGNR at 80 m alt.</td>
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<td>3.</td>
<td>Antidesma montanum Blume</td>
<td>Distribution in W.C.E.-Java at 1 – 200 m. In GTGNR at 103 – 131 m alt.</td>
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<td>4.</td>
<td>Antidesma tetrandrum Blume</td>
<td>Distribution in W.C.E.-Java at 100 – 2400 m. In GTGNR at 80 m alt.</td>
</tr>
<tr>
<td>No.</td>
<td>Species</td>
<td>Distribution Note</td>
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</tr>
<tr>
<td>5.</td>
<td>Aporosa frutescens Blume</td>
<td>Distribution in W Java at 5 – 800 m. In GTGNR at 148 m alt.</td>
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<td>6.</td>
<td>Aporosa lucida (Miq.) Airy Shaw **</td>
<td>In GTGNR at 250 m alt.</td>
</tr>
<tr>
<td>7.</td>
<td>Baccaurea javanica (Bl.) M.A.</td>
<td>Distribution in W.C.E.-Java at 0.25 – 1000 m. In GTGNR at 110 – 172 m alt.</td>
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<td>8.</td>
<td>Baccaurea racemosa (Reinw. ex Bl.) M.A.</td>
<td>Distribution in W.C.E.-Java at 1 – 1000 m. In GTGNR at 113 m alt.</td>
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<td>9.</td>
<td>Bischofia javanica Bl.</td>
<td>Distribution in W.C.E.-Java at 1 – 1500 m. In GTGNR at 110 m alt.</td>
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<td>10.</td>
<td>Botryophora geniculata (Miq.) Beumee ex Airy Shaw ** **</td>
<td>In GTGNR at 190 m alt.</td>
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<td>13.</td>
<td>Claoxylon polot (Bur. f.) Merr.</td>
<td>Distribution in W.C.E.-Java, Madura at 0.5 – 2500 m. In GTGNR at 70 m alt.</td>
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<td>14.</td>
<td>Codiaeum variegatum (L.) Bl.</td>
<td>Distribution in W.C.E.-Java, Madura at 5 – 1500 m. In GTGNR at 70 m alt.</td>
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<td>15.</td>
<td>Drypetes longifolia (Bl.) Pax &amp; Hoffm.</td>
<td>Distribution in W.C.E.-Java at 150 – 1500 m. In GTGNR at 210 m alt.</td>
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<td>16.</td>
<td>Drypetes minahassae (Boerl. &amp; Kds.) Pax &amp; Hoffm.</td>
<td>Distribution in W Java at 1200 – 1500 m. In GTGNR at 250 m alt.</td>
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<td>17.</td>
<td>Euphorbia hirta L. = E. pilulifera L.</td>
<td>Distribution in W.C.E.-Java, Madura at 1 – 1400 m. In GTGNR at 70 m alt.</td>
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<td>Euphorbia plumerioides Teysm. ex Hassk.</td>
<td>Distribution in C.E.-Java. In GTGNR at 70 m alt.</td>
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<td>Galearia filiformis (Bl.) Pax</td>
<td>Distribution in W.E.-Java at 10 – 700 m. In GTGNR at 80 m alt.</td>
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<td>Jatropha curcas L.</td>
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<td>Jatropha multifida L.</td>
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<td>Mallotus blumenus M.A.</td>
<td>Distribution in W Java at 50 – 1000 m. In GTGNR at 101 – 120 m alt.</td>
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<td>24.</td>
<td>Mallotus mollissimus (Geiseler) Airy Shaw ** **</td>
<td>In GTGNR at 15 – 117 m alt.</td>
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<td>27.</td>
<td>Mallotus peiltatus (Geisel) M.A.</td>
<td>Distribution in W Java at 50 – 900 m. In GTGNR at 130 – 172 m alt.</td>
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28. Manihot esculenta Crantz
   Distribution in W.C.E.-Java, Madura at 5 – 1100 m. In GTGNR at 15 m alt.

29. Omalanthus populneus (Geisel) Pax
   Distribution in W.C.E.-Java at 10 – 1800 m. In GTGNR at 120 m alt.

30. Pedilanthus tithymaloides (L.) Polt.
   Distribution in W.C.E.-Java, Madura at 1 – 700 m, especially below 50 m. In GTGNR at 15 m alt.

31. Sumbaviopsis albicans (Bl.) J.J.S.
   Distribution in W.C.E.-Java at 50 – 200 m. In GTGNR at 110 – 160 m alt.

32. Wertia insignis (Steud.) Airy Shaw
   In GTGNR at 20 – 160 m alt.

Note:  * Supported by Airy Shaw (1975, 1980) ***** Supported by Sierra & Welzen (2005)
  *** Supported by Webster (1994)

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Lasiococcineae (Homonoia, Lasiococca, Spathiostemon) and Clonostyli, Ricinus, and
P-SE14

Morphological characteristics of *Frankia* isolated from the Root Nodules of *Casuarina equisetifolia* var incana.

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*Casuarina equisetifolia* var incana, called as Cemara Udang is one of pioneer tree in coastal area. These tree associate with *Frankia* that can fixed nitrogen from the air. The seedlings can be raised only in nursery. The aim of this research is to obtain the superior isolate as inoculum for the seedlings.

Root nodules of *Casuarina equisetifolia* var incana, were collected from the coastal areas of Lombang, Tepus and Samas. *Frankia* were isolated from 21 nodule samples from 7 different trees as replicates. The observations was conducted every weeks during 2 months. Several *Frankia* isolate were tested for their growth ability on a medium of soil extract. These were classified into fast and slow growing isolate. The result showed that most of all isolate produced different colour. Some of *Frankia* isolates produced spores. *Frankia* isolate from Lombang Madura perform the best one.

**Key words:** *Frankia*, Root Nodule, *Casuarina equisetifolia* var incana.

**INTRODUCTION**

One of favorite species for coastal cultivation is Casuarinas spp., especially *C. equisetifolia* because it endures even at pH levels of 9.5. Casuarina are very versatile, endure with drought, salinity, waterlogging, and wind velocity. It can be established in marginal lands, sandy lands, inland soils, coastal dunes and in lands where no other tree species can survive (Rao and Dommergues, 1998).

The seedlings of Causarina can be raised only in nursery and needs maintenances until its high achieve at least 40 cm. There was a problem for preparing high quality seedlings which its root contains of nitrogen fixing bacteria in a short time and low cost.

The nitrogen fixing bacteria in Casuarina called as *Frankia*. It is a kind of Actinomycetes fungi which has special characteristics. In these research, the characteristics of *Frankia* isolated from the root nodules of Casuarina were investigated, included common characteritics such as growth rate, spores formation, colonial morphologies, and common characteristics of hyphae to get the best *Frankia* isolate.
OBJECTIVES

The objectives of these research were (1) to obtain Frankia isolate from any locations, (2) to investigate isolate characteristics on soil extract media, and (3) to obtain the potential inoculum for Frankia isolate.

METHODS

The nodules was collected from 7 randomly trees from coastal areas of Lombang Madura, Tepus Gunung Kidul and Samas Bantul. Immediately after collection, each nodule sample isolated by streaking onto soil extract agar medium, the isolation method refers to bacteria root nodules isolation (Somasegaran and Hoben, 1985). The surface of root nodules was sterilized by H$_2$O$_2$ 5% for 5 minutes, and then it was washed by aquadest repeatedly. The sterilized root nodules streaked onto soil extract agar (Glukosa 10 g, K$_2$HPO$_4$ 0,5 g, stock solution soil extract 100 ml, Tap water 900 ml, agar 16 g) surface. Stock solution soil extract were made from 1000 g soils, 1000 ml tap water and CaCO$_3$, it autoclaved for 30 minutes and filtered by the double filter. Inoculated agar plates were incubated at room temperature, and each colony transferred into the same media until it achieved in a pure culture. Frankia isolate were growth on solid and liquid extract media. The observation was conducted for growth rate, spores formation, colonial morphologies, and common characteristics of hyphae every weeks during 2 months. The cultural characteristics of Frankia pertain to its macroscopic appearance on different kinds of media. The identification of molds is based on surface color, backside color, hyphae structure, and types of spores (Brown, 2005). The observations were carried out in the laboratory of Microbiology Faculty of Forestry, Gadjah Mada University.

RESULTS AND DISCUSSION

The root nodules were gained from 20 years old Casuarina from Lombang-Madura, 10 years old Casuarina from Samas-Bantul, and 5 years old Casuarina from Tepus-Gunung Kidul. Almost all of Frankia was isolated from root nodule can growth in soil extract media. Approximate 10% of nodules were contaminated, 1 nodule from Tepus and 1 nodule from Samas. Isolation process gained 16 pure culture, gram-positive. There were 11 isolates from Madura, 4 isolates from Tepus, and 1 isolate from Samas. On the liquid media, 3 Frankia isolates from Madura had slow growth rate (more than 7 days incubation), whereas the 8 other isolates had fast growth rate (less than 7 days incubation). Frankia isolate from Samas and 2 isolates from Tepus and Madura had a slow growth rate on solid media. Isolate which
grow in solid media had penetrating hyphae on the substrates. Almost isolate produced branching aerial hyphae with spores, and colored colony.

The isolation of *Frankia* from Casuarina nodules is well establish that the growth of *Frankia* is very slow (generation time: 2-5 days), creating a high risk of overgrowth by contaminants which always grow faster than *Frankia* during the isolation procedure (Diem and Dommergues 1983). In most strains of Actinomycetes the surface of the colony is covered with dry, powdery aerial mycelium giving them a distinctive appearance. The vegetative and aerial mycelium of some Actinomycetes possess distinctive colors (Anonim, 1997).

**CONCLUSIONS**

Isolates were classified into fast and slow growing. The result showed that almost all produced different color. Almost *Frankia* isolates produced spores, hyphae and gel. *Frankia* isolate from Lombang Madura perform the best one.

**REFERENCES**


### Topic 4: Physiology and Developmental Biology

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SURVIVABILITY OF COMMERCIAL PROBIOTICS IN PORCINE GASTRIC JUICE

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Abstract
Probiotic bacteria normally have to be administered orally. It is known that stomach has
extreme low acidity. Therefore, we examined in vitro the survivability of commercial probiotic strain
(Lactobacillus casei) using porcine gastric juice. Treated bacteria were incubated in gastric juice with 4
different pH adjustments at pH 1,2,3,4 at 37° C for 90 minutes. Collected samples (100µl) from
different time point (0, 10, 15, 30, 90 minutes) were grown in MRS agar media at 37° C for 48 hours
under anaerobic condition. As a result is at pH 1 and 2 no colony was observed, while at pH 3 and 4
only few colonies were obtained during the 90 minutes of incubation time. Conclusion: The survivability of L.casei is above pH 3. The authenticity of species was confirmed by PCR colony
method, using specific primers which amplify 540 bp tuf gene of L. casei.

Key Words: Gastric juice, probiotic, survivability

INTRODUCTION
Probiotics are live microorganisms which when administered in adequate amounts
confer a health benefit to the host. Bacteria of probiotics are included in lactic acid bacteria
group for example Lactobacillus spp. and Bifidobacterium spp. Probiotic microorganisms can
give benefits to host if they can survive in intestines, then colonize, and multiply ¹ ². Probiotic
microorganisms which live in human digestive system pass through the oral cavity go down
through the throat, stomach, and reach the intestines. In general, the condition within mouth
cavity and throat does not give any meaningful resistance for their survivability. Stomach has
an acid condition between pH 1 to 4, and here probiotic microorganisms begin to be
threatened to survive. Although probiotic microorganisms have been consumed as food for
centuries, the information concerning their survivability through the digestive system in
stomach is limited. One of the reasons is that the possibility of examination by using human’s
gastric juice is difficult to be conducted. Therefore, in this regard animal such as porcine can
be used and also suitable as an animal model because of its similarity with human’s
digestive system and gastric juice composition³. Thus, in this study, we evaluate the survival
rate of commercial probiotics in vitro using porcine gastric juice.
MATERIALS AND METHODS

Sample of probiotic microorganism which is used in this study is commercial *Lactobacillus casei*. For gastric juice preparation: 30 ml of gastric juice was collected by using syringe from the stomach of slaughtered swine. The acidity of gastric juice was adjusted to pH 1, 2, 3 and 4 by using 2M HCl solution. Gastric juice was then sterilized by passing through a 0.45 μm cellulose acetate membrane filter. Probiotic was then added to porcine gastric juice by a ratio of 1:50. Examination of probiotic survival rate was done up to 90 minutes. During the incubation at 37°C, 100 μl samples were collected at time 10 second (0 minute) 10, 15, 30, and 90 minute. Collected samples then immediately mixed with media of MRS agar at 50°C. Plated agar media were incubated in a chamber under anaerobic condition at 37°C for 48 hours. Colony was counted using total plate count method. Colonies of *Lactobacillus casei* which have grown on MRS media were examined with technique of colony PCR with forward primer, RHA (5’-CGTACAGTTGTTGTTG-3’) and reverse primer, CPR (5’-CAATGGATNGAACCTGGCTT-3’). PCR products were separated in 1% agarose gel, and then, agarose gel was stained with ethidium bromide to visualize the bands under UV light.
RESULTS AND DISCUSSION

*Lactobacillus casei* which has been treated in porcine gastric juices with a selected pH was grown in MRS agar media with pour plate method. Each bacterium which survives will grow and be able to form one colony (CFU) in MRS agar media. As the results (Fig. 1), the bacteria which were treated with porcine gastric juices at pH 1 and 2 can not survive because there is no colony of bacteria at pH 1 and 2 in 10 minutes of incubation times. While at pH 3 and 4 bacteria survive upto 90 minutes of incubation. Declining total CFU at pH 3 can be depicted with a equation of line \( y = 5E+06e^{-0.156x} \) and \( R = 0.9586 \). While declining total CFU at pH 4 can be depicted with a equation of line \( y = 3E+06e^{-0.0464x} \) and \( R = 0.9971 \). It seems pH 3 is a critical point for the survival of *Lactobacillus casei* as we do not know whether other substances in porcine gastric juice would also affect its survival, than if it was a human gastric juice. Generally, bacteria have a specific range of pH to live. *Lactobacillus casei* have an optimum of pH 5.5. The exceedingly low acidity will disturb metabolism process and bacteria’s physiology. As shown in Fig. 1, extreme low pH affects negatively on growth and will kill bacteria.

To verify that bacterium which was grown in MRS agar media was *Lactobacillus casei*, technique of colony PCR was applied to amplify a fragment of the *tuf* gene. The *tuf* gene has been used in identifying *Lactobacillus* in phylogenetic analyses. As shown in Fig. 2, specific primers of *tuf* gene could amplify *Lactobacillus casei* specifically and produced a size of 540 kb, but not other *Lactobacillus* sp. Thus, the grown colonies were confirmed as
Lactobacillus casei. Results of this study suggest that porcine gastric juice could be used to evaluate survivability of probiotics. *Lactobacillus casei* has a better survivability at pH >3.

REFERENCES
P-PD02

REDUCTION OF THE TOXIC COMPOUND AZIDE BY [Cu-S-Mo]- CARBON MONOXIDE DEHYDROGENASE AND SUPEROXIDE DEPENDENT NITROGENASE

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Abstract
Carbon monoxide dehydrogenase (CODH) is the key enzyme in the aerobic and anaerobic utilization of CO as the sole source of carbon and energy for a variety of carboxidotrophic bacteria. CODH catalyzes the oxidation of CO with H$_2$O yielding CO$_2$, two electrons and two H$^+$. The crystal structure of CODH from two bacteria Oligotropha carboxidovorans (an aerobic bacterium) and Carboxydothermus hydrogenoformans (an anaerobic bacterium) have been solved recently at atomic resolution. CODH is a dimer of LMS heterotrimers. Each heterotrimer is composed of a molybdoprotein (L, the large subunit), a flavoprotein (M, the medium-sized subunit) and an iron-sulfur protein (S, the small unit). CODH is a copper containing molybdenum-iron-sulfur (Cu-S-Mo) flavoprotein. In the present study we report that the CODHs of Streptomyces thermoautotrophicus and O. carboxidovorans are able to reduce the toxic compound azide (N$_3^-$) to ammonium (NH$_4^+$). In addition, we also demonstrate that S. thermoautotrophicus harbors the enzyme nitrogenase which also reduces azide to ammonium. Azide serves as a new substrate for CODH’s of S. thermoautotrophicus and O. carboxidovorans, as well being a new substrate for the nitrogenase of S. thermoautotrophicus.

Keywords: Carbon Monoxide Dehydrogenase, Azide degradation, alternative substrate

INTRODUCTION
Carbon monoxide dehydrogenase (CO dehydrogenase) is the key enzyme in the aerobic utilization of CO as a sole source of carbon and energy by carboxidotrophic bacteria [1-2]. CO dehydrogenase catalyzes the oxidation of CO [CO + H$_2$O $\rightarrow$ CO$_2$ + 2 H$^+$ + 2 e$^-$] or or H$_2$ [H$_2$ $\rightarrow$ 2 H$^+$ + 2 e$^-$]. Crystal structures have been reported of the CO dehydrogenases Oligotropha carboxidovorans and Hydrogenophaga pseudoflava [6]. CO dehydrogenase is a dimer of LMS heterotrimers. Each heterotrimer is composed of a molybdoprotein (L, the large subunit) which accommodates the active site, a flavoprotein (M, the medium-sized subunit), which contains FAD, and an iron-sulfur protein (S, the small subunit), which carries two types of [2Fe-2S]-clusters [2, 5].

The active site of CO dehydrogenase contains a [CuSMoO$_2$]-cluster, which is coordinated by the enedithiolate of the molybdopterin-cytosine dinucleotide (MCD) cofactor and the $S_\gamma$ of Cys$^{388}$ [5]. S. thermoautotrophicus is a thermophilic bacterium, however the
properties of its CO dehydrogenase show remarkable similarity to the CO dehydrogenase from O. carboxidovorans.

In the course of studies to exploit compounds interacting with the [CuSMoO2]-cluster in CO dehydrogenase from different bacterial origin it was realized that sodium azide is getting reduced and produce ammonia (NH₃) and dinitrogen (N₂). The reaction of azide at the metal cluster was studied employing the CO dehydrogenase from the mesophilic gram-negative α-Proteobacterium O. carboxidovorans [1] and the thermophilic gram-positive S. thermoautotrophicus [3].

Azide is known as an inhibitor of various metalloenzymes such as SOD, oxidases and anaerobic CO dehydrogenase. Structure of Fe(III) and Mn(III)-SOD azide complexes reveal azide bound as a sixth ligand with distorted octahedral geometry at the metal [5].

MATERIALS AND METHODS

O. carboxidovorans and S. thermoautotrophicus were cultivated in a fermentor of 70 l total volume supplied with a mineral medium [1] under the chemolithoautotrophic conditions detailed before [2]. CO dehydrogenases were isolated and purified following published procedures [2]. CO oxidation activity was assayed photometrically with 1-phenyl-2-(4-iodophenyl)-3-(4-nitrophenyl)-2 H-tetrazolium chloride (INT)/1- methoxyphenazine methosulfate (MPMS) as artificial electron acceptor [1]. One unit is defined as 1 µmol of CO oxidized min⁻¹ mg⁻¹ protein at 30° C (O. carboxidovorans) or 50° C (S. thermoautotrophicus).

Azide reduction by CO dehydrogenase was assayed at 60° C in serum-stoppered wheaton vials (9 ml total volume) containing 1 ml of 50 mM KH₂PO₄/NaOH-buffer (pH 6.5) supplied with 2-10 mM sodium dithionite (Na₂S₂O₄), 0.8 to 1.5 mg of CO dehydrogenase and 10 mM NaN₃. The gas atmosphere in the vials was pure helium.

The ammonium formed was analyzed photometrically with the indophenol test [3]. N₂ was analyzed employing a gas chromatograph (model CP 9000; Chrompack, middelburg, the Nederland) equipped with a thermal conductivity detector (model 903 A), a Hayeseq Q column (2.5 m) and a molecular sieve 13 x column (1.8 m). For the assay of azide, protein was removed from samples by ultrafiltration (30 kDa cutoff; Microsep, Pall Co.), and spectrophotometrical analyses were performed on the small molecular mass fraction by reacting azide to ferric azide [4]. For cyanide inactivation the CO dehydrogenase(2.2 mg.ml⁻¹ in KH₂PO₄/NaOH buffer, 50 mM; pH 7.2) was incubated with 10 mM potassium cyanide under anoxic conditions [2].

RESULTS AND DISCUSSION
Under appropriate assay conditions, purified CODHs from *S. thermoautotrophicus* and *O. carboxidivorans* were able to reduce N\(_3^-\) to NH\(_4^+\). For the reduction of N\(_3^-\) to NH\(_4^+\), dithionite was an essential requirement. In controls, where dithionite or CODH were omitted, N3 reduction did not occur. It has been reported that azide can be reduced to ammonium according to the following three equations [4]:

1. \(N_3^- + 3 H^+ + 2 e^- \rightarrow N_2 + NH_3\)
2. \(N_3^- + 7 H^+ + 6 e^- \rightarrow N_2H_4 + NH_3\)
3. \(N_3^- + 9 H^+ + 8 e^- \rightarrow 3 NH_3\)

Using a gas chromatograph equipped with a thermal conductive detector this gas was identified as N\(_2\). We were able to show that N\(_3^-\) had been reduced by the CODHs of *S. thermoautotrophicus* and *O. carboxidivorans* in a stoichiometry (Fig. 1) of 1 mol of N\(_3^-\) : 1 mol NH\(_4^+\) : 1 mol of N\(_2\). This result is consistent with equation 1 and indicates that for this reaction 2e\(^-\) were required.

![Graph](image)

Fig. 1: Stoichiometric reduction of azide catalyzed by CODH of *S. thermoautotrophicus*. Reaction mixtures contained 4 ml of KH\(_2\)PO\(_4\)/NaOH buffer (50 mM, pH 7.5), 5 mM dithionite and 1.2 mg CODH, 4 µ mol or 8 µ mol N\(_3^-\). Assays were performed under a pure He atmosphere at 60°C. Symbols: ○ N\(_2\), ■ NH\(_4^+\), ■ CODH + 8 µ mol N\(_3^-\) + dithionite, ● CODH + 4 µ mol N\(_3^-\) + dithionite, □ N\(_3^-\) + dithionite, ○ control.

It is known that in the presence of cyanide, all cyanoxylyzable sulfur can be entirely removed from CODH leading to an inactivation of the enzyme. Fig. 2 demonstrates that cyanide treatment not only leads to the complete inactivation of CO oxidation, but also N\(_3^-\) reduction activity was substantially (86%) decreased. Fig. 2 also shows that the amount of removal of cyanoxylyzable sulfur as SCN\(^-\) from CODH was inversely proportional to the above mentioned activities. This result suggests that for both activities, cyanoxylyzable sulfur plays an important role.
Fig. 2: Inactivation of CODH by cyanide and release of thiocyanate. CODH (2.2 mg.ml-1) was incubated with 10 mM potassium cyanide under anoxic conditions. The time course of specific CODH activity and NH₄⁺ forming activity were measured in aliquots after removal of reactant by gel filtration on Sephadex G-25. Thiocyanate was analyzed as Fe-thiocyanate [Fe(SCN)₃].

The inset shows the visible spectrum of the iron-thiocyanate complex of a sample CODH of *S. thermoautotrophicus* and *O. carboxidivorans* which normally act as oxidative enzymes, have the capability to reduce azide according to the following equation:

\[ N_3^- + 3 H^+ + 2e^- \rightarrow \text{NH}_3 + \text{H}_2\text{O} \]

By destroying the Cu-S-Mo center the oxidative and reducing activities of CODH from both bacteria used in this work were affected. This suggests that CO and N₃⁻ bind at the same catalytic site after 40 h of incubation of the enzyme with cyanide. Symbols: • CODH activity, ▲ NH₄⁺ forming activity, ▲ thiocyanate.

ACKNOWLEDGMENT

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[CuSMo(=O)OH)] cluster in CO dehydrogenase resolved at 1.1 Å resolution. *Proc. Natl. Acad. Sci.* 99:15971-15976
P-PD03

THE EFFECTS OF A-NAPHTALENE ACETIC ACID ON THE SUCCESSFUL POLLINATION AND FRUIT RIPENING OF Phalaenopsis Orchids

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Abstract
The effects of a-naphtalene acetic acid (NAA) in promoting successful pollination and fruit ripening of Phalaenopsis orchids have been evaluated. The research was carried out by applying lanolin paste containing NAA on the base of flower stalk of self pollinated Ph.delestris, self pollinated Ph.amabilis, cross pollinated of Ph.delestris X Ph.amabilis and cross pollinated of Ph.amabilis X Ph.delestris. Three levels of NAA were used, namely 0 ppm (control), 200 ppm and 500 ppm. The lanolin paste containing NAA was applied after flowers were fully opened and 3 flowers were used for each self and cross pollination of Phalaenopsis.

The result revealed that NAA of 500 ppm gave a successful pollination of Ph.delestris to 100%, however for self pollinated Ph.amabilis, application of NAA up to 500 ppm did not show any significant effects to the successful pollination. The successful pollination of cross pollinated of Ph.delestris X Ph.amabilis was obtained without any application of NAA. On the contrary, successful pollination of Ph.amabilis X Ph.delestris can not be obtained even NAA 500 ppm has been applied. The ripening times for cross pollinated Ph.delestrisX Ph.amabilis fruits was from 77 to 84 dap (days after pollination), in comparison to self pollinated Ph.delestris (49 to 56 dap) and self pollinated Ph.amabilis (up to 120 dap)

Keywords: a-Naphtalene Acetic Acid, pollination, fruit ripening, Phalaenopsis orchid

INTRODUCTION
Indonesia is home of huge number of natural orchids that terms as megabiodiversity, for more than 5000 species (Irawati, 2002). The orchid flowers exhibit wide range of variation in shape, color, and fragrant compare to the other flowering plants. Therefore, orchids is a valuable product in florist-trade as cut flowers, pot plants, or as food additives. Hybridization is very important to increase the orchid genetic variation and to create higher quality of the hybrids than that of the parentals.

Pollination initiates maturation of the ovary and the differentiation of ovules prior to fertilization. Auxin has important role to promote the ovary development after pollination and
to stimulate the ethylene production. Ethylene promotes fruit ripening and flower senescence, it also regulates the early development of ovary. The α-naphtalene acetic acid (NAA) is the most effective auxins to induce the development of ovary into mature fruit (Zhang and O’Neill, 1993).

In this study, the effects of α-naphtalene acetic acid (NAA) in promoting successful pollination and fruit ripening of Phalaenopsis and Doritaenopsis orchids has been evaluated.

MATERIALS AND METHODS

Plant Materials

Orchids plants of the genus Doritaenopsis were obtained from Royal Orchid, East Java while Phalaenopsis amabilis were obtained from local people at Curug Sewu, Sukorejo, Central Java. The orchid plants were maintained under optimal growth condition in a greenhouse at Faculty Biology, Universitas Gadjah Mada, Yogyakarta. Three flowers were used for each self and cross pollination of Doritaenopsis and Phalaenopsis.

Pollination

The fully opened flowers of P. amabilis and Doritaenopsis were self- and cross-pollinated to produce fruits and seeds. Before pollination, the flower stalk were treated by lanolin paste containing three levels of NAA (Sigma) i.e. 0 ppm, 200 ppm and 500 ppm.

Ovary Growth Measurements

After pollinated, flowers increased in size of ovary. At each seven days, the diameter of ovaries of self- and cross-pollinated orchid were measured at the middle of the ovary (Fig. 1a) by using calipers (Zhang and O’neill, 1993). The succesful pollination was determined by comparing the numbers of pollinated flowers that produce fruits and the numbers of pollinated flowers.

RESULTS AND DISCUSSION

The result shows that 500 ppm of NAA gave a succesful pollination of Doritaenopsis to 100%, however for self pollinated P.amabilis, application of NAA up to 500 ppm does not show any significant effects to the successful pollination. The successful pollination of cross pollinated of Doritaenopsis X P. amabilis was obtained without any application of NAA. On the contrary, succesful pollination of P. amabilis X Doritaenopsis can not be obtained even 500 ppm of NAA has been applied. Figure 1b-1d ilustrates morphological changes following pollination as indicated by the increase of fruit diameter. The morphological changes based on fruit diameter among the pollinated orchids are occuring in different time and reflecting the fruit ripening in orchid. The ripening times for cross pollinated Doritaenopsis X P. amabilis fruits was from 77 to 84 dap (days after pollination), in comparison to self pollinated
Doritaenopsis (49 to 56 dap) and self pollinated P.amabilis (up to 120 dap, data not shown in Fig.1b).

![Image of orchids and graphs]

Figure 1. The increase of orchids fruit diameter following pollination. The morphology of Doritaenopsis (upper) and P. amabilis (bottom) and fruit diameter measurement, d ; the increase of orchid fruit diameter in P. amabilis (b), Doritaenopsis (c), and Doritaenopsis X P. amabilis (d).

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FUNDING

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P-PD04

THE OVIPOSITION RESPONS OF *Aedes aegypti* (DIPTERA; CULICIDE) IN OVITRAP WITH LARVACIDES OF TEMEPHOS AND ATTRACTANT OF HAY INFUSION

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Abstract

The research has been held in the aim to know the oviposition responses of *Aedes aegypti* (Diptera : Culicide) in ovitrap with larvicide’s of Temephos and Paspalum grass infusion. The procedure of this research is to give the female gravid with the several of substance which contain the attractant (Paspalum grass infusion 10% and 20%), the larvicide’s (Temephos) and combination of both. The result shows that the oviposition percentage in the ovitrap which contain single larvicide’s almost same with control. The opposite result is in the combination between Paspalum grass infusion and larvicide’s which the percentage of oviposition is rise up to 80%. In those ovitrap we could collects until 15.2 to 19.2 eggs per ovitrap. From this data we can suggest that the combination between attractant and larvicide’s will rise the percentage of oviposition from gravid female. This information is very valuable for controlling the population of *Ae. aegypti* by using of lethal ovitrap.

Keywords : oviposition responds, larvicide’s, attractant, *Aedes aegypti*

Introduction

Dengue hemorrhagic fever (DHF) is one of the most serious public health problems in Indonesia as well as in many other tropical countries around the world. Dengue virus is transmitted by the mosquito *Ae. aegypti* adapted to living near areas of human habitation (Dario et al., 2008). These mosquito species coexist in man-made containers in urban, suburban and rural settlements in tropical and subtropical regions (Adson et al., 2005). In recent years, ovitrap surveys for monitoring the *Ae. aegypti* population have found greater acceptability, as this have been found to be sensitive even at times when vector are at low levels (Kwanda et al., 2007). Temephos has been used as larvicide’s and being accepted for larval control program in several country. Some of researchers suggest that the use of temephos in water container quite effective for control the growth of mosquito larval but the smell of this chemicals compound has been rejected for some people. This condition is became serious in the area where fresh water became valuable resource. This research has the aim to know the preference of female *Ae. aegypti* gravid to oviposition media which contain of larvicide’s and atractant.
Materials And Methods

Traps were consisted of 250 ml plastic glass in cylinder of black plastic tube, 14 cm tall by 12 cm wide. The oviposition substrate was a 12 cm long and 1 cm in diameter of a plastic pipe and covered by coarse grade filter paper. A Paspalum grass infusion (HI) of Paspalum conjugatum in concentration of 10% and 20% was used to attract female mosquitoes. The grass stock infusion was prepared by adding 250 gr dried grass to two liters of tap water and fermented for seven days. We use the commercial abate with active compound as Temephos 1% as larvicide’s. Traps were prepared with 175 ml of water and added by larvicide’s or attractant respectively. The combination of Temephos 1% as a larvicide’s and Paspalum grass infusion as attractant with appropriately labeled papers before transportation to the field.

To evaluate the effect of Temephos 1% on the Aedes aegypti oviposition response, some of ovitraps were placed in shaded area of living quarters, under six different experimental conditions, i.e. (Aquadest, Temephos 1%, Paspalum 10%, Paspalum 20%, Paspalum 10% + Temephos 1%, Paspalum 20% + Temephos 1%). A total of 60 ovitraps were set out and exchanged between 09.00 – 12.00 hours, the time of lowest oviposition activity and left for three days. Egg counts were made using a magnifying glass.

Results and Discussion

We found 832 mosquito eggs and in field experiments, female mosquitoes did not avoid ovipositing in ovitraps that contained of Temephos 1% as larvacides than in the controls although there was choice on the oviposition media contain with the Paspalum grass infusion (20%) attractant (Table 1). In the pure water we just find 5.2 eggs. The addition of Paspalum grass infusion in the ovitraps enhances significantly the number of eggs collected, thus increasing the trap efficacy (Dario et al., 2008).

<table>
<thead>
<tr>
<th>No</th>
<th>Treatment Concentration</th>
<th>Ovitraps</th>
<th>Egg</th>
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<td></td>
<td>Total</td>
<td>Oviposition Rate</td>
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<tr>
<td>1</td>
<td>Control</td>
<td>15</td>
<td>33.3%</td>
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<tr>
<td>2</td>
<td>Temephos 1%</td>
<td>15</td>
<td>40%</td>
</tr>
<tr>
<td>3</td>
<td>Paspalum 10%</td>
<td>15</td>
<td>53.3%</td>
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<tr>
<td>4</td>
<td>Paspalum 20%</td>
<td>15</td>
<td>80%</td>
</tr>
<tr>
<td>5</td>
<td>Paspalum 10% + Temephos 1%</td>
<td>15</td>
<td>80%</td>
</tr>
<tr>
<td>6</td>
<td>Paspalum 20% + Temephos 1%</td>
<td>15</td>
<td>73.3%</td>
</tr>
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</table>

Although the presence of Temephos 1% appears to influence the choice of ovitraps as an oviposition the adding of Paspalum grass infusion as attractant has a effect to rise the oviposition rate. It is possible that female mosquitoes are not able to detect the larvicide’s of a
component of the test product when used with high infusion concentrations (Adson et al., 2005).

We suggest that the adding of attractant on various ovitrap was found to influence the oviposition responses of the gravid females, indicating thereby, the role of some other factor released by various substances used in the ovitraps which influenced the oviposition responses (Dario et al., 2008). The study finding indicate that the present of attractant will make the oviposition media more attractive to gravid mosquitoes as compared to same media without it; indicating presence of certain other chemical responses offered by the organics mater and also the microorganisms and perceived by the female as an encouraging signal for oviposition (Kwanda et al.,2007). The finding from the study have implication on the monitoring of of Aedes breeding wherein these attractant substances may be utilized in ovitrap instead of plain water. However, the community based application of these laboratory generated finding needs to be established by appropriate field studies.

References
P-PD05

MECHANISMS UNDERLYING SOMITE BOUNDARY FORMATION

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Abstract

In vertebrate development, the most prominent metameric structure is somites, which give rise to vertebrae, ribs, skeletal muscles and dermis. Somites are generated by periodic segmentation of anterior of the presomitic mesoderm (PSM), where several genes displays oscillatory expression associated with somite segmentation. It is believed that the oscillatory gene expression works as a segmentation clock, which controls the periodicity of somite segmentation. Retinoic acid (RA) signaling plays important roles in the somite formation and segmentation. However, the dynamic influence of RA on individual somite segmentation in real time frame remains largely unknown. Here we blocked the endogenous RA production in zebrafish embryos by injection of a morpholino (MO) against retinaldehyde dehydrogenase 2 (raldh2), encoding an enzyme which catalyzes the production of RA and performed real-time imaging of somite segmentation to understand the role of RA during dynamic segmentation. raldh2-morphant showed extension of somite segmentation period and variation of length time of individual somite segmentation. The final somite and vertebrae number of raldh2-morphants are less compare to the control, and the vertebrae defects were also observed. However, morphants embryos have almost normal oscillatory expression of her1 cyclic genes. Taken together, these results suggest that Retinoic acid is maintaining constancy and acts as a linker of the segmentation clock and somite segmentation machinery in somitogenesis.

Keyword: Zebrafish, somitogenesis, segmentation clock, retinoic acid

INTRODUCTION

In vertebrate development, the most prominent metameric structure is somites, which gives rise to vertebrae, ribs, skeletal muscles and dermis. Somites are generated by periodical segmentation of anterior extremity of the presomitic mesoderm (PSM) in a sequential manner, where several genes displays oscillatory expression associated with somite segmentation. It is believed that the oscillatory gene expression works as a segmentation clock, which controls the periodicity of somite segmentation. Retinoic acid signaling plays important role on the somite formation and segmentation. Retinoic acid deficiency or the absence of retinoic acid signaling lead to the abnormalities of somite (1), and lack of coordination of somite formation between the left and right side of the mouse
embryo and zebrafish (2,3,4). The objective of this research is to reveal the role of RA signaling during dynamic segmentation of somites.

MATERIALS AND METHODS

Zebrafish embryo

Wild-type zebrafish strains maintained and staged following standard protocols were used in this study.

Gene Knockdown

Depletion of RA was obtained by injection of Morpholino oligos against raldh2 which than be compared to control Morpholino.

Whole-mount and two color in situ hybridization

Whole mount in situ hybridization for Myod, Uncx4.1, cb1045, probes and two-color in situ hybridization for mespb and her1, were conducted following standard protocols.

Skeletal staining

Alcian Blue-Alizarin Red skeletal Staining was used to determined the vertebrate number of raldh2-morphant and control-morphant

Time Lapse imaging

Multi point time-lapse imaging was conducted to observed real-time somite segmentation in raldh2-morphants and control

RESULTS AND DISCUSSION

1. Effects of RA signaling on somite formation

Multiple time point in situ hybridizations revealed that raldh2-morphants and control shows almost same end time point of somite formation at 29 hours post fertilization (hpf). However, the number of somites in the raldh2-morphants is decreased to 2 (two) somites as compared to that of control.

Treatment of RA into raldh2-morphants partially restored defects in the loss of somites, suggesting that the defects in raldh2-morphants depend on the depletion of RA. Consistent with the loss of somite number, the number of vertebrae in raldh2-morphants was also decreased. These results indicate that RA signaling regulates somite numbers in zebrafish embryos.

2. RA signaling may regulate constancy of segmentation period

Although the decrease of somite number seems to be induced by prolonged time of segmentation period, it is not clear whether RA signaling could modulate the period time of the segmentation. To answer the question, we performed real-time imaging of somite segmentation in raldh2-morphants. The period of segmentation in control embryos was
constant (32±1.2 min), whereas the period in raldh2-morphants was highly variable (36±2.4min). These results suggest that RA signaling controls constancy of period time of somite segmentation.

3. RA signaling may not affect the link of somite segmentation clock to boundary formation

In the time-lapse experiments, raldh2-morphants showed loss of constancy of somite segmentation but they displayed constant size of somites. Since the size and periodicity of somite in zebrafish were determined by mespa/b and her1/her7, respectively, we performed two-color in situ hybridization for mespb and her1 and tested whether RA signaling regulates the coupling of somite segmentation clock with boundary formation. We could not detect the difference of their expression between control- and raldh2-morphants, suggesting that RA signaling doesn’t affect the coupling. However, it is still possible that RA signaling regulate the pace of somite segmentation clock to keep constant periodicity.

In summary, raldh2-morphant shows extended of somite segmentation period and variation of length time of individual somite segmentation. The final somite and vertebrae number of morphants are less compare to the control, and the vertebrae defects were observed on the morphants. However, morphants embryos have almost normal oscillatory expression of her1 cyclic genes and mespb genes as in control. These results suggested that Retinoic Acid may play important role as a linker of the segmentation clock and somite segmentation machinery in somitogenesis.

REFERENCES


824
P-PD06


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ABSTRACT

Recently, there has been a great interest in the use of cord blood as a source to obtain stem cells. There are several methods available for stem cell isolation. The manual method uses centrifugation procedure to separate the stem cell from the red blood cells and plasma. The centrifugation then followed by plasma reduction using a plasma extractor. The nucleated cells which contain stem cells then transferred into the collection bag through a sterile tubing system. This method requires a highly-skilled staff which sometime results in a slight variation in the cell composition among different individuals. In this study, we compared the cell yield, hematocrit traces, and the length of time needed for stem cells isolation in the manual and automated method. We found that the cell yield between manual and automated method was comparable. The cell yield in manual method was 19.76 ± 8.42 (10^6) cells/ml whereas in the automated system was 16.3 ± 10.17 (10^6) cells/ml. The hematocrit trace was higher in the automated system with level of 29.44 ± 3.629 % compared to the manual method 12.44 ± 5.419 %. The length of time required in the automated system was 3-4 hours, alike to the manual method which also required 3-4 hours. The automated system requires minimal involvement of staff which allows the individual to perform other activities. Moreover, automated system provides a standardized procedure compared to the manual method. In a busy laboratory the automated system can be operated using 2-4 devices within the same time frame. We conclude that in a low scale laboratory it is still more efficient to use the manual method.

Keywords: umbilical cord blood, stem cell, automated system, manual isolation

INTRODUCTION

Umbilical Cord Blood Mononuclear Cell (UCBMNC) which is derived from Umbilical Cord Blood (UCB) is another source of stem cells. Compared to the other sources of stem cells, UCBMNC offers some benefits, including the easy-to-perform collection procedure through the umbilical vein on the placenta’s side.

Also, allo-transplantation using UCBMNC posseses less risk graft-versus-host disease (GVHD). Aliquots of UCBMNC units can be subjected for HLA typing, sterility tests, and cell
counting, make them readily available for transplantation to suitable host. Because of the limited volume of UCBMNC and the high cost of cryopreservation, it is crucial to find efficient method to isolate mononuclear cells within UCB. Several methods have been developed to obtain UCBMNC, such as density gradient separation using centrifugation technique, sedimentation of red blood cells using gelatin, and differential centrifugation. Within the last few years there have been an attempt to generate automated systems to isolate UCBMNC. The automated systems aimed to facilitate less time and labour of UCB processing. This study compared the efficiency of manual and automated system to isolate UCBMNC based on three parameters including cell yield, hematocrit traces, and the length of time required.

**MATERIALS AND METHODS**

In this study, we compared 5 units in each group i.e. manual and automated system. For this preliminary comparison study, only five UCB samples, 5 for each method, were compared. The three parameters used to determine the efficiency between the systems were cell yield, hematocrit traces, and the length of time needed for stem cells isolation. To calculate the stem cells isolated, we used trypan blue exclusion method. The hematocrit traces were measured by hemacytometer. The time length was counted from transferring UCB from the blood bag to the processing bag up to time when UCBMNC was placed to special container and ready to cryopreserved to the liquid nitrogen tank. All data were averaged then compared between the manual and the automated system.

**RESULTS AND DISCUSSION**

Using the above methods, we found that the hematocrit trace was higher in the automated system with level of 29.44 ± 3.629 % compared to 12.44 ± 5.419 %. The allowed level of hematocrit for stem cell transplantation is no more than 38%. Therefore, despite the higher levels of hematocrit found in the automated system, it was still within the allowed range. The cells yield in manual method was 19.76 ± 8.42 (10^6) cells/ml whereas in the automated system was 16.3 ± 10.17 (10^6) cells/ml.

Table 1 Hematocrit level and total of mononuclear cells between automated and manual processing

<table>
<thead>
<tr>
<th></th>
<th>Automated System (mean ± stdev)</th>
<th>Manual System (mean ± stdev)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematocrit (%)</td>
<td>29.44 ± 3.629</td>
<td>12.44 ± 5.419</td>
</tr>
<tr>
<td>MNC (10^6 cells per ml blood volume)</td>
<td>16.30 ± 10.17</td>
<td>19.76 ± 8.42</td>
</tr>
</tbody>
</table>

Based on the time calculation, time spent in the automated process was 3-4 hours, alike to the manual process which also required 3-4 hours. The significant difference is that...
automated system requires minimal involvement which allows individual to perform other activities simultaneously. Moreover, automated system provides a more standardized procedure compared to the manual method. For big scale laboratory with high number of samples, automated system can be operated using 2-4 devices within the same time frame.

REFERENCES
P-PD07

THE EFFECTS OF SHOOT PRUNING AND LEGIN INOCULATION ON GROWTH AND YIELD OF PEANUT (Arachis hypogaea L.)

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Abstract

This study was carried out to determine the effects of shoot pruning and legin inoculation on the growth and yield of peanut (Arachis hypogaea L.). Legin is rhizobium inoculum which could increase number of nodules and availability of nitrogen for plant growth. The experiment used factorial completely randomized design with five replications for each combination treatment. The first factor was shoot pruning, i.e. without shoot pruning, shoot pruning on the 2nd and 3rd stem internode. Second factor was concentration of legin applied, i.e. 0, 5, 10, 15, and 20 mg/g seed. The growth parameters observed were number of branches, plant biomass, root shoot ratio, number of nodules and number of pods. Data were analyzed using Univariate Analysis of Variance and followed by Duncan’s Multiple Range Test with significantly level of 5%. The results revealed that shoot pruning and legin inoculation give significant effect in all parameters studied. Shoot pruning resulted in delaying of generative phase. Legin inoculation increased the number of nodules and improved peanut growth. The highest yield of peanut were obtained on the treatment of 10 mg/g legin inoculation without shoot pruning.

Keywords: shoot pruning, legin, growth, peanut (Arachis hypogaea L.)

INTRODUCTION

The role of nitrogen fixing legumes, particularly in tropical regions, is becoming increasingly important both in terms of providing essential dietary protein as well as improving the quality of the soil. Peanut (Arachis hypogaea L.) is an annual plant belongs to familia Papilionaceae. It is capable of fixing and utilizing atmospheric nitrogen in symbiotic association with Rhizobium. The formation of root nodules can be stimulated by Rhizobium inoculum, the strains of which must be suited to the plant [1]. The use of appropriate Rhizobium strain may reduce the use of commercial nitrogenous fertilizer and increase the profitability of production. Legin is Rhizobium inoculum which could increase number of nodules and availability of nitrogen for plant growth [2]. Shoot pruning was used to manage plant architecture so that most of ginophore could reach soil. In this present study an effort was made to determine the effects of shoot pruning and legin inoculation on the growth and yield of peanut (Arachis hypogaea L.).
MATERIALS AND METHODS

The study was conducted at green house of the Faculty of Biology, Gadjah Mada University. The experiment used factorial completely randomized design with five replications for each combination treatment. The first factor was shoot pruning, i.e. without shoot pruning (P0), shoot pruning on the 2nd (P1) and 3rd stem internode (P2). Second factor was concentration of legin applied (L0= 0 mg/g; L1= 5 mg/g; L2=10 mg/g; L3=15 mg/g , and L4=20 mg/g of seed). Basal fertilizer consisted of 100 mg of urea, 200 mg of TSP and 100 mg KCl was added into the pot containing 5 kg of soil. Legin inoculation was applied before planting and shoot pruning was done when the plants were 4 weeks old. Data collected were number of branches, plant biomass, root shoot ratio, number of nodules and number of pods. Data were analyzed using Univariate Analysis of Variance and followed by Duncan’s Multiple Range Test with significantly level of 5%.

RESULTS AND DISCUSSION

Physiological characters of peanut (Arachis hypogaea L. cv. Gajah) were studied under green house condition with different levels of legin and shoot pruning treatment. The results revealed that shoot pruning and legin inoculation give significant effect in all parameters studied (Table 1).

The inoculation of legin increased the number of nodules, which will further enhance nitrogen fixation. Thus the elements of nitrogen is available to plants that can enhance growth and yield of peanut. Nodules that have been effectively inoculated and are actively fixing nitrogen are pink on the inside. This study found that the biomass production of peanut increased significantly when inoculated with legin, whereas shoot pruning did not affect biomass production. Shoot pruning tends to delay the generative phase. The highest yield of peanut were obtained on the treatment of 10 mg/g legin inoculation without shoot pruning.

Peanut pods are the productivity of economically valuable plants. The number of peanut pods proportional to the number of flowers formed (Fig.1). Even so the number of pods formed is also influenced by the ability to penetrate the soil ginophore [3]. From the results it is known that there is significant difference between the treatment of the amount of peanut pods are produced.
Table 1. Physiological characters of peanut (Arachis hypogaea L.) after treated with legin inoculation and shoot pruning

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of branches</th>
<th>Number of nodules</th>
<th>Number of pods</th>
<th>Dry weight (g) shoot</th>
<th>Root shoot ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>POL0</td>
<td>3.8±0.45</td>
<td>39.6±19.06</td>
<td>6.4±1.52</td>
<td>7.94±2.63</td>
<td>0.04±0.02</td>
</tr>
<tr>
<td>POL1</td>
<td>4.2±0.45</td>
<td>58.8±17.25</td>
<td>8.0±1.00</td>
<td>7.54±0.70</td>
<td>0.06±0.1</td>
</tr>
<tr>
<td>POL2</td>
<td>3.6±1.14</td>
<td>58.2±20.25</td>
<td>7.6±1.82</td>
<td>7.10±1.20</td>
<td>0.06±0.02</td>
</tr>
<tr>
<td>POL3</td>
<td>4.2±0.45</td>
<td>50.2±17.82</td>
<td>7.4±2.07</td>
<td>7.84±1.36</td>
<td>0.06±0.02</td>
</tr>
<tr>
<td>POL4</td>
<td>4.0±0.70</td>
<td>64.6±4.83</td>
<td>7.0±1.22</td>
<td>8.08±0.65</td>
<td>0.05±0.03</td>
</tr>
<tr>
<td>P1L0</td>
<td>4.0±0.00</td>
<td>41.2±18.57</td>
<td>4.8±0.84</td>
<td>6.92±1.14</td>
<td>0.04±0.01</td>
</tr>
<tr>
<td>P1L1</td>
<td>4.4±0.55</td>
<td>44.8±16.10</td>
<td>5.8±1.92</td>
<td>7.01±1.44</td>
<td>0.06±0.04</td>
</tr>
<tr>
<td>P1L2</td>
<td>4.2±0.45</td>
<td>49.6±26.02</td>
<td>5.2±1.46</td>
<td>6.05±1.13</td>
<td>0.05±0.05</td>
</tr>
<tr>
<td>P1L3</td>
<td>4.0±0.00</td>
<td>49.4±9.04</td>
<td>6.4±1.14</td>
<td>5.35±1.47</td>
<td>0.05±0.05</td>
</tr>
<tr>
<td>P1L4</td>
<td>4.4±0.55</td>
<td>46.0±10.12</td>
<td>5.4±1.10</td>
<td>6.90±1.49</td>
<td>0.05±0.05</td>
</tr>
<tr>
<td>P2L0</td>
<td>4.4±0.55</td>
<td>56.2±15.16</td>
<td>6.2±0.84</td>
<td>7.04±1.62</td>
<td>0.04±0.04</td>
</tr>
<tr>
<td>P2L1</td>
<td>4.6±0.55</td>
<td>57.0±10.61</td>
<td>6.2±0.84</td>
<td>7.73±1.15</td>
<td>0.05±0.01</td>
</tr>
<tr>
<td>P2L2</td>
<td>4.8±0.83</td>
<td>50.4±12.86</td>
<td>6.2±1.10</td>
<td>7.25±1.07</td>
<td>0.05±0.01</td>
</tr>
<tr>
<td>P2L3</td>
<td>4.4±0.55</td>
<td>50.0±6.04</td>
<td>5.6±1.34</td>
<td>6.35±0.73</td>
<td>0.05±0.02</td>
</tr>
<tr>
<td>P2L4</td>
<td>4.2±0.45</td>
<td>43.2±13.66</td>
<td>6.2±0.84</td>
<td>6.96±1.36</td>
<td>0.05±0.02</td>
</tr>
</tbody>
</table>

Means within the same column followed by different letters differ significantly at the 5% probability level.

Figure 1. Relationship between number of flower and pod of peanut after legin inoculation and shoot pruning treatment.

Summarizing the results obtained we could conclude that legin inoculation increased the number of nodules, thereby increasing growth of peanut.

REFERENCES


P-PD08

Cytotoxic Assay of Chloroform and Methanol Extract of
Brucea javanica L. (Merr) on Cervix Cancer (Hela Cell)

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ABSTRACT

B. javanica fruit contain useful material as medicine. Recently, B. javanica fruit have been used traditionally to treat antitumor and anticancer. The scientific studies of anticancer activity of B. javanica fruit on the growth of cancer are relatively very few. Therefore the objectives of this research were to know the cytotoxic assay chloroform and methanol extract of B. javanica fruit on cell line of cervix cancer. These studies using B. javanica from Gunung Palung National Park, West Kalimantan. The cancer cell line used were HeLa cell. B. javanica fruits were extracted by chloroform and methanol using soxhletation method. The cytotoxicity of those extract from these plants were determined using MTT assay. The potential compound were identified by TLC analysis using many kind of detection reagents. The result showed that the higest cytotoxic assay was chloroform extract (LC₅₀ 148,076 µg/ml) than methanol extract (LC₅₀ 199, 165). Based on TLC analysis using many kind of detection reagents, the bioactive compounds obtained was terpenoid group.

It could be concluded that the cytotoxic compound in the chloroform extract of B. javanica fruit on HeLa cell cervix cancer was terpenoid group.

Keywords : extract, cytotoxic, Brucea javanica, HeLa cell.

INTRODUCTION

Plants have an almost limitless ability to synthesize bioactive substances. Most are secondary metabolites, of which at least 100,000 have been isolated with terpenoids and alkaloids as major groups, a number estimated to be less than 10% of the total. Current research is based on this traditional use of plants for their secondary metabolites (Nugroho and Verpoorte, 2002). Brucea javanica L. (Merr) is a traditionally medicine plant which is widely distributed in Sumatera, Jawa, Sulawesi and Kalimantan (Kim and Park. 2002). Its seeds have been used for the treatment of malaria, tumor and cancer and are known to be a source of quassinoids (Fukamiya et al., 1992; Yoshimura et al., 1985; Greenwood et al., 2002). Recently, some quassinoids from seed of B. javanica exhibit such interesting biological activity as antimalaria, antitumor and anticancer activities.
The scientific studies of anticancer activity of *B. javanica* fruit on the growth of cancer are relatively very few. Therefore the objectives of this research were to know the cytotoxic assay chloroform and methanol extract of *B. javanica* fruit on cell line of cervix cancer.

**MATERIALS AND METHODS**

**Plant material.**

The seeds of *B. javanica* were collected from Gunung Palung Park, West Kalimantan in 2008 and botanical origin was determined by Herbarium Bogoriense, Research and Development Centre for Biology LIPI, Indonesia.

**Extraction.**

Dried and ground seeds of *B. javanica* (150 g) were soxhletation with chloroform (250 mL). The solvent was removed in vacou to give a residu. The residu were soxhletation with methanol (250 mL). The potential compound were identified by TLC analysis using many kind of detection reagents.

**In Vitro Assay of Cytotoxic.**

Anticancer activity on cervix cancer cell line of those extracts from these plants were detected using 3-(4,5-dimethylthiazol-2-yl)-2,5diphenyl tetrazolium bromide assay to determine LC$_{50}$. The cancer cell line used were HeLa cell.

**RESULTS AND DISCUSSION**

The result extraction of chloroform and methanol were used for TLC preparative for detecting the potential activity of fractions on cervix cancers. The result showed that the higest cytotoxic assay was chloroform extract was LC$_{50}$ 148,076 µg/mL than methanol extract was LC$_{50}$ 199,165 µg/mL and both of those LC$_{50}$ were lower than LC$_{50}$ of anti cancer drug, Doxoruricine that was 66.70 µg/ml (Moeljopawiro *et al.*, 2007). Curve of relationship between viability of cell and concentration extract after gift of methanol and chloroform extract can be seen in Figure 1.

Based on TLC analysis using many kind of detection reagents, the bioactive compounds obtained was terpenoid group.
Fig. 1. Curve of relationship between viability of cell and concentration extract after gift of methanol and chlorofom extract

REFERENCES
P-PD09

Optimization of Degradation Antibiotic Residue in Fresh Cow Milk by β-Lactamase Enzyme

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\end{itemize}

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1. Abstract

Milk industry still have some problem, one of the problem is antibiotic residue in fresh cow milk. One research indicates that penicillin antibiotic residue and Streptomis in cow milk heated with temperature to 100°C and sterilization in autoclave still can be detected. β-Lactamase can hydrolyse binding of cyclic in molecule β-lactam (for example, penicillin and cephalosporin). If ring of β-lactam at penicillin is hydrolysed by β-lactamase so that will be resulted conspecific compound which is not has antibiotic activity, that is penicililato compound with the stoichiometry composition. The aim of this research is to know that antibiotic residue in fresh cow milk can be degraded by commercial enzyme of β-lactamase and to know how is the optimum condition of dose and treatment of enzyme, so that can be obtained optimum degradation the antibiotic residue in fresh cow milk. The methods of this research is designed as follows: degradation of antibiotic residue by commercial β-lactamase enzyme, looks for optimum condition of the treatment of enzyme in degradeate of antibiotic, and determines optimum dose of commercial β-lactamase in degradeate of antibiotic residue. The result of this research indicates that antibiotic in fresh cow milk can be degraded by commercial β-lactamase. The optimum condition (dose and treatment) of enzyme is at dose twice recipe that was described at packaging lable on the bottle of commercial β- lactamase enzyme with 30 minutes. Hopefully this results can be aplicated to solve the problem of antibiotic residue in milk industry in Indonesia.

Key words: β-lactamase enzyme, antibiotic residue, fresh cow milk.

Introduction

Recently development of biochemistry very fast, especially biotechnology. Many problem in Industry that solved by biochemistry. In Facts many problem in Industry that could not solve, for example is some problem in cooperation of milk wich handling fresh cow milk from cow milk breeder. When their fresh cow milk is contaminated by atibiotic so could not sale to milk industry. that is ironic condition because of Indonesia is one of the biggest country that produce fresh cow milk and milk industry in the world.
Many of cooperation in Indonesia that handling fresh cow milk, one of them is Koperasi SAE Pujon. Koperasi SAE Pujon is the biggest cooperation in Malang (Bintariadi, B., 2002). Koperasi SAE Pujon supply fresh cow milk to Nestle milk industry while Koperasi SAE Pujon take the fresh cow milk from cow milk breeder. Some of antibiotic have been added to cow feed as drugs and stimulate increasing weight of cow. The bad effect from using antibiotic is antibiotic residue will be in tissue that can cause antibiotic resistant and poison that will be dangerous for human (Yuningsih, 2006).

Base on Harlia E., et al. (2006), cow breeder always have problem about health of cow. One of the common illness of cow is mastitis. Mastitis is one of the illness that cause of bacteria. To kill that bacteria usually use β-lactam antibiotic, for example is cephalosporine. β-lactam antibiotic is the common compound as antimicrobial (Silpak B., et. al., 2008). Using antibiotic can result antibiotic residue in fresh cow milk. Another problem is bacteria can be resistant to some antibiotic, but this problem can solve by biotechnology. That is identification antibiotic resistance proteins (ARPs) so we can usefull for new antibiotic discovery (Zhang, H.L., et. al., 2008).

Result from the research show that when antibiotic residue of penicilllin and streptomycin in fresh cow milk heated more than 100°C and sterilized by autoclave still can detected (Harlia E., dkk., 2006). That cause after fresh cow have proceed in milk industry may be still contain antibiotic so milk industry like Nestle did not recieve the fresh cow milk if cantaining antibiotic. That is a big problem because if one tank containing antibiotic so that fresh caow milk must be thrown. The cooperation must be lossy. Containing antibiotic residue in fresh cow milk cause milk industry import fresh cow milk from another country that because of quality and the tax just 0-5% (Poultryindonesia.com, 2006). If we show from health side, the base reason why milk industry refuse fresh cow milk if cantain antibiotic is because of antibiotic danger for healthy. For example is cephalosporin can kill another bacteria that usefull for our body (http://www.susukostrum.com, 2008).

The objective of this research is to know is antibiotic residue can be degradated by β-lactamase enzyme and what is the best condition (dose and treatment) so can resulting optimal degradation.

Antibiotic in microbiology show since 1928. Base on Selman Waksman, antibiotic is chemical substantion that take from microorganism, in watery solution antibiotic have capability to retain growing and kill microbacteria (Suwandi, U., 1993). In biochemistry has discovered enzyme that can degrade antibiotic, that is β-lactamase enzyme. The first activity of β-lactamase has written in 1940 by someone who discovered penicillin (Morin dan Gorman, 1982). β-Lactamase is type of enzyme (EC 3.5.2.6) that produced by some bacteria tha resistant to antibiotic like penicilllin, cephalosporin, cephamycin dan carbaphenem
(Wikipedia). β-lactamase can be produced by *Staphylococcus aureus*, *Bacillus cereus* and *bacillus licheniformis* (Morin dan Gorman, 1982).

The mechanism of β-lactamase is β-lactamase hydrolyse amide cyclic bond in molecule that cantain β-lactam ring like penicillin and cephalosphorin (Morin dan Gorman, 1982). The mechanism of reaction can see in Picture 1.

![Picture 1](image)

**Picture 1.** β-lactam ring in penicillin is hydrolysed by β-lactamase produce the same compound which have no antibiotic activity again, that is penicilloate in stoichiometry. Penicilloate have more one acidic bunch so more acid than penicillin compound. Penicilloate more stable, easy to detected and determine the concentration.

The reaction in cephalosphorine is more complicated because have one system conjugation and substituent in third position in dihydrotiazine ring like in Picture 2 below.

![Picture 2](image)

**Picture 2.** The first product is cephalosporoate which analog with pencilloate, but commonly cephalosphoroate is not stable so it will be decomposition become smaller compound (Morin dan Gorman, 1982). β-lactamase can be detected by much methods. Base on Morin dan Gorman (1982), the common methods are acidimetry, iodometry, microbiology and chromogenic substrates.

1.1. Acidimetry
Hydrolysed one penicillin molecule or cephalosphorin by β-lactamase will produce at least one carbonyl bunch which can be detected by pH indicator (for example is red phenol).

1.2. Iodometry

sefalosporin
The result of hydolysed antibiotic by β-lactamase be redactor that can take iodium from amyllum complex so can reduce purple colour.

1.3. Microbiology
This method base on lossing antibiotic activity after got hydrolysed that can be detected by microbiology technique (use wild type *E. coli*).

1.4. Chromogenic substrates
This method base on the defference of absorbtion spectra before and after hydrolysis. To detected antibiotic can use BetaStar.

**Material and Methods**

**Material**

Material that used in this research is aquades, fresh cow milk, β-lactamase enzyme, cephalosphorin, buffer fosfat solution pH 7 and beta star kit.

**Methods**

The first step in this research is antibiotic degradation test in aquadest solution, then antibiotic degradation test in fresh cow milk solution. Many kind of antibiotics, but in this reasearh use cephalosphorin. Atibiotic detected by beta star kit. For beta star test we must take 200 µl antibiotic in aquadest solution or fresh cow milk solution put in vial for beta star test, then incubate at 47.5 °C (3 min), turn in dipstick in solution has been incubated, incubation again at 47.5 °C (2 min). Take the dipstick and analyse and write the results. After antibiotic degradation test by β-lactamase got success, the second step is determine the the best condition (dose and treatment) so can resulting optimal degradation. The best condition is the smallest dose and the shortest time for incubation which β-lactamase still can degragate antibiotic so in this research we must variation the dose of β-lactamase and time for incubate the solution.

Determination variation of dose an time for incubate in this research base on the procedur in β-lactamase that we use. Activity β-β-Lactamase in this research is one unit β-lactamase can degradate or hydrolyse 1 mol benzyl penicillin and 1.0 mol cephalosphorin a minute at 25°C (*Merck Index* 13, 7164).

**Results**

3.1. Degradation Test of Cephalophorin in Aquadest Solution

Degradation test of cephalophorin in aquadest solution resulted data in Table 1. The optimum concentration of β-lactamase in degradation test of cephalophorin in aquadest solution is two times recipe which written on the bottle of β-lactamase.
Tabel 1. Degradation test of cephalopherin in aquadest solution

<table>
<thead>
<tr>
<th>No. test tube</th>
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<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
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</thead>
<tbody>
<tr>
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<td>2x</td>
<td>4x</td>
<td>5x</td>
<td>0x (Standart)</td>
</tr>
<tr>
<td>The results of beta star test</td>
<td>++</td>
<td>-</td>
<td>++</td>
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</tr>
</tbody>
</table>

3.2. Degradation Test of Cephalopherin in Fresh Cow Milk Solution

Degradation test of cephalopherin in fresh cow milk solution resulted data in Table 2. All of est tube got success degradation of cephalopherin, but the optimum results is in two times recipe which written on the bottle of β-lactamase.

Tabel 2. Degradasi sefalosporin dalam susu oleh enzim β-Lactamase

<table>
<thead>
<tr>
<th>No. test tube</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
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<td>Concentration of β-lactamase (recipe)</td>
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<tr>
<td>The results of beta star test</td>
<td>-</td>
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<td>++</td>
</tr>
</tbody>
</table>

Discussion

4.1. Degradation Test of Cephalopherin in Aquadest Solution

The result of this research show that with 5 minutes incubation did not success. That is may be time for incubation have to longer than 5 minutes. The optimum concentration of β-lactamase in degradation test of cephalopherin in aquadest solution is two times recipe which written on the bottle of β-lactamase. But in four and five times recipe did not success, that is may because of contaminant before beta star test.

4.2. Degradation Test of Cephalopherin in Fresh Cow Milk Solution

All of est tube got success degradation of cephalopherin, but the optimum results is in two times recipe which written on the bottle of β-lactamase. This result same with the result in degradation test of cephalopherin in aquadest solution, so we can say that chemical condition of cephalopherin in aquadest and fresh cow milk solution is not different.

Hopefully this results can be applied to solve the problem of antibiotic residue in milk industry in Indonesia. Bside that this research can be references for the other research in the same field.

Conclusion
Antibiotic residue in fresh cow milk can be degradate by β-lactamase. The optimum condition (dose and treatment) for antibiotic degradation is two times of recipe which show on the bottle label at 25 °C in 30 minutes.

Acknowledgements

The authors acknowledge material support by chemistry laboratory Koperasi SAE Pudjon Kabupaten Malang and chemistry laboratory of Mathematics and Sciences Faculty State University of Malang. The authors acknowledge financial support by Dikti..

References


*Merck Index* 13, 7164.


P-PD10

IN VITRO GERMINATION
OF MOUNTAIN PAPAYA (Carica pubescens  Lenne & K.Koch) SEEDS

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corresponding author: ennisuwarsi@yahoo.com

Abstract

Carica pubescens Lenne & K.Koch (mountain papaya or ‘karika dieng’) has significant potency of economic value and can be developed as the main commodity in Wonosobo regency. It can also be used as source of genetic for tolerance character toward virus that attacks papaya. Its rare existence should be maintained by in vitro storage method. In order to develop the storage method, at the first time, it should be developed in vitro germination method that can produce shoot or seedling as the material which will be saved. The effects of lighting, soaking in gibberelic acid (GA3) before germinating, supplementing of active charcoal, 6-benzyl amino purine (BAP), 2,4-and dichloro phenoxyacetic acid (2.4-D) at different concentrations on Murashige and Skoog’s (MS) medium were examined. The parameter were observed namely the percentage of germination, emergence timing of primary root, and seedling height. The result shows that the germination percentage varied between 5 and 70%. In general, soaking for 24 h in 200 ppm GA3, and supplementing active charcoal increased the percentage and rate of seed germination to 10–90%. An average maximum germination of 90% was obtained after 7–8 days when seed were germinated on MS medium supplemented with active charcoal and 5 mg/l 2.4-D without lighting.

Keywords: Carica pubescens Lenne & K.Koch, seed germination, 2.4-D, active charcoal

Introduction

Carica pubescens Lenne & K.Koch (mountain papaya or ‘karika dieng’) has significant potency of economic value and can be developed as the main commodity in Wonosobo regency, Center of Java. It can also be used as source of genetic for tolerance character toward virus that attacks papaya (Verheij & Coronel 1997). The rare existence should be maintained by in vitro storage method. In order to develop the storage method, at the first time, it should be developed in vitro germination methods that can produce shoot or seedling as the material which will be saved. The effects of lighting; soaking in gibberelic acid (GA3) before germinating; supplementing of active charcoal, 6-benzyl amino purine (BAP), 2,4-and dichloro phenoxyacetic acid (2.4-D) at different concentrations on Murashige and Skoog’s 1962 (MS) medium were examined.
The design was 2x8 factorial experiments in randomized complete block design. The treatments were lighting and supplementing of growth regulators or active charcoal. The lighting consisted of 2 treatments, namely continuous lighting for 24 hours and total darkness for 24 hours. The supplementing of growth regulators or active charcoal consisted of 8 treatments, that were soaking in GA₃ (200 and 0 mg/l), supplementing of BAP (5 and 10 mg/l), 2,4.D (5 and 10 mg/l) and active charcoal (2 and 0 g/l). The experiments were repeated three times, and the experiment unit was culture bottle containing 10 seeds. Culture bottles were incubated for 8 weeks at 10±2°C under light or dark according to the lighting treatment.

Mountain papaya seeds were dried under sunlight for several days, and then were surfaced sterilized by immersing in 70% ethanol and shaking for 5 minutes in rotary shaker at 200 rpm. The alcohol was decanted under sterile condition and seeds were rinse with sterile water three times. The seeds were further treated with a 25% diluted solution of commercial bleach for 10 minutes in a rotary shaker at 200 rpm, followed by washing with sterilized distilled water, and placed on agar solidified germination media in 150 ml culture bottle. For treatment of GA3 soaking, before the latest washing with sterilized water, the seed were soaked in GA3 for 24 hours. The germination media contain 0.3% sucrose in MS medium with addition of growth regulator or active charcoal according to the treatment.

The parameter were observed namely the percentage of germination, the rate of germination, and seedling height at 6 weeks after sowing. Percentage of germination was scored by comparing the amount of germinated seed and the total amount of sowing seeds. The rate of germination was determined by the time (days after sowing) of primary root emergence. Seedling height was measured from the crown to the tip of the shoots. The significance difference between mean values was determined by two way analyses of variance. Comparisons among the mean was detected using DMRT (Duncan’s multiple range test).

The results show the germination percentage varied between 5 and 70%. There were significant interactions between lighting and supplementing of growth regulators and active charcoal for germination percentage, germination rate and seedling height. In general, there were significant differences among kinds and concentration of supplementing growth regulators or active charcoal for germination percentage, germination rate and seedling height. Soaking for 24 h in 200 mg/l GA₃, and supplementing 2 g of active charcoal increased the percentage and rate of seed germination. Seed germination may require gibberel in for one of several possible steps: the activation of vegetative growth of the embryo, the weakening of a growth-constraining endosperm layer surrounding the embryo, and the mobilization of stored food reserves oh the endosperm. Gibberel in application also stimulates
the production of numerous hydrolases, notably α-amylase (Taiz & Zeiger 1998). Active charcoal can absorb chemical compound which inhibit the metabolism, such as phenolic acid that perhaps be produced by seed. Therefore supplementing of active charcoal on the MS medium can promote in vitro germination.

Continuous lighting for 24 h in general decreased percentage and rate of the germination. Germination of most of cultivated seeds do not require light and sometime were inhibited by light because of the blue or far-red present. The far-red wavelengths of sun light are nearly always inhibit metabolism, because they decrease the amount of phytochrom-fr in the seed to a level below that needed for germination (Taiz & Zeiger 1998).

An average maximum germination of 70% was obtained after 7–8 days when seed were germinated on MS medium supplemented with active charcoal or supplemented with 5 mg/l 2.4-D without lighting.

REFERENCES
P-PD11

AN EFFORT for EX-SITU CONSERVATION OF NATURAL RESOURCES USING ENTREPRENEURSHIP ACTIVITIES OF SMA LABORATORIUM UNIVERSITAS NEGERI MALANG STUDENT

Evi Fatmawati, S. Pd
SMA LAB UM Malang

Abstract

Biological resources are animal species have the economically for people. Biological role of teachers is very in shaping a child's mental nations to have any mental conserve biological resources. Conservation of biological resources is not merely for saking of nature, but it should give benefit for people welfare without prejudice the nature, the basic concepts of conservation of natural resources biodiversity, one of which is to be done ex-situ activities through entrepreneurial way with the in-vitro cultivation of orchid species

Keywords: Ex-situ, entrepreneurship, SMA Laboratorium UM

Background of the study

"My teacher, please give us with the valuable inheritance, and don’t give us accident", is a burden if we are able to understand the sentence because teachers are those whom the students “understand” and "imitate", all that we teach will be memorized by our students throughout his life. Since the age of 4 years to 50 years we may still meet with a teacher to draw knowledge. Obviously if the teachers have a responsibility towards the establishment of a major child mental nations. From a teacher's discipline, we hope the nation will be born the son of best-daughter of Indonesia to maintain, preserve and promote the heritage of the nation Indonesia. One of the inheritance that the nation must be guarded is the natural biodiversity of the earth that extends Pertwi Indonesia.

Biological resources are rich and animal species that have the potential usefulness and are known or estimated to provide economic benefits for people. Biology Teachers' role is very large in shaping a child's mental nations to have any mental conserve biological resources that we have. Expected from the draft received by the students, biology teachers as authors hope they have the understanding that the preservation of biological resources, preservation of nature is not merely for the sake of nature, but also for the benefit and welfare of people and there were azaz benefits without prejudice nature. Ex-situ conservation is a way to keep plants and animals from extinction in the original habitat. Ex-situ conservation was conducted in a system of conservation areas such as the botanical garden. Removing the type of plants from the original habitat to other places. Part of this which can
be conserved are all plants: seed tubers, pollen or plant network. Learning ex-situ conservation combined with the entrepreneurial activity, is a learning process that is hit to embed the concept of how they can maintain the heritage of the nation. In this event we will work together with one of the orchid garden of orchids in the in-vitro scale commercial cultivation. As we know one of India including the State's rich biodiversity, including orchid plants, but the existence of the plant began extinct because of Indonesian youngsters themself. For example orchid *Dendrobium Stratiotes* is a native of Papua, now the existence is threatened if there may be a difficult location to reach by people. In fact, it has a high economic value. From the above problems, students are invited by the author to try cultivating dendrobium orchids that have economic value with the in vitro cultivation of beans. Stages of learning in-vitro cultivation orchid species from a seed are:

1. Selfing parent plant: take the pollen to the pistil which is pollenized (this activity is done by one student, other students observe)

![Image of orchid cultivation](image1)

2. Spread orchid seeds in the planting media which have been determined (PLB) activity was done by one student, other students observe.

![Image of PLB activity](image2)

Removing orchid plants from the PLB to the planting medium in a bottle, this activity is done by all students

3. Remove the plant from a bottle of this is done by all students, with the stages as follows:

![Image of plant removal](image3)
- open a closed bottle orchid seeds
- easily fill the water that was issued to
- take the orchid seedlings one by one with the wire form U
- orchid seeds washed in water, and put into rege (a container to filter the seeds from water)
- immerse in water that has been given a few drops bayclean(bleach) (4 drops bayclean to 1 liter of water).
- put seeds in the plastic lining rege newsprint max. for 1 week
- During drained seeds with water every day
- after 4 days out of the seeds with a spray bottle of water + adaptan 1 liter water +1 cc Adaptan7. So seeds with water spray + adaptan once every 4 days.

4. Keeping compote

* move the seeds to a pot in the community (compote = a lot of pot plants) after the root of the plant leaf + strong (1 week after the exit from the bottle)
* Media used: 1 / 2 brick (1) + 1 / 2 short fern or moss
* spray the plants with water fertilizer nitrogen high + 2 times a week
* After the pot is tidah it is enough / not good for growing orchids ( months) to move the plants in a single pot (1 pot a plant)
* At the time the plants are on the single pot plant is ready to use fostered the growth of fertilizer (high N composition).
* flush the plants every morning with the use of clean water
* spray the plants with the fungi insect or when there are attacks of pests or disease.

This activity has not been able to fully be in the high school stage to UM LAB 1. Selfing parent plant; 2. Spread orchid seeds in the planting media which have been determined (PLB); 3. Remove orchid plants from the PLB to the planting medium in a bottle, SMA LAB UM in cooperation with one of the orchid gardens that have lab facilities in-vitro with students’s contribution are charged Rp 15,000, per bottle. Activities is one of the above efforts to introduce the concept of ex-situ concrete on the students. This activity combined
with the entrepreneurial activities in the hope that students are able to think how to make money from activities they do. 15,000 of the money they spend if they can treat it has been issued a bottle and sell it, so they are expected to learn through doing this integrated activities.

There are 3 business segments that can be selected in many orchids. Raising seedlings from bottles so compote (community pot), compote be kept up to individual pot (sedling). Then raise individual orchid pot become teenagers, young people and maintaining orchid blooming and ready to sell.

Especially for high school students choose the authors lab segment plant adolescents with funds flow and profitability of the plant segment adolescents are as follows:

1. seed = Rp 15,000,00
2. 30 pot @ Rp. 300 = Rp 9,000,00
3. Media 30 @ Rp. 300 = Rp 9,000,00
4. Fertilizer = Rp 5,000,00
5. pesticide = Rp 5,000,00
   TOTAL Rp 43,000,00

Income:
Consider 20% of the plants die, the price of plant age 4 months Rp. 7,500,00
   Rp. 7,500 x 24 = Rp. 330,000,00
So profit is Rp. 330,000 - Rp. 43,000 = Rp. 287,000.00.

B. The advantage for students of SMA LAB UM

I think any of us in the school as an instructor will meet students' level of social economy is different. Fortunately students are placed on families who are able, for the cost of education is not concerned with the necessary funds. But the students who are placed on the families who can not afford to think they must be hard to membiyai education. Tergiang still in the ear at the time I ask students in class XII.

Author : "Kid, what will you do after you graduate from SMA, will you continue to universities ?
Student : "No, Mom! My brother needs tuition fee after me!"
This is the author's discussion of biology teachers who want to give a little through the stock ex-situ combined with entrepreneurial activity, the author hopes LAB UM senior high school students at least once can pass;
1. Opening the job itself
2. Working in the laboratory in-vitro in the orchid gardens with skills that have been obtained
3. Having the ability to think that every individual has the obligation to maintain the heritage of the nation, with one guard and preserve the biodiversity of natural resources around us

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P-PD12

GEOGRAPHIC INFORMATION SYSTEM AND P. falciparum MALARIA IN JAYAPURA, PAPUAN PROVINCE

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Abstract

Malaria caused by \textit{Plasmodium falciparum} parasites exacts its greatest toll in Jayapura, Papuan Province, Indonesia, where it is one of the largest causes of morbidity and mortality, creating a significant barrier to economic development. As a step towards reversing this trend, there is growing interest in the mapping and predictive modelling of the geographical limits, intensity and dynamics of the risk of malaria infection, using new tools of surveillance. An unprecedented amount of information on environmental conditions, remotely sensed by satellite sensors, is now available at temporal and spatial resolutions to match our epidemiological questions. Here we showed that how these tools are used to investigate the factors that drive the dynamics of vector populations and malaria parasite transmission on Jayapura, Papuan province. Patterns of infection vary through time owing to extrinsic (for example, climate) and intrinsic (for example, immunity) effects. Studies have already related vector mortality rates and abundance to satellite data, and biological models have been developed for a few vectors and vector-borne pathogens. Extensive satellite coverage coupled with vector-borne pathogen models that are appropriate at a local scale will enable us to build spatially rich, accurate models of vector-borne pathogens. If we can understand transmission dynamics well enough to model the present, we should be able to develop accurate disease early-warning systems in the future. It is clear that the technologies we now have to study these diseases are far better than those available to malarialogists in the early years of the last century.

Keywords: Geographic Information System, \textit{P. falciparum} Malaria, Jayapura

Introduction

Malaria caused by \textit{Plasmodium falciparum} parasites exacts its greatest toll in Jayapura, Papuan Province, Indonesia, where it is one of the largest causes of morbidity and mortality, creating a significant barrier to economic and education development. As a step towards reversing this trend, there is growing interest in the mapping and predictive modelling of the geographical limits, intensity and dynamics of the risk of malaria infection, using new tools of surveillance. An unprecedented amount of information on environmental conditions, remotely sensed by satellite sensors, is now available at temporal and spatial...
resolutions to match our epidemiological questions. Studies have already related vector mortality rates and abundance to satellite data, and biological models have been developed for a few vectors and vector-borne pathogens. Extensive satellite coverage coupled with vector-borne pathogen models that are appropriate at a local scale will enable us to build spatially rich, accurate models of vector-borne pathogens.

Materials and Methods

Satellite sensor designs are rarely ideal for epidemiological studies because of trade-offs between spectral, spatial and temporal resolution, determined by constraints of the earth’s atmosphere, or the original requirements of commissioning agencies on Papuan Province. Diseases caused by vector-borne pathogens vary in magnitude through space and time much more than directly transmitted pathogens, because their innate capacity to increase is usually much higher. This is expressed as the basic reproductive number $R_0: R_0 = ma^2bc_0e^{-TH/ur}$. Predictive accuracy can be assessed using a contingency table that compares the training set data and the suitability category to which the pixels were assigned. From this is calculated the overall percentage of correct predictions, the percentage of false positives and false negatives (that is, false predictions of presence and absence, respectively), and the sensitivity and specificity (proportion of positives or negatives, respectively, correctly identified). The kappa index of agreement, k, measures predictive accuracy compared with a null model (that is, one with no predictive skill); values vary between 0 (fit no better than random) and 1.0 (perfect fit), with a value of more than 0.75 regarded as excellent; confidence intervals can be attached to k values. Once robust and reliable correlations between the satellite and disease data are established, real-time monitoring of environmental conditions by satellites can provide valuable inputs into disease early-warning systems.

Results and Discussion

Here we show how these tools are used to investigate the factors that drive the dynamics of vector populations and malaria parasite transmission. Because mosquito population processes and malaria incubation periods in vectors, for example, vary with temperature and moisture conditions on the ground, remotely sensed images of seasonal climate are powerful predictors of mosquito distribution patterns and average levels of transmission of malaria parasites by these vectors. Patterns of infection vary through time owing to extrinsic (for example, climate) and intrinsic (for example, immunity) effects. The balance of these factors depends upon the levels of malaria transmission in each place and will change over time with resistance to control of parasites and vectors. Early-warning systems, therefore, will require models that incorporate both intrinsic and extrinsic factors.
The hypothetical relationship between the challenge presented to the human population by a vector population and the resulting incidence or prevalence of infection, each assuming stable conditions, is complex and nonlinear (Fig. 1). The humped curve is determined by interactions between transmission rates, the rate of development and duration of temporary acquired immunity in the vertebrate host population, and the age structure of the latter. But the precise shape of the relation, and its implications for malaria control, are controversial, not least because of a shortage of good quality field data available for its determination. The data that do exist for Papuan Province, now being gathered together in the ambitious Mapping Malaria Risk in East Indonesia, may nevertheless be used first to examine the ‘challenge’ axis of Fig. 1.

![Graph showing the relationship between challenge and population prevalence]

**Figure 1.** Hypothetical relationship between the challenge to a host population by a vector-borne pathogen and the risk of the host becoming infected. Challenge is a function of many elements of the vector’s biology; risk is often modulated by host resistance and/or acquired immunity. Fixed age-specific prevalences at each level of challenge with different levels of vertebrate host mortality rate (m in the figure) produce population prevalence/challenge curves of different overall shapes on Papuan Populations.

Extensive satellite coverage coupled with vector-borne pathogen models that are appropriate at a local scale will enable us to build spatially rich, accurate models of vector-borne pathogens. If we can understand transmission dynamics well enough to model the present, we should be able to develop accurate disease early-warning systems in the future. It is clear that the technologies we now have to study these diseases are far better than those available to malarialogists in the early years of the last century. The challenge is to make the science of malaria prediction at least as good.

**References**


P-PD13

EFFECT OF WATER TABLE DEPTH ON THE ROOT SYSTEM OF Acacia crasicarpa IN PEAT SOIL (HISTOSOLS)

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Abstract

Indonesia has 19 million ha of peat soils or organic soils (Histosols). Some parts the soils, especially in Riau and South Sumatera provinces, have been used for planting Acacia crasicarpa as raw material of pulp and paper. As for planting the acacia, water tables must be lowered to make it suitable for the root growth but remain maintaining the humidity in order to avoid fire and prevent emission of CO2, SO2, NO2 gas from aerobic decomposition. The objective of this research was to investigate the difference of water tables on the root systems of the acacia.

The research was conducted on peat soil with different depth of water table, i.e. ≤ 60, 80 and 120 cm from the surface. The research was done in 5 years old acacia plantation of Riau Andalan Pulp and Paper (RAPP) which located in Pelelawan District, Riau Province.

The result showed that most of the roots distributed laterally in the soil less than 25 cm from the surface. In soils with water table of 120 cm, the taproots penetrated to the depth of 80 cm whilst in the soil with water table of 80 cm it only grew to the depth of 60 cm and began to turn laterally at about 15 cm above the water table surface. In the soil with water table less than 60 cm, the taproots did not develop. Growth of the acacia was enhanced by lowering the depth of water table.

Keywords: water table, peat soil, root system, acacia.

INTRAUCTION

Indonesia has 33.39 million hectares of wetlands, mainly in, Kalimantan (11.71 million ha), Papua (10.52 million ha), Sumatera (9.37 million ha) and Sulawesi (1.79 million ha) (Anonim, 2007). Of those 19 million ha are peat soils or Histosols according to Soil Survey Staff (1998). Some parts of the peat soil in Sumatera, especially in Riau and South Sumatera provinces have been used for planting Acacia crasicarpa. The use of peat soils for planting acacias faces various problems. Oxygen (O2), phosphorous (P), potassium (K), calcium (Ca), molybdenum (Mo) are elements most often deficient in peat soils (Fisher and Binkley, 2000). Water tables must be low to make it suitable for the root growth. Root system can be characterized on the basis of (1) rooting habit, which is related to form, direction and distribution of the roots and (2) root intensity, which pertains to the form, distribution and number of small roots. Form of a root system is influenced by local site conditions such as
depth of water table, water saturation (moisture), aeration, bulk density, temperature and soil chemistry (Fisher and Binkley, 2000).

The growth of acacia at the same age (5 years old) varied among the compartments. The main possible cause of the difference was variation of water table depth of the site. The objective of the research was to investigate the effect of water tables depth difference on the root systems of the acacia.

MATERIALS AND METHODS
This research was conducted in 5 years old acacia plantation in RAPP. The site was selected based on the difference of water table, namely ≤ 60, 80 and 120 cm. According to the stage of decomposition, most of the peat was consisted of hemist and saprist with the depth of around 3 m. Investigation was conducted to 5 replication trees per treatment. Parameter measured were plant height, stem diameter and the root system after digging the peat soils using mattock. Root parameter measured were root system diameter, the longest lateral root, the deepest taproot and water content of the peat at the depth of 40 cm from the surface.

RESULTS AND DISCUSSION
The result showed that most of the roots distributed laterally in the soil less than 25 cm from the surface (Fig. 1).

In soils with water table of 120 cm, the taproots penetrated to the depth of 80 cm whilst in the soil with water table of 80 cm it only grew to the depth of 60 cm and began to turn laterally at about 15 cm above the water table surface. In the soil with water table less than 60 cm, the taproots did not develop (Table 1; Fig 2).
This study indicated that the growth of the acacia can be enhanced by lowering the water table because the deeper the water table the deeper the taproot grows. By lowering the water table there are more oxygen available and less water content. This also stimulated the lateral growth of the root hence increasing the diameter of root system (Table 1). Consequently the plants will be less vulnerable to fall down and disease attack.

Table 1. The average of stem diameter, height, root diameter and the longest of root of Acacia crassicarpa (5 yrs old)

<table>
<thead>
<tr>
<th>Water table depth (cm)</th>
<th>Stem diameter (cm)</th>
<th>Height (m)</th>
<th>Root system diameter (cm)</th>
<th>The deepest taproot (cm)</th>
<th>The longest lateral root (m)</th>
<th>Water content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤60</td>
<td>8</td>
<td>12</td>
<td>5.12</td>
<td>nd</td>
<td>2.94</td>
<td>90.34</td>
</tr>
<tr>
<td>80</td>
<td>20</td>
<td>17</td>
<td>10.10</td>
<td>60</td>
<td>5.53</td>
<td>79.43</td>
</tr>
<tr>
<td>120</td>
<td>25</td>
<td>18</td>
<td>16.30</td>
<td>80</td>
<td>8.14</td>
<td>65.38</td>
</tr>
</tbody>
</table>

REFERENCES


P-PD14

SCREENING OF SOYBEAN GERMLASMS THAT TOLERANT TO ACID TIDAL SWAMP

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Abstract

Availability of fertile land in Java decreases due to the shifting agricultural land to non-agricultural land. Hence, there is a need for an extensification of soybean cultivation to suboptimal land areas, such as tidal swamp where the wide area is about 20.192 million hectares. The main problems in this soil are soil acidity, Fe toxicity and water excess. To provide acid tidal swamp tolerant gene resources, glasshouse experiment at ILETRI Malang has been conducted using 185 genotypes of germplasm. The experimental design was factorial randomized completely block design with two factors. The first factor was low pH consisted of two factors, i.e. (1) aerated waterlogging with pH 7 and (2) unaerated waterlogging with pH 4.0. The second factor was genotype which consisted of 185 genotypes. Glasshouse experiment followed by field experiment at Belandean research station, Banjarbaru – South Kalimantan, using the best 17 genotypes selected from glasshouse experiment. The experiment Design was randomized completely block design with two replications. Waterlogging treatment was conducted at one level, i.e. unaerated waterlogging with low pH according to field condition. Results showed that there is variability on responses of each genotypes to acidity and Fe toxicity. Assessment of soybean tolerance to acidity and Fe toxicity based on by root growth. Based on selection criteria, varieties of Lawit and Menyapa which served as check tolerant varieties, having lower growth than the seventeen selected genotypes. While, on field experiment, genotype of MLGG 1087 was identified as the most tolerant one than the twenty tested genotypes. That genotype can served as acid tidal swamp tolerant gene resource because it has highest relative growth rate of root dry weight (not clear), and average of root and shoot dry weight.

Keywords: tidal acid soil, Fe toxicity, soybean, germplasm

INTRODUCTION

Tidal swamp is a marginal land and a fragile ecosystem, because the suitability for agricultural commodities in originally nature condition classified as \textit{marginally suitable} (S3) and moreover included to \textit{presently not suitable} (N1). Many plant growing constraint on tidal area caused by saturated and anaerobic rhizosphere (mud, reduction reaction, oxygen
and H$_2$S gas deficiencies), existing of pyrite or sulfidic materials, Al, Fe Mn toxicities, very acidic reaction of soil, and low natural fertilities (P, N and K deficiencies, and poor bases). While, on waterlogging condition, when water table start to tide to the surface or during rain; increasing pH caused by reduction process leads decreasing Al and Mn toxicities but increase Fe$^{2+}$, H$_2$S, CO$_2$ and water soluble organic acids toxicities. Excess water availability at tide condition also have important role where causes early senescence, which ensues on leaves chlorotic, necrotic and defoliation, nitrogen fixation decreasing and growth termination and seed yield decreasing. Waterlogging at final vegetative phase can decrease seed yield up to 18%; while at early reproductive phase, can decrease seed yield up to 26%. This correlated with plant capacity to survive on oxygen deficiency condition. Finally, tidal period will effect soybean production. Objective of research was to find out soybean gene resources tolerant to acid tidal swamp.

**MATERIALS AND METHOD**

Research was conducted on February – March 2007 at Illetiri’s greenhouse, Malang; followed field experiment at Belandean research station, Banjarbaru – South Kalimantan in May – July 2007. In the greenhouse experiment, plants material were 185 genotypes of soybean seeds from Illetiri collection, included two check adaptive tidal swamp varieties Lawit and Menyapa. Factorial randomized completely block design with two replications used in this experiment. First factor was low pH consisted of two factors, i.e. (1) aerated waterlogging with pH 7 and (2) unaerated waterlogging with pH 4.0. And second factor was genotype, consisted of 185 genotypes. Plants were observed at age 0 and 7 days after transplanting (dat). Relative growth of each organ measured according Nursyamsi (2000).

In the field experiment, plants material were 17 genotypes selected from greenhouse experiment. Design was randomized completely block design with two replications. Stress treatment was conducted at one level i.e. acid waterlogging. Observation was carried out at age 0 and 14 days after transplanting.

**RESULT AND DISCUSSION**

**Greenhouse screening**

Waterlogging treatment with and without aeration was affecting on plant growth. Symptom of toxic plants were chlorosis and leaf twisted (Albano et al., 1996) and also hypocotyls and epicotyls shortening. Oxidative stress also caused the decreasing of total formed chlorophylls (Mehraban et al., 2008). However, the effect of waterlogging was not affected to the plants life (Boru et al., 2003). Rate of relative weight root growth was varied from -0,64 – 13,50 g/7 days with mean1,29 g/7 hari. Negative value from rate growth
showed that root growth of sensitive genotypes were terminated, beside the root was broken so the mass decreased. Genotypes of MLGG 0731 and MLGG 0511 had highest rate of root dry weight growth relative, i.e. 13.50 g/7 days and 11.21 g/7 days respectively. On other genotypes rate of root dry weight growth relative was not as high as those two genotypes. While on shoot dry weight character, MLGG 0647 showed highest rate growth that reached 10.03 g/7 days.

Tolerant plants to acid waterlogging stress were evaluated based on all observed characters simultaneously using selection index (Singh and Chaudhary, 1980). According to the selection index formula with limit of selection criteria >0.75, there were obtained 17 identified genotypes tolerant to acid waterlogging. Genotype of MLGG 0731 had first rank with index value 3.055 followed by MLGG 0511 and MLGG 0170, while genotypes having 15, 16 and 17 ranks were MLGG 0238, MLGG 0061 and MLGG 0592.

**Field Screening**

According to root dry weight criteria, MLGG 1087 genotype had first rank position with root dry weight average 0.024 g; followed by MLGG 0511 and MLGG 0731 with rate 0.016 and 0.013 g respectively. But based on root length average, genotype of MLGG 0592 had first rank followed by MLGG 0511 and MLGG 0009, while root length of MLGG 1087 was shorter than average of all genotypes. On shoot dry weight characters, genotype of MLGG 1087 also having first with average of 0.072 g, where this value was higher than two times of average of all genotypes. On hypocotyls length characters, MLGG 0238 had highest hypocotyls (18.12 cm) followed by MLGG 0592 (17.84 cm) and MLGG 0257 (17.68 cm). Genotype of MLGG 1087 with highest shoot dry weight had lower hypocotyls length than average genotypes. It means that MLGG 1087 had hypocotyls diameter higher than other genotypes.

Genotypes of MLGG 1087 had highest shoot dry weight assumed due to (1) this genotype can eliminate Fe through oxidation in rhizosphere and exudation Fe from plant tissues, or (2) Fe inactivating in plant tissues. Wasiaturrohmah (2008) reported the occurring of faster pH increasing of identified Fe tolerant genotypes in rhizosphere.

There is needed to screen tolerant gene resources using germplasm collection to find out acid tidal swamp tolerant soybean, so probability in developing soybean for tolerance to acid tidal swamp become higher. Genotype of MLGG 1087 can be served as acid tidal swamp tolerant gene resource because having highest root and shoot dry weight.
REFERENCES
P-PD15

The Effect of Aloe vera gel on ethylene emission and post harvest quality of tomato fruit (Lycopersicon esculentum Mill.)

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ABSTRACT

An effort to lengthen the post harvest quality of tomato fruit (Lycopersicon esculentum Mill.) using Aloe vera gel has been carried out. The experiment was aimed to evaluate the effectiveness of Aloe vera gel to supress ethylene emission from tomato fruit in order that the freshness of tomato fruit can be maintained for a longer time.

Tomato fruits were purchased from local market and were selected which have similar size and colour. Fruits were then treated with or without Aloe vera gel. Three replicates were used for each treatment. The emission of ethylene from tomato fruit was determined using Photoacoustic Spectrometer.

The results showed that tomato fruits that were treated with Aloe vera gel have much lower ethylene emission compared to control fruits at the early ripening conditions. The average value of ethylene emission from controls and fruits treated with Aloe vera gel were 788 ppb/g and 158 ppb/g respectively. However, at fully ripening condition there were no significant difference in ethylene emission from both controls and treated fruits. It was concluded that application of Aloe vera gel on tomato fruits inhibit the synthesis of ethylene at early ripening condition and it prolong the freshness of tomato fruits to about three days.

Key words : Aloe vera gel, Lycopersicon esculentum Mill., ethylene.

INTRODUCTION

Newly harvested fruits are still carrying out metabolism process including respiration, and several changes may occur, such as fruit colour, flavor, and taste. The quality of harvested fruit normally decreases when stored in room temperature for long time, and several factors like microbia or other defects in fruit skin, as well as the ethylene releases during the fruit maturation process can accelerate the fruits become rotten. Tomato fruits (Lycopersicon esculentum Mill.) are consumed either raw as fruit or fruit juice, or cooked as tomato sauce. Some efforts to lengthen the post harvest quality of fruits are through storage in cool temperature or by application of gel. Valverde et al. (2005) had reported the potency of Aloe vera gel in prolonging the quality and freshness of grape fruits. It was found that
application of *Aloe vera* gel on Crimson Seedless grapes lengthen the freshness of those grape fruits to 35 days compared to non-treated fruits that only can stay fresh for 7 days. It has been reported that *Aloe vera* gel contain several compounds that play role either as antibiotic or antifungal that can inhibit the growth of microorganisms or fungi spores that stick on the fruit surface (Fumawanthing, 2004). An effort to lengthen the freshness of tomato fruit using *Aloe vera* gel has been carried out. The experiment was aimed to evaluate the effectiveness of *Aloe vera* gel to suppress ethylene emission from tomato fruit in order that the freshness of tomato fruit can be maintained for a longer time.

**MATERIALS AND METHOD**

Tomato fruits were purchased from local market and were selected which have similar size and colour. The fruits were on its early maturing condition. Fruits were washed with water and then treated with or without *Aloe vera* gel for 10 minutes. Three replicates were used for each treatment. The emission of ethylene from tomato fruit at the early maturing and matured conditions were determined for 20 minutes using Photo Acoustic Spectrometer. The speed of fruit maturation and duration of storage longevity were also determined.

**RESULTS AND DISCUSSION**

The results showed that tomato fruits that were treated with *Aloe vera* gel have much lower ethylene emission compared to control fruits at the early ripening conditions. The average value of ethylene emission from controls and fruits treated with *Aloe vera* gel were 788 ppb/g and 158 ppb/g respectively. However, at fully ripening condition there were no significant difference in ethylene emission from both controls and treated fruits.
Aloe vera gel that covered the surface of tomato fruits probably will limit the absorption of oxygen required for cell respiration, in addition certain compounds contained in Aloe vera gel, such as cooper and chromium may also inhibit the binding of ethylene to the responsive cells in the fruit. The inhibition of ethylene emission in fruits treated with Aloe vera gel also slows down the maturation process. As a result, the freshness of fruits treated with Aloe vera gel can be prolonged and the fruits can be stored about three days longer than those of control (Fig.2). In conclusion Aloe vera gel inhibits the ethylene emission of early maturing tomato fruits and it show a potency to be used as an organic material for lengthening the freshness of tomato fruits.

REFERENCES
P-PD16
OPTIMALIZATION OF MULTINUTRIENT FEED SUPPLEMENT ON SORGHUM STOVER AS FEED FOR RUMINANTS

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ABSTRACT

Research was done to obtain complete feed from agricultural waste i.e. sorghum stover with additional supplement to increase the nutrition value. Research using various concentration of Multinutrient Food Supplement (MFS) S1 = 0% MFS, S2 = 2.5% MFS, S3 = 5% MFS, S4 = 10% MFS, dan S5 = 20% MFS. The randomized Complete Block Design has been used for analyzing treatment effect. The parameters which are used digestibility of dry matter and organic matters, gas production and synthesis cell biomass. Result shows that the highest dry matter and organic matters was found at S1 (0% MFS). Gas and biomass production no significant different and treatment, but the trend showed increase at S4 or 10% MFS.
P-PD17

PUERARIN ACTIVITY TO PREVENTS AND RESERVES OF APOPTOTIC AND NECROTIC CELLS EXPRESSION OF HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS (HUVECS) EXPOSED BY LEPTIN IN VITRO

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Abstract

The aim of the research was to know the effect of puerarin an apoptotic and necrosis at Human Umbilical Vein Endothelial Cells (HUVECs) induced by leptin. HUVECs culture had been labeled BrdU during 20 hours, then separated become cluster that were (1) control (without leptin and puerarin), (2) induced by leptin 25 ng/mL, (3) induced by single puerarin 5 ; 25 ; 200 and 525 μM respectively, (4) induced by leptin and puerarin each 5 ; 25 ; 200 and 525 μM. Leptin and puerarin were incubated for 6 hours at 37°C. Apoptotic and necrotic cell were evaluated by ELISA methode for each unit of treatment were replicated 3 times with completely randomized design. The data were analyzed with oneway of ANOVA using SPSS 12.0 for windows program. It was showed that puerarin until 525 μM can not decreased apoptosis and necrosis leptin-induced HUVECs.

Keyword : Apoptosis, HUVECs, leptin, necrosis, and puerarin.
P-PD18

HORMONAL TREATMENT IN SHOOT ORGANOGENESIS OF SUGARCANE (Saccharum officinarum. L) THAT SUITABLE FOR GENETIC TRANSFORMATION VIA Agrobacterium tumefaciens.

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ABSTRACT

Rapid and efficient in vitro regeneration methods that minimize somaclonal variation are critical for the genetic transformation and mass propagation of commercial plant varieties. We have identified some hormones that regulated the development and regeneration of in vitro sugarcane. Axillary bud were isolated from sugarcane stems treated with combination of 1,5 mgL⁻¹ BAP and 0,1 mgL⁻¹ GA₃ were faster to induce development of shoot than the other hormone. Liquid culture was more effective development of shoot than solid culture. Shoot treated with combination of 0,5 mgL⁻¹ 2,4D and 1,5 mgL⁻¹ BAP followed by sub-cultured in free hormone medium produced rapid and high frequency of shoot formation. Average shoot formation were 252 shoots per explants after 30 days. These shoot regeneration system is suitable for agrobacterium-mediated transformation. Agrobacterium-mediated transformation using this explants resulted in higher frequency of putative transforman. The results suggest that production number of shoot is an important aspect in sugarcane transformation.

Keywords: Hormone, Sugarcane, Transformation, Agrobacterium tumefaciens.

INTRODUCTION

The method of plant regeneration through callus cultures increases the risks of somaclonal in sugarcane (Liu,1984; Lee, 1987). Regeneration through axillary buds causes minimal genetic changes, and is routinely used for mass multiplication of plants, including sugarcane (Taylor and Dukie, 1993). Here, we report on the development of the regeneration system and discuss the role of developmental and hormonal factors that regulate shoot of axillary bud in sugarcane.

MATERIALS AND METHODS

The axillary buds of 7-8 months old sugarcane were sterilized with 70% alcohol and 0.05% mercuric chloride (HgCl₂) for 1 min, then washed in sterile distilled water and cultured
either in solid or liquid MS media containing various hormones. The best result of hormonal treatment was then used for transformation using *Agrobacterium tumefaciens*.

**RESULT AND DISCUSSION**

Axillary buds cultured in solid media containing 1.5 mg/l BAP+0,1 mg/l GA3 initiated shoot formation after 4 week incubation under light illumination. While in a liquid media, axillary buds treated with 1,5 mg/l BAP+ 0,1 mg/l GA3 not only initiated shoot formation but also developed their leaves. Hormone treatment of 1,5 mg/l BAP+ 0,1 mg/l GA3, initiated shoot formation for the first time at 6,75 days after planted, but treatment with other hormone such as MS0, 0,5 mg/l 2,4-D+0,1 mg/l GA3; 0,1 mg/l GA3 were 10,6; 16,25 ; 11 days after planted, respectively. Growth of axillary bud showed highest in media containing GA3, but it was not significantly different with the media of 1,5 mg/l BAP+0,1 mg/l GA3. The height of axillary buds in media containing GA3 were 1,76 cm; 1,86 cm and 2,1 cm at 2, 4 and 6 week after planted, respectively (Figure 1). Collectively, the results showed that liquid media containing BAP+ GA3 initiate shoot formation and growth of axillary bud more effective than other media.

![Figure 1: Effect of hormone treatment in an axillary bud growth.](image)

Axillary buds were isolated from sugarcane steam and cultured on MS liquid media containing respective hormones combination. High shoots were monitored at indicated times.

To have a better understanding of hormonal effect on sugarcane shoot multiplication, the developed shoots from auxiliary bud were growth on MS media containing five different auxin-cytokinin combination. MS medium containing 0.5 mg/l of 2,4-D with 1.5 mg/l BAP gave a best hormone combination for shoot formation (Figure 2). However, hormonal treatments caused an increase of phenolic compound that might retard growth and development of the sugarcane explants. Thus, subculture of the explants after hormonal treatment of 0.5 mg/l of 2,4-D with 1.5 mg/l BAP to medium free hormone resulted in
blooming of higher frequency of shoot formation. Average of shoot formation were 252 shoots per explants after 30 days in medium free hormone (Figure 3).

![Graph showing average number of shoots per explant under different hormonal treatments.]

This shoot regeneration system is suitable for Agrobacterium-mediated transformation. Agrobacterium-mediated transformation using explant generated from the sytem resulted in high putative sugarcane transformation.

ACKNOWLEDGEMENT

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REFERENCES

In vitro Activity of Protein Isolated from Melinjo Seeds (Gnetum gnemon L.) Used Antimicrobial Agent Against Phytopathogenic Microbial

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INTRODUCTION

Plant diseases caused by bacteria, and fungi affect crop and are responsible for significant losses and decrease the quality and safety of agriculture product. Their control relies mainly on chemical pesticides. The control of chemical pesticides affect negative to environmental, human, pathogen resistance and plant (1). Therefore was controlled by natural compound as the alternative solution. Antimicrobial proteins are important substances functioning as self-defense against infection by various harmful pathogens. They are isolated from various sources among plants, animals, and have been characterized. The height level of protein in melinjo seeds showed a potential matter that is valuable as an antimicrobial protein source. That’s why a proper solution and purification protein research is needed in order to isolate antimicrobial protein from melinjo seeds. It’s important since the protein could reduce against phytopathogenic microbial growth.

MATERIAL AND METHODS

Materials : melinjo seeds (Gnetum gnemon L.) were obtained from melinjo tree in Jember, East Java, Indonesia. The following bacteria and fungi were Erwinia carotovora, Bacillus cereus, Xanthomonas sp., and Pseudomonas solanacearum, Fusarium oxysporum, and Phytophthora macrophils. Protein Extraction and Purification : mature melinjo seeds (50 g) were homogenized with 100 volumes of 0.05 M NaCl and 0.01 M Tris-HCl buffer (pH 7), the homogenate was filtered through gauze and the filtrate was centrifuged at 12,000 rpm for 20 min. The clear supernatant was concentrated under reduce pressure and saturated with ammonium sulphate (0-30%, final concentration of ammonium sulphate: percentage saturation). The precipitate was dissolve in a small amount 0.15M ammonium acetate buffer Tris-HCl (pH 7) and pass through purification with chromatography ion exchange CM-cellulose (cation exchange) and DEAE-cellulose (anion exchange) and the elution was monitored at 280 nm. The active fraction were pooled and loaded into SDS-PAGE. Electrophoresis : SDS-PAGE containing 12.5% a polyacrylamide separating gel with 5% a stacking gel according to the manufacturer’s instruction. Low molecular mass markers were run simultaneously in the gel. The protein bands were visualized by Coomassie blue staining. Protein concentration was measured by the Bradford method with bovine serum albumin as a standard. In vitro Assay of Antimicrobial activity : antimicrobial activity was measured by a method Radial Diffusion Assay and Filter Paper Disc Assay (for bacteria and fungi) (2). Inoculation of fungi (F. oxysporum) were mixed with appropriate concentration of protein (Gg-AMP) by injecting in tomato fruit (3).
RESULT AND DISCUSSION

The antimicrobial activity of melinjo seeds (50g) were purified by chromatography ion exchange, the active fraction was obtained by DEAE-cellulose. The result showed that protein total and specific activity in DEAE-cellulose was increased 17.22 mg and 934.33 unit/mg, respectively (Table 1). As shown in Fig. 1 Gg-AMP migrated as single band with apparent molecular weight of about 12 kDa. The majority of the antimicrobial protein isolated from other plant relatively small (≤ 10 kDa).

Table 1. Summary of purification of Gg-AMP from Melinjo seeds

<table>
<thead>
<tr>
<th>Step</th>
<th>Total Volume (ml)</th>
<th>Protein Total (μg)</th>
<th>Activity Total (unit)</th>
<th>Specific Activity (unit/mg)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude extract</td>
<td>125</td>
<td>384.33</td>
<td>706.60</td>
<td>199.39</td>
<td>160.00</td>
</tr>
<tr>
<td>Precipitation Am. sulfat 30%</td>
<td>35</td>
<td>76.60</td>
<td>452.16</td>
<td>590.28</td>
<td>21.62</td>
</tr>
<tr>
<td>CM-cellulose</td>
<td>25</td>
<td>58.99</td>
<td>379.94</td>
<td>745.06</td>
<td>14.39</td>
</tr>
<tr>
<td>DEAE-cellulose</td>
<td>15</td>
<td>27.22</td>
<td>254.34</td>
<td>934.33</td>
<td>7.68</td>
</tr>
</tbody>
</table>

The purified protein (Gg-AMP) showed potent antibacterial activity against plant pathogenic gram-negative and positive bacteria (Fig. 2). Antifungal activities of the purified protein against F. oxysporum and P. maydis with 1.37 μg/μl and various volume per disc (Fig. 3). Inhibition activities shown with clear zone around sample hole or paper disc.

![Fig. 2. The effect on growth of bacteria phytopathogenic plant of the purified Gg-AMP. (a) Gram-positive B. cereus. Filter Paper Disc Assay method (b) bacteria Gram-negative P. solanacearum. Radial Diffusion Assay method (c) clear zone inhibition as control contain 0.01M Tris-HCl buffer (pH 7) and protein control with 1μg/μl Bovine Serum Albumin 25μl various concentration applied contain purified Gg-AMP 1,2,3,4,5,6,7, 0.1,0.15,0.2, 0.26,0.32,0.45,0.64 μg/μl respectively and 25μl volume per disc.](image)

![Fig. 3. Inhibitory activity of Gg-AMP on the growth P. maydis (a) and F. oxysporum (b) by radial diffusion assay (RDA), as shown inhibition activities with clear zone around hole sample (c), as control contain 0.01M Tris-HCl buffer (pH 7) and protein control with 1μg/μl Bovine Serum Albumin 25μl Concentration of Gg-AMP 1.37 μg/μl with various volume: a/b1,a/b2,a/b3,a4 and a5; 15μl, 20 μl, 25 μl, 30 μl and 10 μl respectively.](image)
Low concentration of purified Gg-AMP not inhibited growth of hyphae, but the increases concentration with volume same shown inhibition growth of hyphae P. maydis and F. oxysporum. Mode of action the antifungal toward against was first described as involving either fungal cell lysis or interference with fungal cell wall synthesis. The antifungal activity such as many of those isolated from plant to be relative rich in polar and neutral amino acids (4). The antifungal activities of Gg-AMP were investigated on tomato fruit was highly potent against F. oxysporum in a concentration-dependent manner. Figure 4 shows representative result from these infectivity assay of tomato fruit.

Fig 4. Effect of purified Gg-AMP on the growth of hyphae F. oxysporum on tomato fruit. (a) as control (not inoculated with spores and Gg-AMP) (b) purified of Gg-AMP was mixed at concentrations with spores F. oxysporum and inoculated with 1.37 μg/μl on the surface tomato fruit (c) as control with inoculated with spore F. oxysporum without Gg-AMP (d) zoom cropping pictures c. the growth of hyphae without Gg-AMP. Pictures were taken 10 days after inoculation.

References

P-PD20

CHITINOLYTIC FUNGI AS BIOLOGICAL CONTROL AGENTS OF PATHOGENIC FUNGI

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ABSTRACT

The aim of this research was to obtain fungi isolates that have high chitinolytic. The isolation
of fungi was done using minimal medium with chitin as carbon source. All isolates were selected
based on hidrolysis activity by comparing hidrolysis zone diameter with colony diameter and chitinase
specific activity measured by spectrophotometric method.

The result of research found 70 isolates of chitinolytic fungi isolated from chitinous soil,
chitinous waste and rhizosphere. Based on hidrolysis activity, it was obtained 18 isolates with
hidrolysis activity ≥ 2,00. Ten among 18 isolates have higher chitinase specific activity than
Trichoderma viride as reference isolate (210,14 U/mg). KUP2 isolate had the highest chitinase
specific activity 744,20 U/mg. Therefore, it can be concluded that KUP2 isolate is a potential as
biological control agents.

Key words : chitinolytic fungi, chitinase, biological control

INTRODUCTION

Chitinolytic fungi have been reported to be capable on degradation of chitin by
secreting chitinase enzymes. Chitinase enzymes used as biological control agents of
pathogenic fungi. In fact, that chitin is the main structural component of fungi cell wall, which
ranges from 22 – 40% [2]. To inhibit growth of the pathogenic fungi, chitin should be
destroyed. The objective of this study was to obtain fungi isolates that have high chitinolytic
as biological control agents.

MATERIAL AND METHODS

Chitinolytic fungi were isolated from soil, chitinous waste and rhizosphere using
spread plate method on colloidal chitin agar (CCA) medium [1]. All isolates were qualitative
selected based on hydrolysis activity on CCA medium, by comparing clear zone diameter
around colony with colony diameter. Then selected isolates were quantitative assayed term
of chitinase specific activity (chitinase activity per mg protein), which grown in liquid minimal
medium [4], incubated for 7 days on a rotary shaker 150 rpm (rotary per minute). Its
suspensions were centrifuged at 3000 rpm, 4°C for 30 minutes. The supernatant was used as a source of crude enzymes. Chitinase activity was measured on turbidity reduction of colloidal chitin using spectrophotometric method. Protein concentration was determined according to Bradford method.

**RESULT AND DISCUSSION**

Seventy isolates of fungi were able to produce clear zone around colony on colloidal chitin agar medium (Data not shown). Clear zone as indicator of chitin degradation in solid medium by chitinase [1]. Eighteen among 70 isolates had hydrolysis activity ≥ 2.00 (Table 1). Based on quantitative selection showed that 10 isolates were had higher chitinase specific activity compared with *Trichoderma viride* FNCC 6128 (210,14 U/mg) as reference isolate (Table 2). There was no correlation between hydrolysis activity and chitinase specific activity. It was assumed that hydrolysis activity had no correlation with chitinase activity due to the use of different media. The growth of fungi in liquid medium was rapidly than the growth on solid medium [3]. Based on chitinase specific activity, it was concluded that KUP2 isolate had the highest chitinase specific activity 744,20 U/mg, therefore it was regarded a potential as biological control agents.

**Table 1. Hydrolysis activity of chitinolytic fungi on colloidal chitin agar medium**

<table>
<thead>
<tr>
<th>No</th>
<th>Isolate code</th>
<th>Ø Clear zone (cm)</th>
<th>Ø Colony (cm)</th>
<th>Hydrolysis activity</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>KUP2</td>
<td>7.28</td>
<td>2.80</td>
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<td>2</td>
<td>KUP5</td>
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<td>2.50</td>
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</tr>
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<td>3</td>
<td>PIK6</td>
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<td>2.80</td>
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<td>RLF1</td>
<td>3.10</td>
<td>1.40</td>
<td>2.21</td>
</tr>
<tr>
<td>18</td>
<td>RLF2</td>
<td>5.90</td>
<td>2.80</td>
<td>2.11</td>
</tr>
<tr>
<td>18</td>
<td>RLF3</td>
<td>5.90</td>
<td>2.80</td>
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</table>
Table 2. Enzyme specific activity of chitinolytic fungi

<table>
<thead>
<tr>
<th>No</th>
<th>Isolate code</th>
<th>Protein concentration (mg/ml)</th>
<th>Enzyme activity (U/ml)</th>
<th>Specific activity (U/mg)</th>
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<tr>
<td>1</td>
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<td>0.0446</td>
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<td>28.9362</td>
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<tr>
<td>4</td>
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<td>27.7660</td>
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<td>PID2</td>
<td>0.0510</td>
<td>26.9149</td>
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<td>6</td>
<td>LKK1</td>
<td>0.1020</td>
<td>27.1277</td>
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<td>7</td>
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<td>0.0829</td>
<td>32.4468</td>
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<td>8</td>
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<td>444.24</td>
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<td>9</td>
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<td>12</td>
<td>T. viride FNCC 6128</td>
<td>0.0893</td>
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REFERENCES


P-PD21

ANTAGONISM ACTIVITY AND CHARACTERIZATION OF Bacillus subtilis 140B TOWARDS PLANT PATHOGEN IN TOMATO PLANTATION

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corresponding author lie_azzahra@yahoo.co.id

ABSTRACT
A soil bacterium, isolate 140B, was isolated from pineapple garden soil – GGP Lampung Province, showed high antagonistic activity against plant pathogen fungi such as Fusarium solani, Fusarium oxysporum and Curvularia sp. The aims of this experiment are to identify the isolate 140B through 16S rDNA and to know its potency to inhibit the tomato diseases. The DNA sequence analysis indicated that isolate 140B was high similarity with Bacillus subtilis. The role of isolate B subtilis 140B on suppressing tomato diseases was studied in comparison with CC.13, CC.20 and CC.88204 isolates, which was known as biocontrol agents and showed that B. subtilis 140B has a better result to control tomato plant disease. The biochemical characterization of B. subtilis 140B, include the protease and chitinase activity. This B subtilis 140B produced a clear halo region both of on protease-skim milk agar medium plates containing 0.8% skim milk and chitin agar medium plates containing 0.5% colloidal chitin, indicating that it excretes protease and chitinase. The extracellular protease and chitinase from B. subtilis 140B had significant growth-retarding and mycolitic activity on pathogen fungi.

Keywords: B. subtilis 140B, F. oxysporum, biocontrol, tomato disease, protease, chitinase

INTRODUCTION
Rhizosphere bacteria are excellent agents to control soil-borne plant pathogens. Bacterial species like Bacillus have been proved in controlling the fungal disease1. Bacillus subtilis 140B showed high antagonistic activity against plant pathogen fungi such as Fusarium oxysporum, F. solani and Curvularia sp. Bacillus is non pathogenic soil bacteria and offer several advantages over other organisms as they form endospores and hence can tolerate extreme pH, temperature and osmotic condition2. Bacillus species were found to colonize the root surface, increase the plant growth and cause the lysis of fungal mycelia3.F. oxysporum and its various formae speciales have been characterized as causing several symptoms, such as vascular wilt, yellows, corm rot, root rot, and damping-off. The most important of these is vascular wilt4. In general, fusarium wilts first appear as slight vein clearing on the outer portion of the younger leaves, followed by epinasty (downward drooping) of the older leaves. At the seedling stage, plants infected by F. oxysporum may wilt
and die soon after symptoms appear. In older plants, vein clearing and leaf epinasty are often followed by stunting, yellowing of the lower leaves, formation of adventitious roots, wilting of leaves and young stems, defoliation, marginal necrosis of remaining leaves, and finally death of the entire plant. The aims of this experiment were to identify the isolate 140B through 16S rDNA and to know its potency to inhibit the tomato disease.

MATERIALS AND METHODS

B. subtilis 140B was isolated from pineapple garden soil-GGP Lampung Province. Screening for the antagonistic activity was carried out by following the dual plate assay method and incubated at 30 °C for 5 days. Protease activity tested using Calcium Caseinat Agar Medium containing 0.8% skim milk. Chitinase activity tested using Chitin Agar Medium containing 0.5% colloidal chitin. Isolate 140B was identified by its morphological and physiological properties. The role of isolate B. subtilis 140B on suppressing tomato disease was studied in comparison with CC.13, CC.20 and CC.88204 isolates. Determine statistical significance by ANOVA and continued with DMRT 95%.

RESULTS AND DISCUSSION

B. subtilis 140B showed high antagonistic activity against plant pathogen fungi, indicated by its ability to form a clear inhibition zone of 1 cm diameter around the bacterial colony (Fig.1).

![A](image1) ![B](image2)

**Figure 1.** F. oxysporum inoculated on NA medium as control (A); Antagonism activity of B. subtilis 140 B towards F. oxysporum (B).

Result of biochemical test from isolate B. subtilis 140B showed that isolate have a chitinolytic and proteolytic ability. The isolate showed a clear zone on Chitin Agar Medium containing 0.5% colloidal chitin after 3 days incubation and also formed a clear zone on Calcium Caseinat Agar Medium containing 0.8% skim milk after 2 days incubation. Earlier reports showed that microorganisms capable of lysing chitin, which is a major constituent of the fungal cell wall, play an important role in biological control of fungal pathogens. The extracellular protease from B. subtilis 140B had significant growth-retarding and mycolytiic activity on pathogen fungi. Identified isolate 140B by its morphological showed that isolate
was a Gram-positive and rod-shaped bacteria. The 16S rDNA sequence analysis indicated that isolate 140B was high similarity with *Bacillus subtilis* (100%).

The role of *B. subtilis* 140 on suppressing tomato disease has a better result than control, CC.13, CC.20 and CC.88204 isolates (Fig.2 and Table 2).

![Figure 2](image)

**Figure 2.** Percentage of wilt leaves on tomato in an *in vitro* experiment using *B. subtilis* 140B, CC.13, CC.20 and CC.88204 isolates compare with control.

**Table 1.** Effects of treatments with isolates on height, leaves and fruit total on tomato after 14 days and 28 days observation. Values in each column with the same letter are not significantly different (P<0.05).

<table>
<thead>
<tr>
<th>Treatments</th>
<th>14 days</th>
<th>28 days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Height</td>
<td>Leaves total</td>
</tr>
<tr>
<td>Control</td>
<td>15.35a</td>
<td>6.60a</td>
</tr>
<tr>
<td>CC.13,CC20,CC.88204</td>
<td>22.80b</td>
<td>7.90b</td>
</tr>
<tr>
<td><em>B. subtilis</em> 140B</td>
<td>21.40b</td>
<td>7.80b</td>
</tr>
</tbody>
</table>

The result of ANOVA showed that *B. subtilis* 140B was significantly influenced the growth of tomato and has a better result than control and other isolates. It was obvious that application of *B. subtilis* 140B improved height and leaves and fruits total of tomato than control.

**REFERENCES**


Saleem B., and U. Kandasamy. 2002. Antagonism of *Bacillus* species (strain BC121) towards *Curvularia lunata*. *Current Science.* **Vol. 82:**1457-1462


P-PD22

REGENERATION IN VITRO OF TOMATO (Lycopersicon esculentum) FROM Various Explants and Combination Growth Regulators

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Abstract
In vitro culture was assessed in tomato (Lycopersicon esculentum) for optimized callus induction and plantlets regeneration. The study examined effect of various explants and plant growth regulators combination for in vitro culture, growth and regeneration of tomato, derived hypocotyls and cotyledon from aseptically seedlings. Experimental purpose is studying regeneration response both of explants and use of various plant growth regulators combination. The result showed the difference regeneration ability explants response and shoot formation from various plant growth regulators combination. Callus induction was achieved between 8 to 10 days, after 18 to 24 days plant regeneration was observed. Addition of 2 mg L⁻¹ IAA, 2 mg L⁻¹ BA in MS medium known can produce 86% and 20% shoot formation from both of explants. The composition of 0.25 mg L⁻¹ BA, 0.25 mg L⁻¹ GA essential for shoots elongation and 0.15 mg L⁻¹ NAA induced roots formation.

Keywords: Lycopersicon esculentum, plant growth regulators, explants regeneration

Introduction
Tomato is one of the frequently studied higher plants because it is an important crop species with several advantages for genetic, molecular and physiological studies (Chaudhry et.al., 2004 ; Gubis et.al.,2004). Several protocols for tomato regeneration in vitro are nowadays referred. There is a need to improve this tomato using biotechnological approaches and to develop a high frequency regeneration system.

The in vitro morphogenetic responses of cultured plants are affected by different components of culture media. Media with combination growth regulators used in regeneration media affect the formation of adventitious buds and shoots (Gubis et.al.,2005 ; Plevnes et. al., 2006).

Various explant sources are reported for callus induction and regeneration on different media in tomato. Explants like hypocotyls segments, cotyledons, leaf disc, roots, shoot tips and anthers are also reported for callusing and regeneration (Sheeja et.al.,2004 ; Chaudhry et.al., 2004 ; Gubis et.al.,2004). Development of high frequency regeneration
system was not achieved for many explants and many media of tomatoes grown in Indonesia. The experimental work was conducted to establish a reproducible protocol for regeneration in tomato by using specific combinations of growth regulators and various explant.

**Material and methods**

Table 1 Different hormonal combinations used in regeneration medium of tomato

<table>
<thead>
<tr>
<th></th>
<th>G</th>
<th>M1</th>
<th>M2</th>
<th>M3</th>
<th>M4</th>
<th>E</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td>MS</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Sucrose (g l⁻¹)</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>30</td>
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<td>Agar (g l⁻¹)</td>
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<tr>
<td>IAA (mg l⁻¹)</td>
<td>-</td>
<td>0.2</td>
<td>0.5</td>
<td>2</td>
<td>2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>BAP (mg l⁻¹)</td>
<td>-</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>0.25</td>
<td>-</td>
</tr>
<tr>
<td>NAA (mg l⁻¹)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2</td>
<td>-</td>
<td>0.15</td>
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<tr>
<td>Kinetin (mg l⁻¹)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>GA3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.25</td>
<td>-</td>
</tr>
</tbody>
</table>


**Results and Discussion**

Two explant sources i.e. cotyledon and hypocotyl were used. Cotyledon and hypocotyl showed variable response by use of different growth regulators. Their response was dependent on the different combination of growth regulators. Hypocotyl showed 100% of callus induction at all hormone combination, whereas cotyledon exhibited 93.33% at the hormonal combination 0.5 mg l⁻¹ IAA, 2 mg l⁻¹ BAP; and at the hormonal combination 2 mg l⁻¹ IAA, 2 mg l⁻¹ BAP, 2 mg l⁻¹ NAA, 4 mg l⁻¹ kinetin (Table 2).

Results of this experiment confirm the positive influence of growth regulator addition on the number of shoots regenerated from tomato cotyledons and hypocotyls. For all explants, combination growth regulator 0.2 mg l⁻¹ IAA, 2 mg l⁻¹ BAP (M1 medium) supplemented media to give the higher number of shoot per explant at four week ages.
Table 3. Percentage of explants producing callus, shoot primordia and number of shoot per explant of the used four medium (four weeks age)

<table>
<thead>
<tr>
<th>Medium</th>
<th>(explants producing callus) (%)</th>
<th>(explants producing shoot primordia) (%)</th>
<th>No. of shoot per explant</th>
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</thead>
<tbody>
<tr>
<td>Cotyledon</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>M1</td>
<td>100</td>
<td>86</td>
<td>7.67</td>
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<tr>
<td>M2</td>
<td>93.33</td>
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<td>M3</td>
<td>100</td>
<td>73.33</td>
<td>4.67</td>
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<tr>
<td>M4</td>
<td>93.33</td>
<td>6.67</td>
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<td>Hypocotyl</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>M1</td>
<td>100</td>
<td>20</td>
<td>5.33</td>
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<tr>
<td>M2</td>
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<td>M4</td>
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</table>

References


P-PD23

ANATOMY OF PROTOCORM-LIKE BODIES (PLBs) FORMING FROM IN VITRO CULTURE OF Phalaenopsis Blume LEAVES

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corresponding author: p_marianingsih@yahoo.com

ABSTRACT

Induction of protocorm like-bodies (PLBs) in every orchid explants has been done a number of times, especially on the genus Phalaenopsis. However, there is very little known on cells and tissue that produces PLBs. The objective of the study is to examine the cells and tissue of Phalaenopsis leaf explants potential in producing PLBs by anatomical microscope slides observation. The slides were prepared by using the paraffin method or paraffin embedding and safranin-fastgreen stain. Result of the anatomical structure observation shows that the sub-epidermal and epidermal cell of Phalaenopsis leaves has the potential to form PLBs. Observation also indicates that mesophyll cells have meristematic characteristic, even though cleavage has not yet been formed. Morphological and anatomical structure observation shows that PLBs tend to form more in adaxial leaves.

Keywords: anatomy; Phalaenopsis leaves; in vitro culture; Protocorm, PLBs

INTRODUCTION

Phalaenopsis is one of orchid Genuses which is mostly fonde, mainly as cutting flowers or potted plants (Chen & Chang 2004: 290). An efficient artificial propagation for orchid is by in vitro culture (Arditti 1977: 205). Vegetative explants of orchid, cultured by in vitro, will form protocorm-like bodies (PLBs) before it becomes plantlets (Soedjono & Kamidjono 1992: 27). Based on the research of orchid by in vitro culture, PLBs are formed by subepidermal cell of leaf segments Doritaenopsis (Park et al. 2002: 49) and from epidermal cell of leaves derived from flower-stalk cuttings Phalaenopsis "little steve" (Kuo et al. 2005: 455). The objective of the research is to examine cells and tissues of Phalaenopsis leaves explants which potential in producing PLBs by anatomical microscope slides observation. The result of the research hopefully could be a direction for determining the potential of cell forming PLBs and as a basis knowledge to make priority for cells or tissues which will be induced appropriately with the purpose of the culture.
MATERIALS AND METHODS

Plant material
Plant materials are leaves segments of Phalaenopsis, it derived from in vitro culture of flower-stalk cuttings. Then, it is sub-cultured by using half-strength MS (Murashige & Skoog) medium supplemented with growth hormone.

Microscope slides prepared by using Sass (1964) paraffin method
For microscope slides, samples are collected during different culture periods. After that, the samples were fixated in a FAA (Formaldehyd:acetat acid:alcohol) and aspirated in aspirator. Then, the samples were dehydrated in an alcohol-tertier butyl alcohol series, infiltrated and embedded in paraffin. Next, the samples were cutted with microtome becomes the series of slides. Then, the slides were stained by combination of safranin-fastgreen.

RESULTS AND DISCUSSION

Seven weeks cross-section of leaves segments culture show that mitotic divisions had already began at sub epidermal cell (Fig. 1A). The mitotic cell is marked by smaller in size and darker colour than the other cells. The sub epidermal cells will continue divided and will form globular structure which is called PLBs (Fig.1B).
Nine weeks cross-section of leaves segments culture show that mitotic divisions occur at sub epidermal and epidermal cells (Fig. 2). Mesophyll cells also shows the meristematic characteristic, even though the cell divisions not really significance. It is marked by darker in colour and the nucleus can be seen clearly.
The anatomical structure observation show that the Phalaenopsis leaves’s cell; sub epidermal, epidermal and mesophyll cell; could be induced to divide and has the potential to form PLBs. The source of explant material are the 5-month-old leaves, that young enough, derived from in vitro culture of flower-stalk cuttings. According to George & Sherington (1984: 171), the young cells explants are still active to divide. Moreover, Pierik (1987: 45) stated that cell division and growth activity of explants, which culturing in vitro, could be induced by growth hormone that supplemented in medium.
In addition, Morphological and anatomical structure observation shows that PLBs tend to form in adaxial leaves.

**Fig 1. Anatomical structure of cross-section Phalaenopsis leaves after 7 weeks culture**

Ep=epidermal cell, Sb.ep=subepidermal cell

**Fig 2. Anatomical structure of cross-section Phalaenopsis leaves after 9 weeks culture**

Mr=meristematic mesophyll

**REFERENCES**


P-PD24

POLYPEPTIDES COMPRISING MYCORRHIZAL SPECIFIC ESTERASE BANDS ON MAIZE Roots INOCULATED WITH FOUR DIFFERENT AMF

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Abstract

Isozymes analysis in spores are used to determine inter and intra-generic species, or even isolates of AMF species. Detection of mycorrhiza in planta of some AMF species was proved by performing a mycorrhizal specific isozymes (MSI) of malate dehydrogenase (MDH), esterase (EST), and glutamat oxaloacetate transferase (GOT) on mycorrhizal plant roots. MSI of esterase bands of AMF species in roots may vary depending on plant age.

Maize inoculated with each of four AMF species, *Glomus manihotis*, *Glomus etunicatum*, *Gigaspora rosea* and a local isolate of *Glomus* sp., were used to detect MSI esterase in comparison to an un inoculated plant which were grown for 11 weeks. Esterase staining following native PAGE electrophoresis was performed initially to localize the esterase MSI band. The MSI gel bands were separated using SDS-PAGE after electrophelusion to detect their comprising polypeptides.

Similar Rf value (0.72) of esterase MSI band of maize roots inoculated with each AMF species are shown. These consist of four to six polypeptides with combination of 50.5, 52.7, 62.8, 65.6, 74.8, 78.2, 88.9, 110.9, 126.5, 131.8, 150.3, and 171.4 kDa.

**Key-words:** *G. manihotis*, *G. etunicatum*, *Gi. Rosea*
P-PD25

**MYOGLOBIN EXPRESSION IN TURTLE HATCH Chelonia mydas MULTIPLE TISSUES**

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2Department of Biochemistry and Molecular Biology, Faculty of Medicine – University of Indonesia.
3Department of Biochemistry, Faculty of Medicine – Universitas Kristen Indonesia. Jakarta, Indonesia.
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**Abstract**

An understanding of the underpinning physiology and biochemistry of animals is essential to properly understand the impact of anthropogenic changes and natural catastrophes upon the conservation of endangered species. An observation on the tissue location of the key respiratory protein, myoglobin-Mb, now opens up new opportunities for understanding how hypoxia tolerance impacts on diving lifestyle in turtles. The respiratory protein, myoglobin, has functions other than oxygen binding which are involved in hypoxia tolerance, including metabolism of reactive oxygen species and of the vascular function by metabolism of nitric oxide. This project seeks to determine the location of myoglobin the green turtle hatch and to confirm the hypothesis that reptiles also have a distributed myoglobin expression which is linked to the hypoxia-tolerant trait. Turtle hatch Chelonia mydas - Myoglobin sequence 114bp expressed in cDNA from brain, heart and liver tissues by using Iguana iguana primers. Furthermore, it will form the basis for completing the sequence and generating an in situ hybridization - ISH probe for analysis of cell location in expressing tissues as a one technique of myoglobin disposition in multiple tissues.

**Keywords** : hypoxia, tolerance, hatch, Chelonia mydas, cDNA, ISH,
IN VITRO SEED GERMINATION OF THE BLACK ORCHID 
(Coelogyne pandurata Lindl.)

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ABSTRACT
Black orchid (Coelogyne pandurata Lindl.) is an endemic orchid of the Province of East Kalimantan, Indonesia, that threatens to be extinct. The over-collection and difficulties cultivation through conventional methods are the big problem of this orchid. To undergo the successful cultivation, in vitro seed germination is the key step. In order to obtain the optimal condition for in vitro seed germination of this orchid, we explored the various culture medium. Seeds from fully ripening fruit (five-months-old) were sown on various culture media: Knudson C (KC), Vacin & Went (VW), New Phalaenopsis (NP), and Murashige & Skoog (MS) in half, full, and full concentration of macro-elements with 150\textsuperscript{mm} \text{ coconut} water. The cultures were incubated at 25°C, in the dark condition for four weeks first, then continuing with 1000 flux continuous light. The growth steps of embryo during seed germination were classified into four steps based on the growth phases, namely phase1/yellowish embryo, phase2/green embryo, phase3/bipolar embryo and phase4/Single leaf formed embryo. Based on our criterion, the result showed that the optimal culture medium was MS medium in half concentration (\(\frac{1}{2}\)MS). The time courses observation of embryo development showed that the color of embryo started to be changed from yellowish (in the first phase) into green (the second phase) was occurred at three weeks after sowing. At four weeks, the green embryo formed bipolar (the third phase), with one side is dark. The darker pole of embryo changed into leaf primordia (the fourth phase) at the fifth week.

Keywords: \(\alpha\)-Naphtalene Acetic Acid, pollination, fruit ripening, Phalaenopsis orchid

INTRODUCTION
Black orchid (Coelogyne pandurata Lindl.) is an endemic orchid of the Province of East Kalimantan, Indonesia, that threatens to be extinct. The over-collection and difficulties cultivation through conventional methods are the big problem of this orchid (Arditti, 1992). To undergo the successful cultivation, in vitro seed germination is the key step (Arditti & Ernst, 1993). In order to obtain the optimal condition for in vitro seed germination of this orchid, we explored the various culture medium.
MATERIALS AND METHODS

Seeds from fully ripening fruit (five-months-old) were sown on various culture media: Knudson C (KC), Vacin & Went (VW), New Phalaenopsis (NP) (Islam et al., 1998), and Murashige & Skoog (MS) in half, full, and full concentration of macroelements with 150mg/l coconut water. The cultures were incubated at 25°C with 1000 flux continuous light.

RESULTS AND DISCUSSION

The growth steps of embryo during seed germination were classified into six steps based on the growth phases, namely phase1/yellowish embryo, phase2/green embryo, phase3/bipolar embryo phase4/first leaf formed embryo, phase5/second leaf formed embryo and phase6/third leaf formed embryo. The time courses observation of embryo development showed that the color of embryo started to be changed from yellowish (phase 1) into green (phase 2) was occurred at three weeks after sowing. At four weeks, the green embryo formed bipolar structure (phase 3), with one side is darker than the other. The darker pole of embryo changed into leaf primordia (phase 4) at fifth week. Second leaf formed at seventh weeks. Third leaf formed at eleventh week.

![Phase 1 Phase 2 Phase 3 Phase 4 Phase 5 Phase 6](image)

Figure 1. Growth Phase of Seed Germination. Bar: 0.5mm

<table>
<thead>
<tr>
<th>Variation of Medium</th>
<th>Number of Sowed Protocorm</th>
<th>Percentage of growing embryo at each phase</th>
<th>Death protocorm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Phase 1       Phase 2       Phase 3       Phase 4       Phase 5       Phase 6</td>
<td></td>
</tr>
<tr>
<td>1/2 KC</td>
<td>215</td>
<td>0.00%         0.00%         0.00%         57.21% (123)   27.91% (60)    0.00%         14.88% (32)</td>
<td></td>
</tr>
<tr>
<td>KC</td>
<td>174</td>
<td>0.00%         0.00%         0.00%         17.24% (30)    70.69% (123)   0.00%         12.07% (21)</td>
<td></td>
</tr>
<tr>
<td>KC+CW</td>
<td>175</td>
<td>0.00%         0.00%         0.00%         41.14% (72)    2.86% (5)      0.00%         56.00% (98)</td>
<td></td>
</tr>
<tr>
<td>1/2 VW</td>
<td>109</td>
<td>0.00%         0.00%         0.00%         59.63% (65)    40.37% (44)    0.00%         0.00%</td>
<td></td>
</tr>
</tbody>
</table>

Table 1. The growth of Black Orchid’s Embryo in Various Medium at Twelve Weeks After Sowing
<table>
<thead>
<tr>
<th>Treatment</th>
<th>VW</th>
<th>VW+CW</th>
<th>W</th>
<th>NP</th>
<th>NP+CW</th>
<th>MS</th>
<th>MS+CW</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>134</td>
<td>0.00%</td>
<td>0.00%</td>
<td>0.00%</td>
<td>7.46%</td>
<td>40.30%</td>
<td>0.00%</td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>0.00%</td>
<td>0.00%</td>
<td>0.00%</td>
<td>9.33%</td>
<td>25.33%</td>
<td>0.00%</td>
</tr>
<tr>
<td>1/2 NP</td>
<td>193</td>
<td>0.00%</td>
<td>0.00%</td>
<td>0.00%</td>
<td>13.47%</td>
<td>86.53%</td>
<td>0.00%</td>
</tr>
<tr>
<td>NP</td>
<td>112</td>
<td>0.00%</td>
<td>0.00%</td>
<td>0.00%</td>
<td>18.75%</td>
<td>57.14%</td>
<td>4.46%</td>
</tr>
<tr>
<td>NP+CW</td>
<td>105</td>
<td>0.00%</td>
<td>0.00%</td>
<td>0.00%</td>
<td>1.90%</td>
<td>72.38%</td>
<td>8.57%</td>
</tr>
<tr>
<td>1/2 MS</td>
<td>52</td>
<td>0.00%</td>
<td>0.00%</td>
<td>0.00%</td>
<td>5.77%</td>
<td>84.62%</td>
<td>9.62%</td>
</tr>
<tr>
<td>MS</td>
<td>262</td>
<td>55.73%</td>
<td>0.00%</td>
<td>0.76%</td>
<td>20.99%</td>
<td>9.16%</td>
<td>0.00%</td>
</tr>
<tr>
<td>MS+CW</td>
<td>73</td>
<td>0.00%</td>
<td>0.00%</td>
<td>0.00%</td>
<td>32.88%</td>
<td>50.68%</td>
<td>1.37%</td>
</tr>
</tbody>
</table>

After twelve weeks observation, based on the growth rate of embryos, the data revealed that 1/2 MS medium is the best medium to support and accelerate the growth rate of black orchid's embryos. This was indicated by 84.62% protocorms can grow up to phase 5 and 9.62% can grow up to phase 6 with the third leaf has emerged from the shoot tip. This results indicate that half strength concentration of complete elements containing medium is needed for the black orchids seed germination.

ACKNOWLEDGEMENT

The research was supported by Indonesian DGHE Research Competition grant HB XVII 2009 No. LPPM-UGM/604/2009. We thank to Bunga Rintee Orchid Nursery for the gift of fruit of the Black Orchid and to Mr. Wirakusumah for valuable discussion on black orchid culture techniques.

REFERENCES

P-PD27

CONSERVATION BIOLOGY OF MYCOHETEROTROPHIC ORCHID: CASE STUDY OF IN-VITRO CULTURE OF DYDIMOPLEIXIS PALLENS (ORCHIDACEAE)

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ABSTRACT

Didymoplexis pallens is terrestrial orchid species, and almost associated with clumps of Bamboo particularly Gigantochea spp. Although it is widely distributed, the orchid rarely attracts attention as it leafless and is erratic species in terms of flowering and fruiting. In Bogor Botanic Garden D. pallens grows in a restricted area, in a bamboo clump along particularly in one location. The site is highly vulnerable and there is little known of the biology and ecology of the species in terms of the growth and development of the species. Growth and development of this orchid in aseptic condition was observed. However, information gained from invitro culture in the laboratory did not necessarily represent condition occurred in the wild. Thus, long term survival of this species could not depend on this method alone. More detail study involving seed baiting experiment in its habitat is required to assess population dynamics of this species. This is important to determined conservation management strategy of this species.

INTRODUCTION

Bogor Botanic garden is a natural habitat for several epiphytic and terrestrial orchids including Dydimoplexis pallens. The presence of this orchid in Bogor Botanic Garden had been known since early 20th century and was always associated with bamboo (Smith, 1905; Burgeff, 1959). Observation on the life cycle of mycoheterotrophic orchid like Didymoplexis pallens in its habitat was limited by their cryptic habit. In Bogor Botanic Garden this orchid can only be seen in particular site at a limited period during the changing season from wet to dry season (March to July). During flowering time, this ephemeral species emerge from the ground among bamboo leaf-litter, with white flower 15 – 30 cm height. Long term survival of this species is vulnerable as their presence rarely attract any attention and human activities became a significant threat. In-vitro culture of D. pallens was conducted to get more understanding on the life history of this orchid. This is important to assess and determined conservation management strategy of this species.
MATERIALS AND METHODS

Fruit capsules of *D. pallens* were harvested from the habitat in Bogor Botanic Garden. Seeds were surface sterilized and sown on modified Hyponex (25:5:20) agar medium. The cultures were incubated at 26°C ± 2°C, illuminated for 16 hours. Subculturing to a new fresh medium was carried out for further development. Observation on the development of the orchid was recorded.

RESULTS AND DISCUSSION

Germination of *D. pallens* in Hyponex medium occurred after 83 days. This was showed by seed enlargement with *trichome* surround the seeds surface. Rhizome and bulbs formation were recorded on day 256. Bulb is important organ as a food reserve to maintain its existence during dormant period before or after flowering time. However, flowering stage did not occur during the course of this experiment (466 days). Instead of bulb formation, flowering stage was observed in similar experiment by Irawati (2005) using Knudson C medium. It was not clear whether medium has contribution to this. This experiment only depicted the life cycle of the orchid in controlled condition and was not necessarily occurred in the wild. Early stage of orchid seeds development in nature was defined by the presence of its mycorrhizal partner (Rassmusen, 1995). The level of dependency of leafless species to its partner is higher than green orchid since they have no photosynthetic capacity. Achlorophyllous species is totally depend on mycorrhizal association for water and nutrient supply (Leake, 2000).

Glucose, amino acid, phosphate, vitamins and proteins were transferred to orchid by mycorrhizal fungi (Arditti & Ernst, 1984). The ability of *D. pallens* to grow in invitro culture indicated that nutrient supply required by this species might have represented on medium used in this experiment.

The presence of *D. pallens* in restricted area in Bogor Botanic Garden is an indication of it preference to specific habitat. Thus, although several bamboo clumps are spread in Bogor Botanic Garden, not all clumps of bamboo can accommodate population of *D. pallens*. Study of fungi associated with bamboo by Burgeff (1959) suggested that *Marasmius coniatus* can support the live of *D. pallens* through symbiotic culture in the laboratory. However, in a long run conservation of this species could not depend on laboratory work alone. The risk of contamination, human error, shortage of nutrition supply could occur in artificial medium.

Study on population dynamic of *D. pallens* need to be carried out in its natural habitat by seed burial experiment as suggested by Rassmusen & Whigham (1993) and Matsuhara & Katsuya (1993) to get further understanding. Isolation and storage of orchid
symbiotic fungus is a further step to ensure its availability in the laboratory for conservation purposes such as research, relocation, recovery or reintroduction program, not only for *D. pallens* but also other rare Indonesian orchid species which require support form its mycorrhizal partner.

REFERENCES


P-PD28

ESTIMATION OF GROWTH AND BIOMASS PRODUCTION OF Rhizophora apiculata IN WEST AREA OF INDONESIA BY ALLOMETRIC METHOD

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ABSTRACT

Mangrove trees have a very significant impact to the coastal environment; carbon fixation capacity of mangrove forests is higher than that of the terrestrial forest. Therefore it has been considered as an important carbon sink in coastal ecosystems. This paper reported a study to investigated 1) growth and biomass production of R. apiculata mangrove as a function of time, 2) and the relationship with soil environment fertility. The study was conducted during the period of Juli 2007 to June 2009 in 4 locations in west area of Indonesia: (1) reforested 2, 3, 4, 6 year old R. apiculata at Sungai Assam Riau; (2) reforested 5, 6, 7, 28 year old stand of R. apiculata at Cilacap Central Java; (3) reforested 12 year old stand of R. apiculata at East Lampung; and (4) 12 and 14 year old stand R. apiculata at Kuta Bali. Measured parameters were root-, trunk-, and total plant -height, trunk circle, plant canopy area and biomass production. Canopy area and biomass were determined by allometric method. The results showed: 1) Total plant height increased linear until the age of 10 year old, and reached the height of 10 m. Plant height reached the leveling off at the age of more than 10 year old. Maximum of total plant height will reach about 12 m at the age of 20 year; 3) Root height developed until the height of 75 cm at the age of 5 years, and continued to growth until 150 cm at the age of 15 year old. 4) Plant canopy increased fast at period of age 0-10 year; and the development began slowly as the plant reached the age of 20 years; 5) Increase of plant height and trunk diameter followed the sigmoid growth pattern, the increase of height and trunk diameter was linear and very fast at period of age 2-10 years, reached the leveling off at the age 10-20 year old, and it was very slow as the plant reached the age of 20 years; 6) Trunk circle reached 20 cm at the age of 10 years, and reached 30 cm at the age of 20 years. Trunk circle size of 30 cm, when the mangrove usually harvested for wood and house material, occurred at the 20-25 year old, and already had total plant height of 12 m, trunk height of 6 m, plant canopy area of 8 m²; 7) Biomass production of mangrove forest determined by allometric method increased with the increase of plant age. Biomass production increased linear until the age of 10 years, biomass accumulation began to decrease as the plant reached the age of 15 yeras, and the accumulation was very low after the plant reached the age 20 years: 8) Biomass production of 2, 6, 28 year old mangrove were 8.6; 49.4; 95. 9 dan 119. 8 ton dry weight/ha; 9) Net CO₂ fixation capacity of mangrove forest, measured by annual growth increment, increases with the increase of plant age. There were 8.99, 20.64, ton/ha/year in 2 and 6 year old mangrove plants, began to decrease to 8.83 ton/ha/year after 12 years, and continued to decrease and reached 1.49 t ton/ha/year as plant reached the age of 28 year old; 10) After the mangrove plants reached the age of more than 20 years, for the purposes of CDM project, the plants should should be felled and re-
planted again, since its net capacity to absorb atmosphere CO$_2$ had already decreased; 11) Growth rate of mangrove in island of Sumatera was faster than that was in Java and Bali. Soil fertility was responsible for this discrepancy, the growth was positively correlated with some soil characteristic: pH, P Bray content, and %-age clay.

**Keywords:** Rhizophora apiculata, growth, biomass, allometric, CO$_2$ fixation.

**INTRODUCTION**

Mangrove trees have a very significant impact to the coastal environment. Carbon fixation capacity of mangrove forests is higher than that of the terrestrial forest. Therefore it has been considered as an important carbon sink in coastal ecosystems.

**MATERIALS AND METHODS**

This paper reported a study of investigation 1) growth and biomass production of *R. apiculata* mangrove as a function of time, 2) and the relationship with soil environment fertility. The study was conducted during the period of July 2007 to June 2009 in 4 locations in area of Indonesia: 1) reforested 2, 3, 4, 6 year old *R. apiculata* at Sungai Asam, Riau; 2) reforested 5, 6, 7, 28 year old stand of *R. apiculata* at Cilacap, Central Java; 3) reforested 12 year old stand of *R. apiculata* at East Lampung; and 4) 12 and 14 year old stand *R. apiculata* at Kuta Bali. Measured parameters were trunk circle (1) root-, trunk-, total plant height, , plant canopy area and biomass production. Canopy area and biomass were determined by allometric method (2).

**RESULTS AND DISCUSSION**

The results showed: 1) Total plant height increased linier until the age of 10 year old, and reached the height of 10 m. Plant height reached the leveling off at the age of more than 10 years old. Maximum of total plant height will reach about 12 m at the age of 20 year; 3) Root height developed until the height of 75 cm at the age of 5 years, and continued to growth until 150 cm at the age of 15 year old; 4) Plant canopy increased fast at period of age 0-10 year; and the development began slowly as the plant reached the age of 20 year; 5) Increasing of plant height and trunk diameter followed the sigmoid growth pattern, the increasing of height and trunk diameter was linier and very fast at period of age 2-10 year, reached the leveling off at the age 10-20 year old, and it was very slow as the plant reached the age of 20 year; 6) Trunk circle reached 20 cm at the age of 10 year, and reached 30 cm at the age of 20 year. Trunk circle size of 30 cm, when the mangrove usually harvested for wood and house material, occurred at the 20-25 years old, and already had total plant height
of 12 m, trunk height of 6 m, plant canopy area of 8 m²; 7) Biomass production of mangrove forest determined by allometric method increased with the increase of plant age (Fig. 1).

![Graph showing growth curve and biomass production relation of R. apiculata forested with allometric method.](image)

Fig. 1: Growth curve of age and biomass production relation of *R. apiculata* forested with allometric method.

Biomass production increased linearly until the age of 10 year, biomass accumulation began to decrease as the plant reached the age of 15 year, and the accumulation was very low after the plant reached the age 20 year: 8) Biomass production of 2, 6, 28 year old mangrove were 8.6; 49.4; 95.9; and 119.8 ton dry weight/ha; 9) Net CO₂ fixation capacity of mangrove forest, measured by annual growth increment, increases with the increase of plant age. There were 8.99; 20.64 ton/ha/year in 2 and 6 year old mangrove plants, began to decrease to 8.83 ton/ha/year after 12 year, and continued to decrease and reached 1.49 ton/ha/year as plant reached the age of 28 year old; 10) After the mangrove plants reached the age of more than 20 years, for the purposes of CDM project, the plants should should be felled and re-planted again, since its net capacity to absorb atmosphere CO₂ had already decreased; 11) Growth rate of mangrove in island of Sumatera was faster than that was in Java and Bali. Soil fertility was responsible for this discrepancy, the growth was positively correlated with some soil characteristic: pH, P Bray content, and %-age clay.

**REFERENCES**


P-PD29

DEVELOPMENT OF LARVAL DIGESTIVE SYSTEM OF GOURAMY (Osphronemus gouramy Lac.)

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corresponding author : Email: lidiatesa_vs@yahoo.com

INTRODUCTION

Larval stage of gouramy was critical period of life. Survival rate was about 30-40 % (Hardaningsih, 1999). Various study were done to reduce the mortality. Most study were related to treatment with external factor such temperature and feeding. Certainly, temperature was effective on developing larvae in subtrropic country (Heming and Budington, 1988). However, those did not reduce significantly of the mortality. It was rare study related to internal factor, such developmental changes of internal organs. Developmental changes of digestive system was important to study because it was concern to food intake and digest for life. This present study look for an involvement development changes of digestive system with larval mortality.

MATERIAL AND METHOD

Fishes were provided by breeder in Bantul and Kulonprogo regency. Spawning at night, female laid eggs in a nest, male spread sperm in nest to fertilize. In the morning fertilized eggs taken out, reared in aerated water aquarium, 24-28°C, 10 cm depth, for 35 days. Feeding post larvae with thread worm (Tubifex). Samples of embryo, larvae were taken daily, fixed by Bouin’s dye, for histological preparation with paraffin method. Serial section were produced, 6 µm, transversal and longitudinal section, stained with hematoxylin-eosin. Microscopic observation was emphasized on developmental changes of yolk, cellular differentiation, morphogenic structures of future alimentary canal and digestive gland.

RESULTS AND DISCUSSION

Microscopic observation on serial section of embryos to post larvae showed significant developmental changes (Fig. 1-9). Gouramy’s yolk belong to group of mesolecithal egg, that was able to stand for 2 week after hatching (Heming and Budington, 1988). At first week, yolk decreased gradually, at second week decreased rapidly, no yolk at 3th week. It was said that larva stage of gouramy within 2 weeks, after that was called post larvae stage. Extinction the yolk leaving a space, the future of belly space (coelom). Because of yolk extinction in 3th week, large number of larvae were death. Initial of digestive system seen as solid endodermal chord at 18 hours embryo (Fig. 1). Canalization of the chord
become primitive gut, the future alimentary canal (Fig. 2-3). The anlage of digestive gland (liver and pancreas) seen at 48 hours embryo, as entodermal cluster beside fore gut. Morphogenic prosses of alimentary canal of gouramy same with other teleostean, that was by keel method (Sagi, 2002). Compared with mammalian digestive system by folding method (Gilbert, 2000).

From hatching to 6th day larvae, primitive gut undergo differentiation become: esophagae, ventricle and intestine. In 7-15th, alimentary canal enlarge, curled and growth, but still empty. Histological changes of epithelial lining of alimentary canal from simple cuboid, columnnerize and epithelial complex. In the ventricle, epithelial folding as rugae became complicated fold in the day after. In post larvae stage, 22-35th day, epithelial structure and muscular structures similar to the mature stage of Chalcalbumus tarichi (Ünal et al., 2001). Development of liver and pancreas after hatching were started from separation of the organs with primitive gut (Fig. 2-3). The future hepatocyt of liver and acinal cells of pancreas differentiated in 4-7th day larva.

Cellular differentiation of the digestive gland went on the 2nd week. Pancreas comprised of acinal cell, parenchymal cell and Langerhans islet at day 11th larva (Fig. 6-7). Liver growth rapidly in 3rd week comprised of parenchymal cell, hepatocyt and sinusoid. Liver were prominent in belly space at 35th day, the hepatic structures similar to mature stage. Takasima and Hibiya (1995) stated that liver and pancreas of teleostean united to be hepatopancreas. At larva stage the two organs separated but at 35th day post larva the pancreas “engulfed” by liver (Fig. 7-9). It was assumed that two organs at the first step to unite.

Larval mortality times occurred from 2nd week to 5th week. Those were coincide with developmental transformation of yolk exist to yolk extinct, indifferent alimentary canal to differentiated one, separated liver (hepar) and pancreas to unite hepatopancreas. It was concluded that larva developmental transformation of digestive system involved with larval mortality because the developing organ was vulnerable against instable condition.

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P-PD30

COMPARATIVE STUDY ON AGRONOMIC CHARACTERS BETWEEN RESISTANT AND SUSCEPTIBLE SOYBEAN TO Cowpea Mild Mottle Virus

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ABSTRACT

This research had been carried out at Indonesian Legume Research Institute (ILETRI) in Malang. Research materials used were five resistant genotypes (MLGG 0006, MLGG 0106, MLGG 0297, MLGG 0315, and MLGG 0599) and five susceptible genotypes (MLGG 0123, MLGG 0379, MLGG 0603, MLGG 0695, and MLGG 0796). All of the ten genotypes were inoculated by CPMMV using Bemisia tabaci as vectors. Research results showed that there were differences related to agronomic characters between resistant genotypes and susceptible genotypes. Genotype of MLGG 0695 was the most susceptible one, indicated by lowest average number of pods and seeds as well as genotype of MLGG 0297 was the most resistant one, indicated by highest average number of pods and seeds. Genotype of MLGG 0297 have highest average number of pods those are 5 unfilled pods and 23 filled pods, and the average number of seeds per plant were 56 seeds. Genotype of MLGG 0603 have lowest average number of pods those are 2 unfilled pods and 4 filled pods, and the average number of seeds per plant were 4 seeds. Hence, MLGG 0297 can be used as gene resource for developing CPMMV resistant soybean varieties.

Key words: agronomic characters, soybean, CPMMV

INTRODUCTION

In Indonesia, soybean is the third main food commodity after rice and maize. Usually, soybean is used as staple foods of tempeh, tofu, soy sauce, and soymilk. However, Indonesian soybean production is low due to many diseases infect soybean plants such as virus diseases, especially Cowpea Mild Mottle Virus (CPMMV). CPMMV is transmitted by the whitefly, Bemisia tabaci (Homoptera: Aleyrodidae), in a non-persistent manner, causes mosaic, chlorosis, necrosis and distortion in a range of indicator host plants [1]. In East Java, CPMMV has infected almost all soybean plants with infection rates up to 100%. The decrease of products related to the infected seeds was in the range of 53.91-99.36%. The
losses due to virus infection have been variously depending on the virus-host vector relationships as well as the prevailing epidemiological factors [2]. Host-plant resistance is currently the most effective methods for the control of cowpea virus disease.

Until now there has been no kind of soybean that are resistant to CPMMV and have high yield, so this study is the stage that conducted to obtain a resistant soybean to CPMMV and have high yield. The aim of this study was to find out some agronomic characters of five susceptible and five resistant genotypes of soybean which can be used to develop superior varieties.

MATERIALS AND METHODS

This research had been carried out at Indonesian Legume Research Institute (ILETRI) in Malang. Research materials were five resistant genotypes (MLGG 0006, MLGG 0106, MLGG 0297, MLGG 0315, and MLGG 0599) and five susceptible genotypes (MLGG 0123, MLGG 0379, MLGG 0603, MLGG 0695, and MLGG 0796). All soybean genotypes were planted in polybags and managed without insecticides for controlling CPMMV vector, so viruses were inoculated naturally by the vector *Bemisia tabaci*. The determination of resistant and susceptible genotype refers to reference of visual methods [3]. We observed agronomic characters include the number of the unfilled pods, filled pods, and number of seeds per plant.

RESULTS AND DISCUSSION

Research results showed that there were the differences related to agronomic characters between resistant genotypes and susceptible genotypes as indicated in Table 1. Genotype of MLGG 0695 was the most susceptible one indicated by lowest average number of pods and seeds, as well as genotype of MLGG 0297 was the most resistant one indicated by highest average number of pods and seeds. As seen in Table 1, genotype of MLGG 0297 has the highest average number of pods those are 5 unfilled pods and 23 filled pods, and the average number of seeds per plant were 56 seeds. Genotype of MLGG 0603 have the lowest number average of pods those are 2 unfilled pods and 4 filled pods, and the average number of seeds per plant were 4 seeds. Hence, MLGG 0297 can be used as gene resource for developing CPMMV resistant soybean varieties.
Table 1. The average data of soybean agronomic traits tested

<table>
<thead>
<tr>
<th>Genotype</th>
<th>No. of Unfilled Pods</th>
<th>No. of Filled Pods</th>
<th>Total No. of Pods</th>
<th>Seed no./plant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resistant</td>
<td>MLGG 0006</td>
<td>2</td>
<td>13</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>MLGG 0106</td>
<td>4</td>
<td>13</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>MLGG 0297</td>
<td>5</td>
<td>23</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>MLGG 0315</td>
<td>4</td>
<td>16</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>MLGG 0599</td>
<td>2</td>
<td>17</td>
<td>19</td>
</tr>
<tr>
<td>Susceptible</td>
<td>MLGG 0123</td>
<td>4</td>
<td>11</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>MLGG 0379</td>
<td>3</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>MLGG 0603</td>
<td>2</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>MLGG 0695</td>
<td>5</td>
<td>3</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>MLGG 0796</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
</tbody>
</table>

ACKNOWLEDGMENTS
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REFERENCES
P-PD31

Integrating Pest Management Value in *Usada Carik* Balinese Script

Dr. Suryadarma IGP.

Abstract

Indonesia is a country with hundreds of ethnics. Each ethnic has a long history of traditional knowledge but recently modern science has taken it over. Traditional pest control is slowly finding a place in modern pest management. Many developed countries start to realize the indigenous pest management principles. It is locally available, culturally acceptable and less expensive. *Usada Carik* is a Balinese traditional pest management knowledge, which people use for maintaining paddy production. That script was written on the leaves of palm tree (*Borrassus flabellifer*). Its Balinese word refers to the practice of using natural resources for pest control. Can pest management principles being adapted into scientific curriculum approach?

The transcript of scripture was collected from the Bali Government Information Centre. That pest information then clarified and transformed into deep ecology philosophy. The interviews were unstructured, open-ended. The pest management information was written in narrative system, that beginning with the problem of paddy maintenance, name of pest causes, characteristic of management. *Usada Carik* explained that the balance interrelationship among organism is a natural pattern. That scripture described many kind of pests that cause paddy production failure. Those pests were consisted of ten species of grasshopper, viruses, bugs, eight species of rats, and birds. Many plants were used and prepared in various ways. Its activities are similar to deep ecology concepts. It principles can be transformed into scientific ecological approach. 1. How people use the voice of cricket to protect their paddy from the rats, because its ringing voice is equal with certain vibration qualities. 2. How the peoples protect their rice paddy from the birds with “orang-orangan” similar to the people presence in the field. 3. How people using the pheromone of wild cats to protect their rice from the rats, because this hormone is similar to the presence of the cats in the rice fields. All of the above activities are similar to integrated pest management and deep ecology principles.

Key words: *Usada Carik* Script, Deep Ecology principles, Integrated Pest Management.

Introduction

Traditional pest knowledge is slowly finding a place in integrated pest management. Some scientist believe that it may help to discover natural pest control. Many developed countries start to realize it, because it is locally available, culturally acceptable and less expensive. *Usada Carik* is a Balinese traditional pest control knowledge, which peoples use for maintaining their paddy production. This manuscript was written on the leaves of palm tree (*Borrassus flabellifer*). The Balinese peoples can not be separate from the rice, because rice is part of their life. Rice is consider as both physical and spiritual foods. “Rice is a special
sacred food, divinely given to humans. The rice plant has a living spirit or soul comparable to that of humans, and the life cycle of the rice plant is equated with the human life cycle. The rice spirit must be honored and nurtured through rituals in order to assure a bountiful harvest” (Hamilton, 2003). Can integrated pest management and deep ecology approach values being adapted into scientific information?

Materials and Methods

The main material is Usada Carik which transcript of the scripture was collected from the Bali Government Information Centre. Data and information was collected from two sources. Firstly dirived from the content analysis of Usada Carik and secondly obtained from key informants. The information content analysis are including structures of scripture, number of pests and plants species being used, how to use the materials, and to restructure those informations into scientific knowledge. That information clarified with deep ecology principles (Arness, 1986) and transformation model adopted from Usada Taru Pramana research (Suryadarma, 2005).

Results and Discussions
1. Structure of Usada Carik

Usada Carik script had written about the method of pest management, the kind of plants species being used, how to prepare and to use them. Its information begins with the plant name, the characteristic of the parts of the plant and its composition. It information similar to traditional Usada Taru Pramana script (Suryadarma, 2005) (Figure 1).

2. Number of paddy’s pests

Usada Carik described several numbers of paddy’s pests. Those pests are consisting ten species of grasshopper, eight varieties of viruses, one bug, eight varieties of rats, and four species of birds (Table 1)

3. Number of plants being used

The number of plants being used is fifteenth species that consist of different of plant’s part, such as leaf, tuber, bark, and seeds (Table 1)
4. How to use material components

Many plants are use in various ways and those plants were prepared mostly in the form of decoctions which made by mortar, stone, knife (Table 1). Almost all of the materials used in unrefined plant products, some combined with material of the animal, like the pheromone of wild cat.

The total content analysis and description were presented in Table 1

<table>
<thead>
<tr>
<th>No</th>
<th>Pests Control function</th>
<th>Plant and or animal use against pests</th>
<th>Composition and technic</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Against Viruses and bugs</td>
<td>1. The tip of younger paddy 2. Bark of Albasia sp</td>
<td>Grinding, mix wit water. Spray every nignt and chatting verses.</td>
</tr>
<tr>
<td>2</td>
<td>Against the algae</td>
<td>1. The kitchen ash</td>
<td>Spray in rice field</td>
</tr>
<tr>
<td>4</td>
<td>Against Viruses or lanas wereng</td>
<td>1. The bark of erythina plant 2. Onion 4. Garlic</td>
<td>Decoctions made by mortar, stone. Put in the in let of water canal</td>
</tr>
<tr>
<td>5</td>
<td>Against imago of insects</td>
<td>Salt, one kg</td>
<td>Spray in the rice field</td>
</tr>
<tr>
<td>7</td>
<td>Against over-grassing by grasshopper (“balang sangit”)</td>
<td>1. The solvent of burnt rice, like ash 2. The leaf of erythina, bundle with three colours thread –red, white, black-, etc 3. Fogging with fire on the coconut shell</td>
<td>Spray in rice field, tern leaf, three time. I hope success.</td>
</tr>
<tr>
<td>8</td>
<td>Against the rats, and mouse.</td>
<td>1 Ceremony for KI Gili Tunnggil- security of rice field Red rice, covered with erythina leaf in each conner of field, etc 2. Ceremony for Rare Angon, the symbol children who love playing with wild cat in the rice field). Raw material for pray consits of rice, eggs salt, bettle leaf, chalk, erythina leaf, etc 3. Sesajji offering for the rats: rice, combines with duck blood, etc 4. Neutralizer of the rats activities Water put in the miane leaf etc 5. Agains the rats, etc</td>
<td>Spray in rice field, turn left, and rigt, three time. Hope for success. Make the voices of mouse and cat, three time.</td>
</tr>
<tr>
<td>9</td>
<td>Many kind of birds</td>
<td>1. Put white rice in the samida leaf, combine with chalk, etc 2. Orang-orangan (scar-crow), spray with onion and garlic three times 2. Put sunari, that made from bamboo pole, etc</td>
<td>The bird will be fear, when the scar-crow is moving, blown by the wind The bird will be fear, when the holes on the bamboo poles making sound or high frequency vibration when blown by the wind</td>
</tr>
</tbody>
</table>
The values of *Usada Carik* approach are similar to deep ecology concepts. These are several samples: 1. How farmers use the voice of cricket to protect their paddy from the rats, because it ringing voice is equal with certain vibration qualities. 2. How farmers protect their rice paddy from the birds with “orang-orangan”, because it is similar to the presence of the peoples in the rice field. 3. How farmers use the pheromone of wild cats to protect their rice from the rats, because this hormone is similar to the presence of the cats in the rice field. The farmers did not kill all rats, because the rat is representing “human desire”. The farmers honour all of the animals, because everything on Earth is connected.

5. Restructure *Usada Carik* Information

The objective to restructure *Usada Carik* is to promote its traditional pest control information into scientific knowledge (Table 2).

<table>
<thead>
<tr>
<th><em>Usada Carik</em> script</th>
<th>Transliterate in English</th>
<th>Local pest</th>
<th>Composition</th>
<th>Nama bahan</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a. liki Usada sawah .ta. pari, sa camanining blalu incuk, woring yeh embong, ma ping 3, sawengi gawe ring sawah, sadakep sira tur ider kiwa ping 3, temu ring pakundan , ma , etc</td>
<td>This is the Usada Sawah (the traditional knowledge to maintain the production of rice. The protection from the pest is as follow: The outer bark of blalu (Albaasia sp), grinding it, mixed with the tip of paddy. Do it every night, three time, move from the left side to right, in the higher field, chanting mantra. Etc</td>
<td>The kind of pest a. Kamal b. Perti c. Bikul d. Kubangkubang e. Balang batu f. Balang kori g. Balang sangit h. Puthan</td>
<td>1. Combinati on a part plant resources 2. Spiritual activities</td>
<td>1. Bark of Albacia sp 2. Tip of younger paddy regeneration (embong)</td>
</tr>
</tbody>
</table>

It restructure would be accommodated different perception, because what people do were depended on what they think about themselves in relation to things around (Toledo, 1992). It principles is similar to the philosophy of permaculture. Permaculture is a philosophy of working with nature rather than against nature and permaculture designs system which arranges what was always there in different way, it works to conserve energy than consumes (Mollison, 1992). We can learn the law of return and its concept associated with platform of deep ecology (Arness, 1986).

References


P-PD32

PLANTLET REGENERATION OF Kalanchoe prolifera Bowie ON LEAVES NATURALLY AND THOSE TREATED WITH COMBINATION OF NAA AND KINETIN

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ABSTRACT

Flower of Kalanchoe prolifera Bowie is generally formed, but seeds can not developed normally. Plant regeneration arises from the notch of the mature leaves, so this plant is called as maternity plant. The aim of this research were ; 1) to observe the initial cells which form the meristematic zone and differentiation of the meristematic zone to form RAM (Root Apical Meristem) and SAM (Shoot Apical Meristem) naturally; 2) to evaluate the effect of plant growth regulator (NAA and Kinetin) on RAM and SAM differentiation, and plant regeneration. To know the natural differentiation of meristematic zone and the effect of plant growth treatment, transversal sections of leaves notch were prepared using paraffin method. The concentration of plant growth regulators were 0,0; 0,01; 0,1; 1,0 and 10 ppm, respectively. The cutting leaves consisted of four notches were cultured on wet cotton in a disk consisting of simple media. The simple medium was added with various concentration of plant growth regulators. The parameters of growth were the velocity of shoot arises, the number of leaves and roots. The result showed that the initial meristematic zone originated from sub-epidermal layer, and these cells divided to form clump of meristematic zone, and differentiated to form SAM and RAM. The differentiation of SAM follows the Tunica-Corpus theory, while RAM differentiation follows the Histogen theory. Application of NAA and kinetin stimulated shoots and roots formation at leaves notches compared to control. Higher ratio concentration of NAA/Kinetin promoted the number of roots formation, while higher ratio concentration of Kinetin/NAA accelerated shoot formation.

Keywords: Plantlet, regeneration, Kalanchoe prolifera, leaves, NAA, kinetin

INTRODUCTION

Naturaly plant regeneration originated from leaf, root, stem, rhizome, and other part of plant (Salisbury and Ross, 1995). Plantlet regeneration of Kalanchoe prolifera Bowie originated from their leaf. These plant called maternity plant, because many plantlet develop from their marginal notches of the leaf. From the notch grew many plantlet, and then grow up as independently new plant (Campbell et al., 2003).
Plant Growth Regulator (PGR) as auxin and cytokinin generally stimulated rooting and shoot formation. Auxin stimulated cell elongation, initiation of root formation on stem cutting, and root differentiation. Cytokinin promoted cell division and initiation of shoot formation. Combination of auxin and cytokinin stimulated cell division on meristematic tissue (Setiti et al., 1996). The aim of this research were: 1) to observed the differentiation of meristematic zone to form Root Apical Meristem (RAM) and Shoot Apical Meristem (SAM); 2) to evaluate the effect of PGR (Auxin and Kinetin) on RAM and SAM differentiation and plantlet regeneration.

MATERIAL AND METHOD

Third or fourth leave were cutting (1cm x 1cm), and treated with combination of NAA (Naphthalene Acetic Acid) and kinetin. The cutting leaf consist of notches, and cultured on simple medium (wet cotton medium). The level of NAA and Kinetin concentration was same, i.e. : 0, 0.01, 0.1, 1.0 and 10 ppm respectively. Each treatment combination has 3 replicates. For anatomical observation, the cutting leaves were prepared using paraffin method.

RESULTS AND DISCUSSION

The results were devided into two groups i.e. : 1) anatomi of original leaf and differentiation of meristematics tissue in the notcharea; 2) the effect of PGR treatment to the morphology and antomy of plantlet.

ANATOMY OF LEAF

Mesophyl of *K.prolifera* consist of parenchyma tissue and protected by epidermal layer, and rich with green chloroplast. There were many stomata in the epidermal layer. Through cross section, below the notch there were clump of meristematic cells. The clump of meristematic cells continue to devided, and differentiated formed a bipolar structure i.e. : root primordium and shoot primordium (Gaj, 2001).

DIFFERENTIATION OF MERISTEMATIC TISSUE IN NOTCH ZONE

Microscopically, differentiation of meristematic tissue to form shoot was: the origin of meristematic zone is sub-epidermal near the notch. The size of meriistematic zone was very small, compare with the surrounding cells. Some meristematic zone to differentiated into shoot, and from these shoot some leaves were form, but at the other part root were formed.
EFFECT OF AUXIN AND KINETIN TREATMENT

Addition of PGR on variation concentration combination showed different responses i.e.: the velocity of organ formation, number of organ (root and leaves), long of root. The area of meristematic zone more wide than control, and differentiated to form primordial root and shoot. Both of the primordium differentiated into many root and leaves. The velocity of shoot arised, the average number of leaves in plantlet, the number of root, and the size of root long caused by treatments see.

CONCLUSION
1) Meristematic zone initiated from sub-epidermal layer, and differentiated to form SAM and RAM
2) Application of PGR initiated more shoot and root compare to the control. High ratio concentration of Auxin/Kinetin promote the number of root, while high ratio concentration of Kinetin/Auxin accelerated shoots morphogenesis

REFERENCES
P-PD33

Vincristine defect on primary spermatocyte and spermatid of grasshopper

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Jl. Surabaya 6 Malang 65145 Indonesia

ABSTRACT

Catharanthus roseus produce vinca alkaloid which are commonly used for anticancer therapy. Vincristine is one of those compounds. This molecule binds to tubulin and prevent microtubule’s assembly. Previous studies reported that 0.005% of vincristine significantly delay anaphase and potencially induce cell damage or defect. This study aimed to examine cell defect caused by vincristine. We soaked the testicles of grasshopper in 0,005% of vincristine in 0,9% Carlson solution for 10 or15 minutes. This research showed that vincristine significantly caused defect of dividing primary spermatocyte and spermatid. The longer soaking 0,005% vincristine in 0,9% Carlson solution increase the amount of defect cells, 10': 12-37%; 15': 16-55%, respectively. The defect including cytokinesis defect, binucleus cell, cell membrane damage, spermatid defect, and cell swelling. From this research we conclude that vincristine affect dividing cell in causes various types of defect.

Keywords: vincristine, dividing cells, cell defect, spermatocyte, spermatid

INTRODUCTION

It has been reported that 0.005% vincristin slow down cell division of grasshopper primary spermatocytes, and stop the movement of chromosomes when the concentration increased to 0.01%1). Further studies reported that 0.005% Vincristin also slow down the movement of chromosomes and anaphase process, caused some cell defects2,3), affect the length of the primary spermatocyte cells at cytokinesis, effectively extend the duration of prophase, metaphase, telophase, and cytokinesis3), and inhibits the polymerization of tumor cells from various organs4). Vincristin disturb the binding of β tubulin, disrupt polymerization and cause depolimerisation of microtubule5,6). Interference caused inhibition of chromosome segregation in mitosis, consequently inhibite cell division4). Vincristine pontecially cause the defect or damage in some cells but it has not yet reported in detail1,2). It this research examined the effect of vincristine on dividing primary spermatocytes and spermatids of grasshopper.
MATERIAL AND METHOD

Spermatocyte isolation was done by cutting grasshopper abdomen diagonally, at 4th segment from distal end using fine scissors. Isolated testicles then soaked in 0,9% Carlson’s solution (control) or 0,005% vincristine in 0,9% Carlson’s solution for 10 or 15 minutes. After determined time testicles were cut open to disperse spermatocytes and spermatids from testicular lobe into to 0,9% Carlson’s solution. Defect and damage cells were observed among 150 cells from each treatments and control.

RESULTS AND DISCUSSION

The result shows that there are various types of defect including cytokinesis defect, bi/multinucleated cells, swelling cells, multiple cleavage furrow, cell damage membrane, spermatid defect. Soaking in 0,005% vincristine in 0,9% Carlson solution for 15’ cause more defect compared to 10’ (fig. 1). The most prominent defect is multiple cleavage furrow (33,3%) after 10’ soaking and cytokinesis defect (55,3%) after 15’ soaking. Swelling cells and damage of cell membrane are caused by the disruption of cytoplasmic microtubule7).

![Diagram showing cell defects caused by 0.005% vincristine](image)

Figure 1: Cell Defects caused by 0.005% vincristine. A. cell membrane damage, B. defect of cytokinesis, C. cell swelling, D. bimultinucleated cell, E. multiple cleavage furrow, F. spermatid defect

Some primary spermatocyte and spermatid failed to complete the cytokinesis and became binucleated cell8) caused by delay or alteration in actin patch redistribution9), error in chromosome segregation occurred during the previous meiotic division10) and polar microtubule overlapping7). Multiple cleavage Furrow caused displacement of the centrosome10). From this research we can conclude that 0.005% vincristine in 0.9% Carlson solution various type defect, and the longer soaking increase the amount of defect cells.
REFERENCES


P-PD34

Resistency *Paramecium caudatum* of Pb, Cd, and Fe

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Abstract

*Paramecium caudatum* is one of protozoa that live in the polluted fresh water by organic matter or inorganic matter pollutant. This pollutant were included heavy metal like Pb, Cd, and Fe. The improvement of the freshwater to fulfill the needs water plant like *Paramecium caudatum* should be conducted. This research was aimed to find out resistency *Paramecium caudatum* of heavy metal. Design of research was used by Completely Random Design with three heavy metals consist of Pb, Cd, Fe and 0;025;0,50;0,75,1,2,3 mg/l concentration. The result show that *Paramecium* resistent of Fe, Pb, and Cd. Resistent level of *Paramecium* of Fe, Pb, and Cd were 0,25-1 mg/l.

Keywords: Resistency, *Paramecium caudatum*, Pb,Cd,Fe.

Background

The water fluctuation in the stream according to Suriawira (1999) experiencing fluctuations where the water bodies are polluted will experience water quality purifications gradually with the help of water unicellular microbe such as bacteria, protozoa, and algae. Natural system purifications in stream are started by water microbe.

Protozoa are micro animal that feed on organic in the water, some of this animals are resistance to pollutant such as protozoa from Ciliata class. These animals also make mutualism interactions with some bacteria. Protozoa consumed bacteria or smaller microorganism. Bacteria metabolisms are also producing organic with high level in the water. Even more, bacteria also produce ammonia in the water. Ammonia in water can be use by micro algae metabolism and water plants in limited scale. According to the previous research, the protozoa that resistant with ammonium level and other high level organics substances in Tambak Oso river, Sidoarjo (1999) are Paramaecium, Vorticella and Didinium. (Budijastuti, 1995). This shows that protozoa are able to survive in polluted conditions. Small, E.B. (1993) find that there is relation between total biomass bacteria and water protozoa in Cheesepeake lake, next the sam researcher are studying ciliate that survive in the polluted river in east Illinois. Meanwhile, Cairms, Lanza and Parker (1995) studying the relation between waters pollutant and the change of freshwater protozoa community. The pollutants
are organics, nitrogen, phosphors, and carbon. From that the researcher are conducting further research by finding the level of resistance of paramaecium on NH₄ is 3 ppm. (Yunita, Budjiastuti, and Rachmadiarti, 2006). And about the range of protozoa resistance on Pb, Cd, Fe heavy metals are not much learned, as those heavy metals are hazardous metals and often polluted the environment such as Mercury (Hg), Lead (Pb), Arsenic (Ar), Cadmium (Cd), Chromium (Cr) and Nickel (Ni), those metals are accumulate in the body in long period as toxic that accumulated. Two type of heavy metals that often contaminate the waters are Mercury and Lead (Kristanto, 2002). Cadmium and Fe in Surabaya river are very high because the industrial wastewater that many that using this substances. For that reason need to more understand about the resistance range of Protozoa of the Ciliata group specially *Paramaecium caudatum* type on Cd, Pb and Fe metals which are known that Pramaecium resistance to NH4 are very high.

To analyze the resistance of *Paramaecium caudatum* on Cd, Pb and Fe metals through the addition of Cd, Pb, and Fe metals on the development of total populations of *Paramaecium caudatum* on straws media and given feed of Pseudomonas fluorescens bacteria.

**Method**

This research is experimental research and using Ecology Lab (Biology Department, FMIPA UNESA) as research area, and also using BTKL DEPKES Lab for heavy metals level analysis.

The subjects of this research are pure/clean culture of *Paramaecium caudatum* that are breed in straw medium and given feed of Pseudomonas fluorescens bacteria. Experiment variables in this research are the adding of heavy metals with concentrations of Fe, Pb, Cd are 0.25; 0.5; 0.75 mg/l respectively. Dan responses variables are the total populations of *Paramaecium caudatum* that being count each day for 10 days observations.

This research design being used are Factorial Block Random Design, this design are being used because in this research have two experiments with two factors. The first factor is concentrations and the second factor is the metal type/organics substances. Data analysis that being conducted are quantitative descriptive.
Result

Table 1. Average growth population of *Paramaecium caudatum* to Fe

<table>
<thead>
<tr>
<th>Day to Treatment</th>
<th>Growth in 0.1 ml/100 ml day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>K</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 2. Analysis of Chemical concentrations of Fe in media after treatment

<table>
<thead>
<tr>
<th>No</th>
<th>Treatment (ppm)</th>
<th>Fe (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.25</td>
<td>0.106</td>
</tr>
<tr>
<td>2</td>
<td>0.50</td>
<td>0.125</td>
</tr>
<tr>
<td>3</td>
<td>0.75</td>
<td>0.130</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>0.126</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>0.119</td>
</tr>
<tr>
<td>6</td>
<td>3</td>
<td>0.146</td>
</tr>
</tbody>
</table>

The resistance of *Paramaecium caudatum* of Fe total population are increasing optimal best in 0.25 ppm and generally are happen time extending that needed (day 7th) in peak phase than control group (day 5th). Susceptibility resistance of *Paramaecium caudatum* shown in 0.25 – 3 ppm although total populations that reach peak are not better than control. Generally, *Paramaecium caudatum* are resistance of Fe because able to absorb Fe.

Table 3. Average growth population of *Paramaecium caudatum* to Cd

<table>
<thead>
<tr>
<th>Day to Treatment</th>
<th>Growth in 0.1 ml/100 ml day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>K</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
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<td>3</td>
<td>0</td>
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<tr>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 4. Analysis of Chemical concentrations of Cd in media after treatment

<table>
<thead>
<tr>
<th>No</th>
<th>Treatment (ppm)</th>
<th>Fe (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.25</td>
<td>0.0</td>
</tr>
<tr>
<td>2</td>
<td>0.50</td>
<td>0.0</td>
</tr>
<tr>
<td>3</td>
<td>0.75</td>
<td>0.0</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>0.0</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>0.0</td>
</tr>
<tr>
<td>6</td>
<td>3</td>
<td>0.0</td>
</tr>
</tbody>
</table>

The *Paramaecium caudatum* resistance to Cd metal, the total populations increases the best at 0.25 ppm to 1 ppm. Even compare to the optimal total of control group, just that
all treatment shows that more time to achieve optimal total (day 6-7) are needed. Graph for treatment above 0.75 is once again increase in the 10th day, so that generally it can be said that *Paramaecium caudatum* is resistant to Cd. The Absorption ability of Cd can be seen after the examination of Cd level that drastically decrease in the sixth treatment.

Table 5. Average growth population of *Paramaecium caudatum* to Pb

<table>
<thead>
<tr>
<th>Day to treatment</th>
<th>Growth in 0.1 ml/100 ml day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>K</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
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<td>5</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 6. Analysis of Chemical concentrations of Pb in media after treatment

<table>
<thead>
<tr>
<th>No</th>
<th>Treatment (ppm)</th>
<th>Pb(ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
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<td>0,025</td>
</tr>
<tr>
<td>2.</td>
<td>0,50</td>
<td>0,029</td>
</tr>
<tr>
<td>3.</td>
<td>0,75</td>
<td>0,019</td>
</tr>
<tr>
<td>4.</td>
<td>1.0</td>
<td>0,065</td>
</tr>
<tr>
<td>5.</td>
<td>2.0</td>
<td>0,132</td>
</tr>
<tr>
<td>6.</td>
<td>3.0</td>
<td>0,233</td>
</tr>
</tbody>
</table>

The *Paramaecium caudatum* resistance to Pb metal, the total populations increases the best at control group that closing the control group is the 1 ppm treatment. All the treatments shows longer time needed to achieve the optimal total (days 6th – 7th). And it seems that the ability to absorb Pb is existed in all treatments. This shows that *Paramaecium caudatum* is still resistant to Pb before the tenth day that showing death signs.

**Conclusions**

The results of this research can be concluded as below:

1. The *Paramaecium caudatum* resistant to Fe have range of 0.25 – 3 ppm and the total populations until peak phase is not better than control but *Paramaecium caudatum* are able to absorb Fe.

2. The *Paramaecium caudatum* resistant to Cd have range of 0.25 ppm to 1 ppm and the total populations until peak phase is better in control and also Paramaecium caudatum are able to absorb Cd very well.

3. The *Paramaecium caudatum* resistant to Pb are 1 ppm and the total populations until peak phase is approaching control and also Paramaecium *caudatum* are able to absorb Pb.
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P-PD35

Competency of Wolffia arrhiza of Pb, Cd, and Fe Absorbtion

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Abstract

Wolffia sp is aquatic plant that live in polluted fresh water by organic matter or inorganic matter polutan. This polutan were included heavy metal like Pb, Cd, and Fe. The improvement of the freshwater to fulfill the needs water plant like Wolffia sp should be conducted. This research was aimed to find out heavy metal can optimum absorbtion by Wolffia sp. Design of research was used by Factorial Completly Block Random Design with three heavy metals consist of Pb, Cd, Fe and 0,1,2,3 mg/ concentracion. The result show that there are no significan differences between heavy metals of Cd and Fe. However, there are significan difference of Pb, between 2 and 3 mg/l concentracion significan differences with 0 and 1 mg/l concentration. Competency of Wolffia sp of heavy metal absorbtion were followed by Pb, Fe, and Cd.

Keywords: Competency, Wolffia sp, Pb,Cd,Fe,Absorbtion.

Background

Pollutions by heavy metal in the environments can affect the decrease of plants’ development and productivities, and also can cause death in several cases. The plants’ growth is depending on the conditions of external environment especially root’s systems and seeds. The decrease of development and productivity in plants can make the plants became cretinous and chlorosis (Connel and Miler, 1995). The levels of metal toxicity are depending on the availability of the metal and the interaction between water and metal, nutrient status, and plants’ substances. Although some plants species able to tolerate metal toxicity, some other species that have sensitive genotypes will suffer metal poisoning, even though in small concentrations (Darmono, 1995).

The development of plants population that tolerate with the heavy metal pollutions are because there is direct contact with the heavy metal and populations that are resistance with the pollutions are the results of a selection in specific time period. The mechanisms that have built by the plants to resist the heavy metal toxicity are achieved by releasing the metal form condensed areas, forming special enzymes that anti-metal and obstructing the metabolism path. Plants that sensitive with copper (Cu), zinc (Zn), lead, cobalt nickel, cadmium (Cd) and Manganese (Mg) containing enzymes with cluster function/compound of sulfhidril and karboksilat that react with the metal in different ways (Connel and Miler, 1995).
Water vegetations are popularly used for nutrient processing and the organics substances that exist in the domestic wastewater, but according to some researches, water vegetations are also known to have ability to accumulating heavy metals in it surroundings, therefore it further develop in order to be use in heavy metals treatment that existed in the wastewater such as develop by domestic waste.

The *Wolffia arrhiza* plants are member of Lemnaceae group (Magnoliophyta Division, Lliopsida Class, Arecidae Subclass and Arales Order) which are vegetations that floating above the water surfaces (Floating Plant), doesn’t have roots, only posses single leave or two leaves that are connected and contain tissues that carry nutrients. This plant doesn’t need soil as the growing media so it can be place on water surfaces. *Wolffia* can be seen abundance in shallow waters, rice fields, swamps, ponds, and lakes. The spreading of this plants are very wide especially on tropical areas and areas that have warm temperature (Marianto, 2001).

*Wolffia arrhiza* plants can grows and develops without control because of the supporting environment factors such as the abundance of nutrients and sunlight on suffused/still waters. *Wolffia* can grow rapidly especially on waters that contain high nitrogen and phosphate. Not like other vegetations, *Wolffia* are able to survive in bad environment, which indicate there is potential of *Wolffia*. For example, it can tolerate on various pH levels (5-9), but the best growth is in 6.5 – 7.5 pH., also it can survive in high salinity conditions (up to 4000 mg/liter). It also able to gather metals residues in industrial wastewaters therefore it will produce waters that suitable to release in streams or irrigations so that in the same time it can give economic solutions in restoring the precious resources, when if it not manage good enough could polluted the environment (Leng, et al., 1995).

This research using *Wolffia* plants to find out it’s ability in absorbing heavy metals. These plants can be seen in many places and sprouts up in waters and the member of Lemnaceae group which according to preliminary research, can be use as bio-indicators in monitoring the polluted water environments. The preliminary research had been conducted on Lemnaceae especially minor Lemna as bio-indicator plant of heavy metals. The research results shows that minor Lemna can decrease the heavy metals level in wastewater that contained Fe 2.273 ppm, Cu 5.529 ppm, and Pb 1.523. Minor Lemna can decrease the heavy metals level up to 50% for Fe, 50% for Cu and 30% for Pb (Safiludin, Fida R., Yuliani, 2005). Thus, it’s hoped from other plants type in the Lumnacease group posses the same ability.

The heavy metals being used in this research are Pb, Cd and Fe. These three heavy metals are common use in many applications, therefore it is routinely produces in industrial scale. The usage of those heavy metals in everyday activities directly or indirectly can pollute the environment (Fardiaz, 1992). Those metals have accumulative characteristic in the body
in long period as toxic that accumulated. Thus, it is needed to develop water plants that can absorb and lowering the heavy metals level in waters so it can be expected that can expand the usage of various type of the appropriate water plants for monitoring and solving the waste pollution especially heavy metals pollution in waters.

This research is aim to understand the resistance of Wolffia on heavy metals of Pb, Cd, Fe with assessing the level of heavy metals in the Wolffia plant, the level of heavy metals in plant media and calculating the percentage of decrease of those three heavy metals in plant media of Wolffia.

Methods
This research is experimental research with research subject of *Wolffia arrhiza*. The experiment variables in this research in the adding of different heavy metals concentrations, which are heavy metals level of Fe, Cd and Pb in 1, 2 and 3 mg/liter. The responses variables are a) the concentrations of Fe, Cd and Pb heavy metals in Wolffia (before and after the treatment) b) the concentrations of Fe, Cd and Pb heavy metals in water plant media (before and after treatment,) c) the percentage of heavy metals decrease in plant media. The research design being use are *Factorial Block Random Design*, this design are being used because in this research have two experiments with two factors. The first factor is concentrations and the second factor is the metal type/organics substances.

This research are consist of three stages, the first stage is (a) water plant acclimation procedures: growing Wolffia in water media and for every two days the water are replaced, plant acclimation are conducted for 10 days and analyzing the early heavy metals level in the plants, (b) making plant media: giving the plant media the metals of Pb, Cd, Fe with levels according to the experiments design and placing plant media in 5 liter container. Preparing plant media that are controlled without any concentration of Pb, Cd and Fe (aqua media), (c) maintaining the plants for 10 days and analyzing the Pb, Cd, Fe in the plants. Beside that are also conducting pH and temperature measurements as supports.

Data analyses of this research are using two directions ANOVA that will be continued using DMRT to see the differences.

**Results**
1. Early concentrations data of Pb, Cd, Fe (ppm) in Wolffia plant and in Pacet water source, Mojokerto.

Wolffia plants that being used are obtained from Sukolilo areas, Surabaya, in those plants, existed heavy metals although below maximum level such in the next table:
Table 1. Research Result of Early Level of Pb, Cd and Fe (ppm) in Wolffia

<table>
<thead>
<tr>
<th>Plant type</th>
<th>Heavy Metals</th>
<th>Level (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wolffia arrizha</td>
<td>Lead (Pb)</td>
<td>0.012</td>
</tr>
<tr>
<td></td>
<td>Cadmium (Cd)</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Iron (Fe)</td>
<td>0.020</td>
</tr>
</tbody>
</table>

For this research, the water are obtained from Pacet, Mojokerto with heavy metals analysis results presented below:

Table 2. Research Result of Early Level of Pb, Cd and Fe (ppm) in Pacet water source, Mojokerto

<table>
<thead>
<tr>
<th>Material</th>
<th>Metals</th>
<th>Level (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pacet water source, Mojokerto</td>
<td>Lead (Pb)</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>Cadmium (Cd)</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>Iron (Fe)</td>
<td>0.11</td>
</tr>
</tbody>
</table>

From tables 1. and 2. obtained that Fe metal are exist in big amount than those two metals, both in water and in the plant, nevertheless, its still below maximum level, this can be understand because Fe is essential metal for vegetations as part of catalyst process from many reduction oxidation enzymes.

2. Data of Pb, Cd and Fe Heavy Metals concentrations in Wolffia after treatment

The result of variant analysis shows that treatment of heavy metals with various type and concentrations are effected the concentrations of heavy metals (Pb, Cd and Fe) that being absorbed by Wolffia. Thus, the interaction of metals type and concentrations are affecting the heavy metals absorption in Wolffia plants. The heavy metals concentrations in Wolffia plants after treatment for 10 days are presented in table 3 as follows:

Table 3. The absorption ability of Wolffia Plant on Pb, Cd, Fe heavy metals

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Heavy metal contents in the Plant types (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heavy metal type</td>
<td>Concentration (mg/l)</td>
</tr>
<tr>
<td>Pb</td>
<td>K_0 (0 mg/l)</td>
</tr>
<tr>
<td></td>
<td>K_1 (1 mg/l)</td>
</tr>
<tr>
<td></td>
<td>K_2 (2 mg/l)</td>
</tr>
</tbody>
</table>
BNT test result in 0.05 significant levels on Wolffia shows, that in Cd and Fe metals there are no significant differences but different with Pb. For the K2 and K3 concentrations have significant different with the K0 and K1 concentrations.

3. Heavy metals concentrations data on Wolffia growth media after treatment
After planting for ten days, the Pb, Cd and Fe heavy metals concentrations in growth media are being analysis using AAS, the results are shown in table 4.

Table 4. The concentrations level of Pb, Cd, Fe heavy metals in growth media after treatment

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Heavy metal contents in the plant medium (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heavy metal type</td>
<td>Concentration (mg/l)</td>
</tr>
<tr>
<td>Pb</td>
<td>K0 (0 mg/l)</td>
</tr>
<tr>
<td></td>
<td>K1 (1 mg/l)</td>
</tr>
<tr>
<td></td>
<td>K2 (2 mg/l)</td>
</tr>
<tr>
<td></td>
<td>K3 (3 mg/l)</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
</tr>
<tr>
<td>Cd</td>
<td>K0 (0 mg/l)</td>
</tr>
<tr>
<td></td>
<td>K1 (1 mg/l)</td>
</tr>
<tr>
<td></td>
<td>K2 (2 mg/l)</td>
</tr>
<tr>
<td></td>
<td>K3 (3 mg/l)</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
</tr>
<tr>
<td>Fe</td>
<td>K0 (0 mg/l)</td>
</tr>
<tr>
<td></td>
<td>K1 (1 mg/l)</td>
</tr>
<tr>
<td></td>
<td>K2 (2 mg/l)</td>
</tr>
<tr>
<td></td>
<td>K3 (3 mg/l)</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
</tr>
</tbody>
</table>

Note: Numbers that followed by the same letter in the columns are not significantly different with 5% BNT test level.
Overall, from the average of heavy metals level on growth media shows that in the growth media of Wolffia plants shows that for Cd metal are more absorbed than Pb and Fe.

4. Decrease percentage data of Pb, Cd, Fe heavy metals in water media of Wolffia

The decrease percentage of heavy metal in growth media are measured based on early metals level in water media with metals level after treatment.

<table>
<thead>
<tr>
<th>Heavy metal type</th>
<th>Initial concentration of heavy metal (mg/l)</th>
<th>Concentration of final water condition (mg/l)</th>
<th>% Heavy metal decrease on the concentration (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Lemna major</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pb</td>
<td>1.0</td>
<td>2.0</td>
<td>3.0</td>
</tr>
<tr>
<td>Cd</td>
<td>1.0</td>
<td>2.0</td>
<td>3.0</td>
</tr>
<tr>
<td>Fe</td>
<td>1.11</td>
<td>2.11</td>
<td>3.11</td>
</tr>
</tbody>
</table>

In Wolffia, shows that for Pb absorption is increasing but inline with the increase the absorption concentration became more stable. the more high concentration of Fe the more high metal absorption, and for Cd shows stable absorption. The absorbed heavy metals are Cd, and then followed by Pb and Fe, respectively.

The results shows that Wolffia posses the ability to absorb heavy metals of Pb, Cd and Fe in growth water media. Although the Wolffia ability to absorb Cd is bigger than absorbing Pb and Fe.

The vegetations that live in polluted area have its own adjustment mechanism that make the pollutant became non active and being stored in old tissues so it cannot harm the life and development of these plants. According to Fitter and Hay (2001) suggest four main mechanisms so that process occurs, which are:a) Penology avoidance (escape), if there a stress that occurs in the plans that periodically, the plants can adjust it self, so it will growth in climate/season that appropriate; b) Exclusion, the plants can recognize toxic ion and prevented to be taken so it wont experience toxicity; c) Amelioration, the plants can absorb ions, but acts in such in order to minimize it effects. the acts including forming chelae/khelat, localizations, or even excretions; d) Tolerate, the plants develop metabolism system that can be functions in potential toxic concentrations, with molecules and genetic works.

The decrease of iron (Fe), cadmium (cd), and lead/timbale (Pb) in the growth water media it because there is cordages process of iron, cadmium, and lead in the plants tissues. Iron, cadmium, and lead in the growth media are rarely in form of single atom but usually
being bond by other compound that eventually form salt substances (Darmono, 1995). The roots can absorb heavy metals in form of dissolved ions in the water such as organic substances that entered along with the water flows. Metals accumulations in the roots through the help of metals transport in roots membranes, that eventually will form complex metals transport that will penetrated the xylem and goes all the into the leaves cells, once it reach the leave it will past through plasmalema, cytoplasm clan vacuole, where the metals will accumulated in the vacuole that will not connected with the physiology process of the plant cells. The environment that fills with heavy metals will make the protein regulator in those plants conducting gene expression in order to form bonding compound that called phytochelatine. Phytochelatine is peptide that contain 2 -8 systein amino acid in the molecules center and glutamate salt and glassine/glisin in opposite end. Phytochelatine is containing many cluster-functions of SH-, S^2-, RS-. Those clusters is exist in systein amino acid that compound of phytochelatine. Phytochelatine will make sulfide bonds in the sulphuric ends in the systein if meet with iron, cadmium, lead and other heavy metals, and form complex compounds. Thus, the heavy metals will carry into the plants’ tissues (Salisbury and Ross, 1995).

Wolffia posses the biggest absorption ability than the other two plants, Wolffia doesn’t have roots, posses only single or two leaves that connected each other that contain nutrients-carrying tissues, the absent of roots will make leave of Wolffia have important role in the process of heavy metals absorptions, thus, the heavy metals are no longer bond in the roots but directly absorb by the leave where the metals will accumulated in the vacuole that will not connected with the physiology process of the plants.

Conclusions
From those results, it can be concluded as follows:

1. Wolffia plants posses’ resistance to Pb, Cd, and Fe heavy metals.
2. The concentrations of the heavy metals in Wolffia shows that the bigger concentrations (1 and 2 gram) the absorption are also bigger, but more bigger concentrations (3 gram) experiencing decrease. The biggest absorptions of heavy metals are for Pb, then Fe and Cd.
3. The concentrations of Pb, Cd, and Fe heavy metals (ppm) in growth water media after the treatment shows that for Cd metals are more absorbed than Pb and Fe. Cd level in growth water media are 0.01 ppm. Pb level are 0.07 ppm, and Fe level are 0.24 ppm.
4. Wolffia plants have the ability to absorb Pb, Cd, and Fe heavy metals in the growth water media. The biggest absorptions are for Cd heavy metals, and then followed by Pb and Fe.

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Industrial Water Treatment Know. Journal of Science and Technology in December 1999 special edition
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DAMPING-OFF OF CHILLI (Capsicum annum L.) MANAGEMENT BY USING BIOCONTROL AGENTS OF Bacillus brevis AND Bacillus pantothenicus

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ABSTRACT
Bacillus brevis and Bacillus pantothenicus were evaluation under greenhouse condition for efficacy in suppressing Rhizoctonia solani damping-off incidence and plant growth promoting bacteria in chilli (Capsicum annum L). B.pantothenicus was the most effective in reducing of the disease incidence (51%) followed by B.brevis (35%) more effective than control. Both of them gave significant effect on the chilli height and no significant effect on dry weight of the chilli. Two of these Bacillus species were indicated significant effect on the reducing of the number of R.solani colonies in rhizosphere soil.

Key words; B.brevis, B.pantothenicus, Capsicum annum L., damping-off and plant growth promoting bacteria

INTRODUCTION
Biological control is an alternative to the management of diseases caused by soil-borne microorganisms. Rhizoctonia solani is the main causal agent of damping-off disease of seedling as well as root and stem rot in young transplants of several plant species. R.solani is widely distributed pathogen affecting different economically important crops. Bacillus spp. are non-pathogenic, easy to cultivate, protein and metabolite secretors. These characteristics make them appropriate for the formulation of stable and viable biological products that could be used for soil-borne disease management. The aim of this research was to observe the effectivities of two biocontrol agents; B.brevis and B.pantothenicus application in suppressing of R.solani damping-off under greenhouse experiment.

MATERIAL AND METHODS
Microorganisms.
Bacillus brevis and B.pantothenicus
Soil treatments. The soil was sieved and air dried. One kilogram soil was put into a sterilizable polybag and autoclaved for 60 minutes at 121°C four times at 12 hours intervals.
Inoculation of R.solani into soil. Fifty milliliters of steril PDP medium was inoculated with 5mm plug of R.solani, incubate for one week. Then the mycelial mats, on the surface of
the medium were homogenized with blender. 150 g of homogenized mycelia of *R. solani* inoculated into the soil and the pots were incubated in greenhouse for two days.

**Application of *B. brevis* and *B. pantotheinicus* into soil.**

Each of them (*B. brevis, B. pantotheinicus*) was incubated for about 20 hours in LB medium using water bath shaker (30°C, 130 rpm), and then 1 ml of the culture broth was inoculated into 100 ml of No. 3 medium. The flasks were shaken for 5 days at room temperature. 150 ml the culture broth was mixed with 1 kg soil containing *R. solani* 2 days incubated times, then the pots were incubated for 3 days in greenhouse.

**Plant growth**

After incubation (see procedures above), three of chilli seedling were planted into each of the pots, and keep its in greenhouse for three weeks.

**Counting of *R. solani* viable cell number**

The number of *R. solani* colonies was counted as described by Ko and Hora (1971) Analysis data.

The results were analyzed by Randomized Completely Block Design (RCBD). The treatment were: 1. Control (only chilli, without fungi and bacteria), 2. inoculated with *R. solani*, 3. inoculated with *R. solani* and *B. brevis*, 4. inoculated with *R. solani* and *B. pantotheinicus*. 

**RESULTS AND DISCUSSION**

**The effect of *B. brevis* and *B. pantotheinicus* on Rhizoctonia solani damping-off chilli.**

In greenhouse study, when chilli inoculated with *B. brevis* or *B. pantotheinicus* decreased damping-off chilli significantly. By application of *B. pantotheinicus* the diseases percentage was about 53.7% and by using *B. brevis* was 35.0% (see Table). The application of *B. subtilis* RB14-C culture broth alone, reduced the damping-off tomato was about 38.9%

5. Probably it was due they produce bioactive compounds for suppressing *R. solani* growth.

Table. The effect of *B. brevis* dan *B. pantotheinicus* on Rhizoctonia solani damping-off chilli

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Percentage of deseases</th>
<th>Chilli height (cm)</th>
<th>Dry weight (mg)</th>
<th>Colonies number /g of soil (x10^-1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.0°</td>
<td>10.10°</td>
<td>990 *</td>
<td>0.0°</td>
</tr>
<tr>
<td>3. <em>R. solani</em></td>
<td>86.3°</td>
<td>7.76°</td>
<td>893.3 *</td>
<td>4.2°</td>
</tr>
<tr>
<td><em>R. solani</em>+<em>B. brevis</em></td>
<td>53.7°</td>
<td>10.00°</td>
<td>756.6 *</td>
<td>2.4°</td>
</tr>
<tr>
<td><em>R. solani</em>+<em>B. pantotheinicus</em></td>
<td>35.0°</td>
<td>11.40°</td>
<td>930 *</td>
<td>1.5°</td>
</tr>
</tbody>
</table>

**The effect of *B. brevis* B. pantotheinicus on chilli height**
When *B. brevis*, *Bacillus pantothenicus* were applied into the soil, the chilli height were significantly higher than those of non-treated chilli infested with *R. solani* (Table). A microorganism that colonizes root is ideal for use as biocontrol agent against soil-borne diseases, and consequently in improving plant growth.  

**The effect of *B. brevis* dan *B. pantotheicinus* on dry weight of chilli**

*B. brevis*, and *B. pantotheicinus* were not resulted significant effect on the weight of chilli. Probably it was the observation was performed only for three weeks (vegetative growth).

**The effect of *B. brevis* dan *B. pantotheicinus* on colonies number of *R. solani***

When *B. brevis*, *B. pantotheicinus* were inoculated into the soil, colonies number of *R. solani* decreased significantly. The explanation for these results were the possible mechanisms of the biocontrol agents against plant pathogens such as antibiosis, nutrient competition, lytic enzymes and siderophore production.

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P-PD37

PLASMODIUM, ANOPHELES GENOMICS AND MALARIA ON NORTHERN PAPUAN POPULATIONS

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Abstract

The Plasmodium spp. parasites that cause malaria are transmitted to humans by Anopheles spp. mosquitoes. Scientists have now amassed a great body of knowledge about the parasite, its mosquito vector and human host. Yet this year there will be 300–500 million new malaria infections and 1–3 million deaths caused by the disease. We believe that integrated analyses of genome sequence, DNA polymorphisms, and messenger RNA and protein expression profiles will lead to greater understanding of the molecular basis of vector parasite interactions and provide strategies to build upon these insights to develop interventions to mitigate human morbidity and mortality from malaria. The genome sequences of many of the key species in the life cycle of the malaria parasite will be available. This includes: the 25–30 million base pairs of the genomes of \textit{Plasmodium falciparum}-the parasite responsible for more than 95% of all malaria deaths-the most important mosquito vector of \textit{P. falciparum} in Northern Papuan Populations, where malaria causes the most deaths; and the 2,900 and 2,600 Mb of the human and mouse genomes, respectively. Here, we summarize the current status of the \textit{Plasmodium}, Papuan human and \textit{Anopheles} genome sequencing projects on Papuan Samples. We show how a number of fields of biology, based on genomics, enabled and integrated by bioinformatics, and often referred to collectively as ‘functional genomics’, are being brought to bear on malaria research, and how we think these approaches will improve the chances of success in our battle against malaria on Papuan Province.

\textbf{Key Words}: Plasmodium, Anopheles Genomics, Malaria, and Northern Papuan Populations

Introduction

The Plasmodium spp. parasites that cause malaria are transmitted to humans by Anopheles spp. mosquitoes. Scientists have now amassed a great body of knowledge about the parasite, its mosquito vector and human host. Yet this year there will be 300–500 million new malaria infections and 1–3 million deaths caused by the disease. We believe that integrated analyses of genome sequence, DNA polymorphisms, and messenger RNA and protein expression profiles will lead to greater understanding of the molecular basis of vector
parasite interactions and provide strategies to build upon these insights to develop interventions to mitigate human morbidity and mortality from malaria. The genome sequences of many of the key species in the life cycle of the malaria parasite will be available. This includes: the 25–30 million base pairs of the genomes of *Plasmodium falciparum* -the parasite responsible for more than 95% of all malaria deaths-the most important mosquito vector of *P. falciparum* in Northern Papuan Populations, where malaria causes the most deaths.

**Materials and Methods**

Experimental and computational hurdles had to be overcome to complete sequencing and assembly of this genome. Large genomic fragments of *P. falciparum* DNA are not stable in *Escherichia coli*, a problem thought in large part to be due to the skewing of nucleotide content from the 59% adenine and thymine (A+T) of the human genome and 49% of the *E. coli* genome to the 80% found in the *P. falciparum* genome. The presence of A+T repeats/homopolymers has also made gap closure and accurate assembly difficult. New techniques and annotation software had to be developed to overcome these problems. To facilitate interpretation and dissemination of genomic sequence data to researchers worldwide, informatics capabilities that integrate sequence data, automated analyses and annotation data emerging from the *P. falciparum* genome project have been developed.

**Results and Discussions**

The value of genomic sequence data and comparative analyses to identify new drug targets was shown recently with the identification in *P. falciparum* of the metal-dependent RNA triphosphatase protein family, members of which are crucial in mRNA cap formation and eukaryotic gene expression. The structure of the active site and catalytic mechanism of this protein family in *P. falciparum* and fungi are completely different from the RNA triphosphatase domain of the metazoan (human) capping enzymes, and metazoans encode no identifiable homologues of the fungal or *Plasmodium* RNA triphosphatases. The structural similarity between the plasmodial and the fungal RNA triphosphatases raises the exciting possibility of achieving antifungal and antimalarial activity with a single class of mechanism-based inhibitors. Protein families that are expanded in *P. falciparum* and are considered attractive drug targets include the cysteine and aspartyl class of proteases, with both classes constituting a parasite adaptation to its need for haemoglobin digestion. Of special interest is the representation of several members of the recently described metacaspase family of
cysteine proteases in *Plasmodium*. The absence of this class of proteases in humans makes the *P. falciparum* metacaspases particularly attractive as drug targets.

Here, we summarize the current status of the *Plasmodium*, Papuan human and *Anopheles* genome sequencing projects on Papuan Samples. We show how a number of fields of biology, based on genomics, enabled and integrated by bioinformatics, and often referred to collectively as ‘functional genomics’, are being brought to bear on malaria research, and how we think these approaches will improve the chances of success in our battle against malaria on Papuan Province, especially on northern part. The availability of genomic sequence data, use of sequence-based, high-throughput technologies, and advances in bioinformatics to analyse and interpret genomic data will ultimately provide an integrated picture of malarial biology, pathogenesis and epidemiology. We anticipate a time when we will routinely measure real-time biological profiles, including molecular phenotypic expression and genetic polymorphisms of the vector, host and parasite, and clinical outcomes, to create molecular kinetic portraits. Such complex biological systems have properties that cannot be predicted *a priori*. The development of mathematical modelling methods to describe biological systems at the molecular and/or population level, and predict behaviours of these systems in response to various virtual treatments, will be crucial in developing a new generation of interventions to attack malaria. The promise of a genome-sequence-based platform thereby lies in providing an integrated reconstruction of the spectrum of molecular and cellular interactions among parasite, vector and human host, with the ultimate goal of eradicating malaria.

References


P-PD38

SHOOT-TIP CULTURE
OF RARE PAPUAN ORCHID SPECIES *Dendrobium spectabile*

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ABSTRACT

Besides reputed for its beauty, *Dendrobium spectabile* has also known for scarcely flowering species. This made generative propagation of the species a rare occasion. Long term storage of this orchid in term of in-vitro collection is important for its conservation. In this study, bud formation of *Dendrobium spectabile* was induce in *Hyponex* medium supplemented with (0,1,2,3,4,5, and 6 mg/l) BAP. The result showed that new buds were gradually formed after 2 weeks, during 16 weeks observation. The highest number of new buds was obtained in the medium supplemented with 5 and 6 mg/l BAP.

**Keywords**: Shoot-tip culture, *Dendrobium spectabile*, conservation

INTRODUCTION

*Dendrobium spectabile* is one of the Papuan orchid endemic species. Its curly petals had made this orchid named *anggrek kribo* (curly orchid) in Indonesian. Beside its distinctive feature, this orchid also reputed for its rarity in terms flowering and fruiting (Lavarack *et al.*, 2000). *Dendrobium spectabile* in Bogor Botanic Garden was collected during Flora Expedition to Papua in 2004. Seed capsules were already set when this orchid planted for ex-situ collection and harvested at the same year. Under the glass house condition in Bogor, the orchid has never been flowering since then. According to Nash (1994), this species is reputed as a difficult plant to produce flowers. *In vitro* culture of the seeds had been carried out to get mature plant collection. However, stock material is important to be available at all times in Bogor Botanic garden, especially for conservation programs in either short or long term purposes. *In vitro* culture is the most effective method to get large number of seedlings, however, information on medium for its growth and development is limited. Preliminary study showed that *Hyponex* based medium was more effective than MS medium to induce *Dendrobium* culture growth, but optimum concentration level of BAP for shoot induction of this rare species was unknown.
MATERIALS AND METHODS

Explant were obtained from in vitro seedling of Dendrobium spectabile from Bogor Botanis Garden collection. After removing leaves, shoot tip of each planlet were placed on the modified Hyponex (N:P:K = 25:5:20) basal medium with 1g/l sweet potato, 20g/l banana, supplemented with 0, 1, 2, 3, 4, 5, and 6 mg/l 6-benzylaminopurin (BAP). The treatment consisted of 12 replicates with 12 culture vessel contained 15 ml medium, each with 3 explants. All cultures were maintained at 25°C under 16 hours light. Visual observation on new bud formation and number of leaf was carried out in 2 weeks interval during the course of 16 weeks.

RESULTS AND DISCUSSION

The result showed that new buds were gradually formed after 2 weeks, during 16 weeks observation. The highest percentage of explants producing buds (91.7%), with an average of 3.18 buds per explants, was induced by culturing shoot tip on Hyponex medium supplemented with 3.0 mg/l BAP. However, the highest number of new buds (11 buds) was obtained in the medium supplemented with 5 and 6 mg/l BAP (Fig 1). The number of new bud formation depended on the source of explants and the concentration of growth regulators added to the medium. In this experiment, the number of buds were varied even within replicates. This result is better compared to the preliminary study using Murashige and Skoog (MS) medium supplemented with 0; 1,5; 2; 2,5; 3; dan 3,5 mg/l BAP. Number of newly developed bud in that experiment within 12 weeks ranged from 0 - 3 buds only (unpublished data). Similar experiment on D. spectabile using MS medium supplemented with 20% coconut water and 1 mg/l BAP by Silalahi & Noya (2009) only reach 6.4 new buds formation at the highest. At the end of this experiment 23 leaves were also found on the same treatment and this result was correlated with the number of buds.

![Figure 1](image.png)

Fig 1. Buds formation of D. spectabile during 16 weeks observation with 2 week interval observation.
Benzy1 Adenin (BA) is the most effective growth regulator to induce development of many orchid species (Nayak et al., 2002). However, the respond of each *Dendrobium* species to BA were varied according to species and basal medium in used. The newly formed PLBs of *Dendrobium densiflorum* Lindl. ex Wall. proliferated well on the MS basal medium and completely converted into shoots on MS medium containing 2.0 mg/l BAP (Luo, et al. 2008). On the other hand, leaf of *Dendrobium* hybrid from flower stalk node derived explants cultured on half-strength MS medium supplemented with 44.4 μM BA, developed more than seven shoots per explants (Martin and Madassery, 2006). Other previous research indicated that the best growth of *D. nobile* was obtained on MS medium supplemented with 4.95 mg/l BAP (Nayak et al, 2002). In this research 5-6 mg/l BAP is suggested as the most effective concentration of plant growth regulator to induce buds formation of *D. spectabile* when added to modified Hyponex basal medium.

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## Topic 5: Biomedics

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P-BM01

Repetitive 529 bp. DNA Fragment of an Indonesian *Toxoplasma gondii* Isolate: Primary Structure and Sequence Variations

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ABSTRACT

Toxoplasmosis is one of the most common parasitic diseases in humans and other warm-blooded animals. In humans, infections are usually asymptomatic but severe disease can occur in immunocompromised individuals and newborns. The availability of efficient early diagnostic tools is essential. The genome of *Toxoplasma gondii* contains repetitive sequences which constitute ideal targets for genome-based detection methods. We have isolated and amplified a tandem repeat of the repetitive 529 bp. sequence, previously reported to be composed of 529 bp. (R529), from an Indonesian isolate and established that the actual length of the repeat unit is 522 bp. We suggest therefore to call it henceforth R522 and not R529. Sequence comparison with two other sequences of this repetitive sequence showed variations within the repeat units either in a given or in different species, in contrast to the high sequence conservation of the single-copy SAG1 gene.

Keywords: R522, R529 repetitive sequence, sequence comparison, *Toxoplasma gondii* Indonesian isolate

INTRODUCTION

*T. gondii* is an obligate protozoan intracellular parasite that belongs to the phylum Apicomplexa, subclass coccidia, and constitutes one of the most successful protozoan parasites on Earth. While toxoplasmosis is usually innocuous or asymptomatic in most individuals, infection with *T. gondii* during pregnancy may lead to severe, if not fatal, infection of the fetus¹,². In order to take suitable measures, an early diagnosis of the disease is essential and the availability of efficient and sensitive diagnostic tools is of great importance. A certain number of genes are used as targets in *Toxoplasma* detection by *in vitro* amplifications (PCR). The repetitive 529 bp. DNA fragment (R529) which is a non-coding sequence, appears to be the most promising gene target due to its high copy
number, evaluated to be 200-300 copies or even more\textsuperscript{3,4}. This paper described the nucleotide sequence of a tandem repeat of R529 from an Indonesian \textit{T. gondii} isolate. We established that the actual length of R529 is 522 bp. We suggest therefore to call it henceforth R522. Sequence comparison with two other available sequences showed variations within the repeat units.

**MATERIALS AND METHODS**

The Indonesian isolate of \textit{T. gondii}, called IS-1 for convenience was isolated from the diaphragm of a goat at the slaughterhouse Cibadak at Sukabumi, West Jawa, Indonesia. DNA was extracted from tachyzoites isolated using DNAZOL (Invitrogen). The repetitive 529 bp. DNA fragment of the Indonesian \textit{T. gondii} isolate was isolated and amplified by PCR using primers located at its ends, i.e. sense primer S1 and anti-sense primer C1 or internally located sense primer S2 and anti-sense primer C1 (see Results below for primer sequences). Amplification products were analyzed by electrophoresis on a 1% agarose gel. Amplified products obtained with primer couple were further directly cloned in pCR2.1-TOPO (Invitrogen). Positive clones (white colonies) were cultured and the plasmid prepared using "High Pure Plasmid Isolation Kit" (Roche). Nucleotide sequencing was carried out directly on insertion-containing plasmids, on the two strands (MilleGen, France). Nucleotide sequence comparison was done by manually aligning the R529 sequence of IS-1 with two R529 nucleotide sequences available in GeneBank.

**RESULTS**

PCR primers were “manually” designed based on the sequence data in GenBank (Acc. no. AF146527). They are as follows (their localization within the repeat sequence indicated in brackets). The expected length of the amplified fragments is 529 bp. (couple S1/C1), i.e. the whole repeat sequence, and 379 bp. (couple S2/C1), according to the sequence data.
Analysis of the amplification products by electrophoresis on a 1% agarose gel is shown in figure 1.

![Amplification using primer couple S1/C1 (lanes 1, 2) or primer couple S2/C1 (lanes 3, 4); molecular weight markers (M)](image)

Figure 1. Amplification using primer couple S1/C1 (lanes 1, 2) or primer couple S2/C1 (lanes 3, 4); molecular weight markers (M)

Nucleotide sequencing, done on clones containing the ca. 900 bp. DNA insert, clearly showed that the ca. 900 bp. DNA fragment was precisely 900 bp. long and actually contained the repetitive 529 bp. DNA fragment. It is composed of a partial repeat unit (the expected amplified DNA fragment) at the upstream-end linked to a full-length repeat unit at the downstream-end (nucleotide sequence now available in GenBank with Acc. no. EF195646) (Fig. 2). By examining the nucleotide sequence of R529 of the Indonesian isolate, which has been previously determined as belonging to RH strain, some differences were observed when compared to two other R529 sequences available in GenBank (Fig. 2).

A* ctgcaaggg gaagacgaaa gtgttttttt ttcttttgtt ttcttgtttt 60
B* gggacgaaggg aaatgttttt ttcttttgtt ttcttgtttt
C* --- -------- -------- -------- -------- --------
D* --- -------- -------- -------- -------- --------

A* ttgttttttt tgactcgggc ccagctgcgt ctgtcggaag gacaccgcgg agccgaagtg 128
B* tgccagccgg ccagctgcgt ctgtcggaag gacaccgcgg agccgaagtg
C* -------- -------- -------- -------- --------
D* -------- -------- -------- -------- --------

A* cgttcttttt tttgtacttt tttcagcagca gcagctgcgt tcgtcggaag 180
B* cgttcttttt tttgacttt tttcagcagca gcagctgcgt tcgtcggaag
C* -------- -------- -------- -------- --------
D* cgttcttttt tttgacttt tttcagcagca gcagctgcgt tcgtcggaag

A* ccagaggg acagagtcgg caggggccgta gcgcgctcgc cagggggttt 240
B* ccagaggg acagagtcgg caggggccgta gcgcgctcgc cagggggttt
C* ccagaggg acagagtcgg caggggccgta gcgcgctcgc cagggggttt
D* ccagaggg acagagtcgg caggggccgta gcgcgctcgc cagggggttt
A° ggaggaga gatcagact gtatagtgaag gcgagggagtga ggatgagggg gttggtgtgtg 300
B° ggaggaga gatcagact gtatagtgaag gcgagggagtga ggatgagggg gttggtgtgtg
C° ggaggaga gatcagact gtatagtgaag gcgagggagtga ggatgagggg gttggtgtgtg
D° ggaggaga gatcagact gtatagtgaag gcgagggagtga ggatgagggg gttggtgtgtg

A° tggagaagca cgacagactgg agagggagaa gatttttcgg gcttttgtgg 360
B° tggagaagca cgacagactgg agagggagaa gatttttcgg gcttttgtgg
C° tggagaagca cgacagactgg agagggagaa gatttttcgg gcttttgtgg
D° tggagaagca cgacagactgg agagggagaa gatttttcgg gcttttgtgg

A° gggtggaaaa cagacacgcc gaaatcgcac acagacttcgc gacgctttcc tcttgttgtg 420
B° gggtggaaaa cagacacgcc gaaatcgcac acagacttcgc gacgctttcc tcttgttgtg
C° gggtggaaaa cagacacgcc gaaatcgcac acagacttcgc gacgctttcc tcttgttgtg
D° gggtggaaaa cagacacgcc gaaatcgcac acagacttcgc gacgctttcc tcttgttgtg

A° ggcggagaga attgaagagt ggagaagagg gcgaggagaga cagagtcgga gcgttgag 480
B° ggcggagaga attgaagagt ggagaagagg gcgaggagaga cagagtcgga gcgttgag
C° ggcggagaga attgaagagt ggagaagagg gcgaggagaga cagagtcgga gcgttgag
D° ggcggagaga attgaagagt ggagaagagg gcgaggagaga cagagtcgga gcgttgag

A° aaggaggag gagggtagg agaggaatcc agatgcactg tcttgtcag 529
B° aaggaggag gagggtagg agaggaatcc agatgcactg tcttgtcag
C° aaggaggag gagggtagg agaggaatcc agatgcactg tcttgtcag
D° aaggaggag gagggtagg agaggaatcc agatgcactg tcttgtcag

Figure 2. Comparison of the nucleotide sequence of four repeat units of 529/522 bp. Repetitive DNA fragment of T. gondii.

For convenience, the nucleotide numbering (upper, right) is that of AF 146527, taken as reference for comparison. The oligonucleotide (PstI site) “ctgcag” is underlined and the sequence variants are underlined and in bold characters. Accession numbers: A (AF146527; complete repeat unit), B (EF195646; complete repeat unit), C (EF195646; partial repeat unit), D (AF487550; partial repeat unit). B and C are components of the 900 bp. tandem repeat of the Indonesian isolate IS-1, B being located downstream of C.
REFERENCES
P-BM02

IDENTIFICATION AND SEQUENCING OF 16S rRNA GENE OF *Salmonella typhi* ISOLATES FROM THE PATIENT WITH TYPHOID FEVER AT KARITAS HOSPITAL IN THE SOUTH-WEST SUMBA REGENCY NUSA TENGGARA TIMUR

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Abstract

*Salmonella typhi* strains were isolated from patient with typhoid fever by using selective method. Human isolates of *S. typhi* recovered from blood specimens obtained from Karitas Hospital in the South-West Sumba Regency, Nusa Tenggara Timur. The obtained strains were characterised and identified by a combination of conventional and molecular methods. Fifty three isolates obtained from 34 patients with sporadic cases of typhoid fever among which, 5 isolates were taken to represent different geographical origins. The representative isolates along with reference strain of human originated *S. typhi* NCTC 786 were characterised and identified based on phenotypic characteristics and 16S rRNA gene sequences. Phenotypic characteristics were determined by using API 20E and API 50CHE diagnostics while the 16S rRNA gene sequences were obtained by PCR amplification and sequencing. All phenotypic characters existed in one of two mutually exclusive states and were either scored plus (1) or minus (0). The binary data were prepared in Programmer’s File Editor (PFE) software. The data were analyzed by using the Multi Variate Statistical Package (MVSP) Plus-Version 2.0 using the Simple Matching Coefficient ($S_{SM}$). Clustering was achieved using the UPGMA algorithm. The results were presented as dendrogram. The 16S rRNA sequence data were aligned with corresponding available *S. typhi* sequences retrieved from the NCBI data base by using the CUSTAL X software. Phylogenetic trees were generated by using the PHYLIP software package. The results of the study showed that phenotypic identification analysis indicated that all of the isolates were assigned to two distinct multimembered cluster but clearly separated with the reference strain. Molecular phylogenetic analysis showed that all of the isolates belonged to species of *S. typhi* suggesting by their relatedness with the type strain of *S. typhi* (ATCC19430⁵). Therefore, the study demonstrated that the isolates belong to the species of *S. typhi* albeit they formed several different center of diversity within the 16S rRNA gene tree.

**Key words:** *Salmonella typhi* strains, typhoid fever, phenotypic characteristics, 16S rRNA gene sequences.
INTRODUCTION

The problem related to the outbreak of typhoid fever is abundant in the South-West Sumba Regency, one of the regency in the Province of East Nusa Tenggara (NTT), Indonesia. The incidence rate of the diseases is high estimated at 725/100,000 based on the data from the Karitas Hospital, as the only the reference hospital in Weetabula, South-West Sumba Regency, NTT.

Despite the high morbidity, there is limited information about the molecular epidemiology of S. typhi infection in this region. With regard to the molecular epidemiology, the ability to distinguish clinical isolates is a critical tool in epidemiological investigation but the classical method of distinguishing different clinical isolates has low discriminatory potential. Therefore in order to indentify the types of strain, we characterized and identified human isolates of S. typhi recovered from blood specimens by using combination of conventional and molecular methods. Molecular techniques have led to a significant increase in discriminatory power. These approaches have provided useful insights into epidemiological relationships of strains of S. typhi. The results of this identification will be used to classify the types of bacteria for the objective to aid the treatment and prevention of the disease.

MATERIALS AND METHODS

Patient and Specimen Collection

The specimen was collected from the Karitas Hospital in Weetabula under the consideration of the promising and richness of data in this area. Clinical typhoid fever was defined as fever with an axillary temperature ≥ 38.5°C for 3 days with a cutoff titer of ≥1/200 for O agglutinin.

Blood culture and microbiological methods

A 5 ml blood sample for adult or a 3 ml blood sample for pediatrics was collected in a BacT/Alert FAN blood culture bottle (Biomerieux). Cultures were incubated at 37°C for 7 days and checked at 1, 3 and 7 days for growth. When blood culture turns positive, the bottles were further inoculated into selective and differential media for culture and identification according to the standard method from World Health Organization (2003). Suspected colonies were identified by standard conventional biochemical methods. In addition, the API 20E and API 50CHE system (Biomerieux) were used for identification of the bacterial isolate. All phenotypic characters were analyzed by using the Multi Variate Statistical Package (MVSP) Plus-Version 2.0 using the Simple Matching Coefficient (S_{SM}). Clustering was achieved using the UPGMA algorithm.
Extraction of Bacterial DNA, PCR amplification, and DNA sequencing

Bacterial DNA extraction was done according to the protocol's instructions using a Purelink™ Genomic DNA Mini Kit (Invitrogen K1820-00). The bacterial DNA and control were amplified using 0.2 µM primers (Massi et al. 2005) and PCR SuperMix (Invitrogen™ 11306-016). The PCR mixtures were amplified for 40 cycles at 94°C for 1 minute, 55°C for 1 minute, and 72°C for 2 minute, with a final extension at 72°C for 10 minutes in automated Applied Biosystems GeneAmp PCR System 2400. An aliquot of 10 µl of each amplified product was electrophoresed in 1.5% (wt/vol) agarose gel, with a DNA Molecular Weight Marker (Gel Pilot 100bp Ladder 100 Lanes, Qiagen) in parallel.

The PCR product was gel purified using the QIAquick PCR purification kit (QIAGen, Hilden, Germany). The purified PCR product was sequenced with an ABI Prism 3100-Avant Genetic Analyzer according to the manufacture’s instructions (Applied Biosystems, USA) using the PCR primers. Nucleotide sequence data were analysed, edited and assembled with Finch TV 1.4.0 and DNA Baser sequence analysis software. Complete assembled sequences were aligned with corresponding available S. typhi sequences retrieved from the NCBI data base with CUSTAL X software. Phylogenetic analysis were conducted using the PHYLIP software package.

RESULTS AND DISCUSSION

Standard conventional microbiological methods based on blood culture showed that the profile of all suspected colonies reveal a pattern resembling with reference strain S. typhi NCTC 786. All isolates were identified as S. typhi based on their Gram staining characteristics and biochemical reactions using the API systems (20E and 50 CHE). A dendogram summarizing the biochemical relationships among the 5 isolates is shown in Fig 1.
Phenotypic identification analysis indicated that all of the isolates were assigned to two distinct multimered cluster but clearly separated with the reference strain *S. typhi* NCTC 786. PCR of 16S rRNA gene using different pairs of primers showed bands of 428 bp, 484 bp, 483 bp. Complete assembled sequences were analyzed by comparison with corresponding available *S. typhi* sequences and reference organisms from the family Enterobacteriaceae retrieved from the NCBI data base. A phylogenetic tree is shown in Fig 2.
All of the isolates belonged to species of *S. typhi* suggesting by their relatedness with the type strain of *S. typhi* (ATCC19430T). The isolates belong to the species of *S. typhi* albeit they formed several different center of diversity within the 16S rRNA gene tree.

REFERENCES


P-BM03

ANTIBIOTIC PRODUCING STREPTOMYCETES ASSOCIATED WITH THE RHIZOSPHERE OF SEVERAL SPECIES OF MANGROVE PLANTS

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Abstract

Streptomyces associated with the rhizosphere of three species of mangrove plants (Rhizophora apiculata, R. mucronata, and R. stylosa) and mangrove sediments were selectively isolated and purified using pretreatment regime and selective isolation media of starch-casein and raffinose-histidin. Streptomyces colonies were putatively identified based on growth characteristics and colonial morphology. Purified streptomycete isolates were preliminarily classified by using color-grouping method with oatmeal agar (ISP3). Representative isolates were subsequently characterized in terms of gram staining and spore chain morphology by using phase-contrast microscopy method. Representative isolates were screened for ability to produce antibiotics based on the ability to inhibit test bacterial strains of Staphylococcus aureus and Escherichia coli. It was found that 70 isolates of streptomycetes could be obtained from rhizosphere and sediment samples. Isolates could be classified into 5 color groups based on aerial and vegetative mycelium color, and the ability to produce melanoid pigments. In terms of spore chain morphology it was found very diverse, including, flexuous, spiral, curely and folded. Furthermore, 12 isolates were found to inhibit strongly the test bacterial strains, namely S. aureus as well as E. coli. Therefore, it was clearly indicated that the streptomycetes associated with the rhizosphere of mangrove plants could be very diverse and very potential to produce bioactive substances, such as antibiotics.

Key words: antibiotic, streptomycetes, mangrove, rhizosphere
P-BM04

DIGOXIGENIN LABELED PROBE DERIVED FROM R529 REPETITIVE SQUENCE FOR
Toxoplasma gondii DETECTION

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Abstract

Rapid, sensitive and accurate detection method for determination heath status, diagnostic, treatment and management diseases is essential. Toxoplasmosis caused by Toxoplasma gondii, could be fatal for AIDS, emerged some problem: abortus, mental disorder, blindness, and decreasing productivity even life-threatening for humans and animals. Due to toxoplasmosis is not specific and usually asymptomatic, the diagnostic is hard to be accomplished clinically. Therefore, developing initial detection of T. gondii infection is a need. The aim of this study is to develop probe from R529 sequence for detecting T. gondii infection and examining its specificity and sensitivity. R529 sequences were isolated from T. gondii genome and amplified by PCR using reverse and forward primers to derive 238 bp. The sequence was labeled with digoxigenin using PCR Dig Labeling Mix. Probes were successfully synthesized and hopefully able to detect sensitively protozoan DNA of T.gondii.

Keywords: probe, R529, Toxoplasma gondii, dot-blot hybridization

Introduction

T. gondii is an obligate intracellular and an opportunistic protozoan parasite which is a significant human and veterinary patogen¹. Toxosplasmosis is often asymptomatic or has mild symptom, but it can cause serious problem in the immunocompromised patients and in congenitally infected children². In order to take suitable measures, an early diagnosis of the disease is essential and the availability of efficient and sensitive diagnostic tools is a great importance. The 529 bp. DNA fragment (R529), repeated 200- to 300-fold in the genome of T. gondii³, is a promising target for diagnosis puposes³. We utilized this fragment for the development a sensitive and specific probe through dot-blot hybridization.
Material and Method

Template *T. gondii* DNA was prepared from ascetic fluid of intraperitonially inoculated mice using DNAzol (Invitrogen). The R529 Polymerase Chain Reaction was performed using Illustra pure Taq Ready to go PCR Beads (GE Healthcare). R529 fragments were cloned in pCR 2.1 (PCR topo Cloning, Invitrogen) then transformed into *Escherichia coli* DH5α. Probe was prepared by PCR (PCR Dig Labeling Mix Kit, Roche) using a sets of primer and template from pCR2.1 plasmid containing R529 (pCR-529), resulting the 238bp digoxigenin-labeled fragment.

Result

According to comparation sequent (blast analysis, NCBI), the 529 bp. DNA fragment is highly coserved. pCR2.1-529 isolated plasmids (Fig.1.A) were used as template to prepare digoxigenin labeled probes.

![Figure 1. Electrophoresis analysis on agarose gel of A] pCR21 plasmid containing R529 (4.4kbp) B] PCR amplified of R529 (529bp) and C] digoxigenin labeled probe (238 bp). DNA marker are indicated](image)

Probes were successfully synthesized (Fig.1.C.) and hopefully able to detect sensitively protozoan DNA of *T. gondii* through *dot-blot* hybridization. The high copy number will lead to an ultimate level of analytical sensitivity in routine practice.
References
THE ESTROGENIC EFFECT OF ETHANOL EXTRACT OF ADAS (*Foeniculum vulgare* Mill.) IN RAT (*Rattus* sp.)

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Abstract

The research was conducted to study the effect of ethanol extract of adas/fennel fruits (*Foeniculum vulgare* Mill.) in rats. Thirty of female rats, 16 weeks old and 200 gram of body weight were used in this experiment. The animals were grouped into five groups, each consisted of six animals. Group I (negative control) was given aquadest, group II (positive control) was given 9 x 10⁻³ mg/ 200g BW of ethynil estradiol (Lynoral), group III, IV and V were given ethanol extract of adas at the dose of 0,97; 1,94; 3,88 g/200 g BW respectively. Treatments were given orally every day for seven days period. Parameters observed were the length of estrus cycle, uterus vascularisation, the weight and histopathology picture of the ovary and the uterus during pro-estrus phase. The result showed that group IV which was given 1,94 g/200 g BW of extract lengthened the period of estrus cycle significantly compared to negative control group. Group V which was given 3,88g/ 200 g BW of extract showed increase in vascularisation in the uterus, and the ethanol extract of adas at this dose had the similar effect with ethynile estradiol in the increasing of the ovaries and the uterus weight. The highest of the endometrial thickness was found in group V (dose of 3,88 g /200 g BW) and the highest amount of uterus glands was in group I (0,97g/200g BW), however, of all the treated doses showed no effect in increasing follicle developing in the ovary.

**Keywords:** phytoestrogen, ethanol extract of adas, estrus cycle

Introduction

Studies and research on phytoestrogens lately intensified, particularly since the invention of phytoestrogens constituent in soybeans in a significant amount for overcoming problems arising from menopause. Fennel fruits (*Foeniculum vulgare* Mill.) is one of the Indonesian biodiversity that used to be utilized as spice, is known to have phytoestrogen compound of lignan group, which is called trans-anethol (Murkies *et al.* 1998). Several previous studies have been conducted on the properties of fennel are oestrogenic (*Malini et al.* 1985; *Cosge et al.* 2008), however, a thorough exploration of
the fennel still needs to be done, especially related to the type and origin of fennel, its impact on reproductive organs and the other target organs of estrogen in macro and micro perspective that hopefully later can be used as a basis for further studies on fennel. The research was designed to study the estrogenic effect of fennel fruit on the length of estrous cycle, vascularisation of ovary and uterus, change in ovarium and uterus weight as well as its histological picture in female rats.

Materials And Methods

Ethanol extract of fennel fruit was obtained through the process of maceration of 200 g of simplicia fennel powder dissolved with 2 L of ethanol 70% for 24 hours. Collected macerat was then evaporated by using rotary evaporator at 30-40°C until thick extract formed. Thirty adult e female rats, 2 weighing about 200-250 g were used for this research. These animals were grouped into 5 groups with 6 individuals for each group and treated as follows: Group I (Negative Control Group) was given aquabidest, Group II (Positive Control Group) was given ethynil estradiol (Lynoral) with the dose of 4.5 x 10^5 mg/kg of BW, Group III, IV and V were treated with ethanol extract of fennel fruit with the dose of 4.85 g/kg of BW, 9.70 g/kg of BW and 19.40 g/kg of BW respectively. Treatments were given orally every morning for 7 days period, started and finished when the animal was in estrus phase. The phase of estrus cycle was determined through vagina smear examination. At the end of treatments, the animals were killed for ovary and uterus collection. Immediately after collection, organs were put in Buffer Normal Formalin (BNF) solution 10% until processed to prepare materials for histological examination. In general, parameters observed were 1) Length of estrous cycle included the related phases, 2) Vascularisation of ovary and uterus, 3) Change of ovary and uterus weight and 4) Histological picture of ovary and bifurcatio utery of the uterus.

Results and Discussion

Results obtained showed that the length of estrous cycle in Group I was 95 h, in Group II was 105 h, in Group III, IV and V were 90 h, 124 h, and 112 h respectively. When examining from each phase, extended estrous cycle phase resulted from the extension of proestrous and estrous phases. The highest increase in vascularisation as well as ovary and uterus weight were found in Group V (at the dose of 19.40 g/kg of BW). The increase in vascularisation was thought to be due to the estrogenic effect of fennel fruit which at the time of estrus, it increased blood pressure in the capillaries surrounding the reproductive organs, especially the ovaries and uterus (Liu et al. 2000). Based on ovarian histology
observation, it was seen that there was follicle developing process in estrous phase as well as vascularisation of this reproductive organ, nevertheless, the rate of the change in follicle growth was not affected by the Lynoral or fennel extracts in various doses. Primordial, primary, secondary, tertiary and de Graf follicles did not show any significant increase in line with the increase in the amount of extracts given. An increase of estrogen level in estrous phase does not directly affect the length of estrous cycle, but can enhance follicular development so that there are many mature follicles in the ovary and this will cause more estrogen secreted and tends to prolong the estrous cycle (Liu et al. 2000). The highest of endometrium thickness was found in Group V (at the dose of 19.40 g/kg of BW), while the amount of uterus gland was highest in Group I (at the dose of 4.85 g/kg of BW) and tended to decrease with the increase of extract doses. This is in accordance with the statement of Mills (2007) that during the initial period of proliferation, that is in proestrous phase, the rate of tissues development and endometrium glands will increase. The decrease in the amount seemed to be dosage dependent and might be due to the negative feedback mechanism through hypothalamus-hypophyse axis as well as anti-estrogenic effect after the optimum dosage reached. These all finding represented estrogenic effect of ethanol extract of fennel fruit on reproductive organs of female rats in productive age and further research in prepubertal and menopause periode will be such a valuable study to be carried out.

Acknowledgments

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References

Cosge B., Kirahan M., and Gurbuz B. 2008. Characteristics of fatty acids and essential oil from sweet fennel (Foeniculum vulgare Mill. var. dulce) and bitter fennel fruits (Foeniculum vulgare Mill. var. vulgare) growing in Turkey. Natural Product Research 22(12):1011-6.


P-BM06
SCREENING OF ANTIBACTERIAL AND CYTOTOXIC ACTIVITY FROM SPONGES AND SOFT CORALS KEPULAUAN SERIBU, INDONESIA

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ABSTRACT
Living benthic marine organism such as sponge and soft corals are frequently equipped with natural chemical defense compounds to combat the microbial attack. The defense mechanism of these organisms is performed through the production and accumulation of secondary metabolites which has antimicrobial activities. This study was to reveal bioactive compound in methanol extracts from sponges and soft corals which were collected from 3 different sampling locations at Kepulauan Seribu, Indonesia. This study was preliminary work to explore the antibacterial of marine organism especially sponges and soft coral samples with consideration toward the environmental parameters influencing antimicrobial activity. Methanol extracts from forty six samples were screened for antibacterial activity against Escherichia coli, Salmonella typhosa, Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus subtilis, and Streptococcus mutan. The methods chosen for determined the antibacterial activity was agar disk diffusion method using chloramphenicol as the comparative antibiotic standard. The Results showed that several extracts tends to have broad spectrum antimicrobial activity.

Keywords: sponges, soft corals, antimicrobial activity, environmental parameters

INTRODUCTION
Nature has been a source of medicinal agents for thousand of years and an impressive number of modern drugs have been isolated from natural sources (Nair et al., 2005). Indonesia, as in other developing countries, has a very significant proportion of diseases due to microbial infection, and it has been become one of the causal human largest killers (Mukhopadhyay, 2006). The interest natural bioactive compound with antibacterial properties has been revived due to current problems associated with the use of antibiotics with the increased prevalence of multiple drug-resistant strains of pathogenic bacteria (Voravuthikunchai, 2005). The natural bioactive compounds with the antibacterial properties usually having relative lower incidence of adverse reactions compared to
modern conventional pharmaceuticals. Coupled with reduced cost, it is encouraging both the consuming public and national health care institutions to consider natural bioactive compounds as alternatives to synthetic drugs (Nair et al., 2005).

The number of marine animal (over 2,000,000) and microorganism species available for investigations is enormous. With 70% of earth surface and 95% of its tropical biosphere, this marine environment, containing approximately half of the total global species, possessed a biodiversity as extensive as all the rain forest combined with greater diversity at higher taxonomic level. This biodiversity has resulted chemical diversity, combine with the most accessible levels of marine environment has provided unique new compounds exhibiting pharmacologically useful activities. Yet less than 0.5% of the marine organism has received effort to detect their constituents, and even fewer have been examined to discover agents against many infectious diseases (Cuttler et al, 2000).

After the 1960s, the investigation and collection of marine organism, previously very difficult, were facilitated by the availability of SCUBA diving equipment, thus allowing a remarkable development of drug discovery against many infectious diseases (Tringali, 2001). This improvement in underwater life-support systems has provided marine scientist new mechanism for collecting marine organism such as sponge and soft corals from unexplored regions and depths (Cuttler et al., 2000). On the other hand, drug discoveries programs are largely based on the screening of natural products possessing biological activities supported by improvements in bioassay techniques provides leading information regarding the drug discoveries program, especially to combat the diseases due to microbial infection (Tringali et al., 2001).

Living benthic marine organism such as sponges and soft corals are frequently colonized by bacteria. Since this microorganism are ubiquitous in the marine environment and especially on the surface of benthic invertebrates, these organism need to resist microbial colonization by producing chemical defense (Kelman et al., 2001). Inhibition of bacterial growth is one of roles of secondary metabolites taken on in order to have chemically defense in sponge and soft corals (Lenio, 2002). Several recent reports on antimicrobial activity of sponge and soft corals have examined the activity of secondary metabolites against ecologically relevant bacteria in order to elucidate their function in the chemical mediation of interaction between marine invertebrates and bacteria. The controlled diffusion of antibiotic agents in the living tissue of marine invertebrates may increase their efficiency, and may thereby provide a better defense against microbial infections (Kelman et al., 2001).
The objective of this research was to determine the specimen of sample which has potent broad spectrum activity or selective spectrum activity against tested pathogenic bacteria strains. This study also tries to explain the correlation between the potency of antimicrobial activity and the ecological roles of the sample collection place. In the future, it should be needed to conduct another research to determinate and elucidate the antimicrobial compounds from those potent sponges and soft corals samples in order to discover the new marine antibiotic agents against many infectious diseases due to microbial infection.

MATERIAL AND METHODS

Collection of sponges and soft corals samples

Twenty four sponges and soft corals specimens were collected by hand using SCUBA diving from the coral reef of Kepulauan Seribu on Juni 2005, at depths between 10.21 to 15.30 m. Each sample of sponges and soft corals was immediately embedded into 100 ml of methanol and maintain at room temperature and transferred immediately to laboratory Research Center of Marine and Fisheries Product Processing and Biotechnology in Jakarta.

Extraction of methanol active fractions

The sponge sample specimens were weighed at 100 g, each specimen were cut into small pieces and placed at plastic flask. The specimens were then embedded using the same solvent for three days at room temperature, allowing the extraction process of the antibacterial compounds. The solvent were filtered, and removed by rotary evaporator under vacuum at room temperature. The crude methanol extract from each sample then collected and evaporated to dryness under vacuum at 0°C temperature using freeze dried apparatus. Each dried crude methanol extract were weighed and kept under 8°C temperature for further use.

Antibacterial assay

Antibacterial activity was tested against the pathogenic gram negative strains of *Escherichia coli, Salmonella typhosa, Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus subtilis, and Streptococcus mutan*. The bacteria strains were obtained from Laboratory of Marine and Fisheries Biotechnology, Research Center of Marine and Fisheries Product Processing and Biotechnology. Single disk method as described by Vitor (2002) was used, using the Muller Hinton media for bacterial growth. The tested bacterial strains inoculums was measured to know the optical density (OD) at 600 nm
lambda and the absorbance of the inoculums solution must be at range 0.5 – 0.9. Approximately 20 µl of the tested bacterial strains inoculums was put into microbial tube contain 15 ml Mueller Hinton agar at the temperature 45° C, followed by vortex step to homogenize the mixture, and then poured to petridishes. At the same time, sterile paper disc of 6 mm diameter were embedded with 20 µl of the methanol extract from the tested sample at concentration of 10 mg/ml, and also paper disc with the chloramphenicol standard as the comparative antibiotic with same quantity and concentration added to the culture dishes. The antibacterial activity of sponges and soft corals methanol extracts against tested strains was determined after 24 hour incubation at 37° C temperatures by measuring the diameter of the halo around the disc (Nair et al., 2005) and (Casel and Mekalanos, 2001).

Representative halos were measured according to Davis Stout Method with specification: very strong (inhibition zone ≥ 20 mm); strong (inhibition zone: 10 – 20 mm); medium (inhibition zone: 5 – 10 mm); weak (inhibition zone ≤ 5 mm) (Sugiyono, 2003).

RESULT & DISCUSSION

Several strains were used for this antimicrobial assay including gram negative bacteria such as Escheria coli, Salmonella typhosa, and Pseudomonas aeruginosa and gram positive bacteria such as Staphylococcus aureus, Basilus subtilis and Streptococcus mutan. These bacterial strains usually cause many diseases to human body.

Result of the study showed that there was one kind of sponge (Demospongia sp.) from sampling station 1 which has microbial activity against all tested bacterial strain, while two kind of soft coral (Sarcophyton sp and unidentified sp.) had a good result against gram positive bacterial strains tested only two soft corals from sampling station 1 had antimicrobial activity against gram positive and negative bacterial strains tested.

Figure 1. Sponge and soft corals having the broad spectrumantimicrobial activity collected from station 1 (left: Demospongia sp. right: Dendronephthya sp.)
This study was also considered as preliminary study for screening antimicrobial potency as well as to find correlation between ecological roles affecting the antimicrobial potency. From the statistic test using Kruskall Wallis to find the environment parameter affecting the antimicrobial effect from those sponges and corals collected from the three different areas of sampling at Kepulauan Seribu. Specimens had been taken from three locations of sampling station, all of them having same depth with various environment parameter such as water salinity, pH, oxygen contain, temperature, phosphate, nitric, nitrous, ammonia contain as well the sun exposure and the wave through the sampling station area. By using statistic we come to conclusion that the most influencing parameters i.e. pH, water oxygen content, temperature, phosphate, and nitrous contents of the waters significantly affected the antimicrobial potency. The sun exposure of the sampling station did not influencing the antimicrobial activity of the sponges and soft corals inhabiting these area. The organic component of the sponges and corals living in this environment may have great influenced toward chemical compounds initialized for chemical defense against sponge and corals predators.

Table 1: Result of antimicrobial screening of some methanol extracts of sponges and soft corals collected from Kepulauan Seribu waters

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<td></td>
<td></td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>not active</td>
</tr>
<tr>
<td>38-PS-05</td>
<td>Unidentified Sp.</td>
<td></td>
<td></td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>not active</td>
</tr>
<tr>
<td>05*-PS-05</td>
<td>Dendronephthya</td>
<td></td>
<td>8</td>
<td>8</td>
<td>7</td>
<td>9</td>
<td>0</td>
<td></td>
<td>broad spectrum activity</td>
</tr>
<tr>
<td>09*-PS-05</td>
<td>Demospongia</td>
<td></td>
<td>8</td>
<td>0</td>
<td>8</td>
<td>8</td>
<td>0</td>
<td></td>
<td>broad spectrum activity</td>
</tr>
<tr>
<td>18*-PS-05</td>
<td>Demospongia</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>7</td>
<td>broad spectrum activity</td>
</tr>
<tr>
<td>Sampling Location</td>
<td>Marine Biota</td>
<td>Gram negative activity</td>
<td>Gram positive Activity</td>
<td>Activity against gram positive and negative bacteria</td>
<td>Ecological parameters</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Sampling station 1 (the most distance from human population)</td>
<td>Sponges</td>
<td>Demospongia sp.</td>
<td>Demospongia sp.</td>
<td>-</td>
<td>The most base water (pH 8.30)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Soft corals</td>
<td>Sarcophyton sp. (2 samples)</td>
<td>Dendronephthya sp.</td>
<td>Sarcophyton sp.</td>
<td>The highest oxygen content (5.60 ppm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>The highest phosphate (0.150 ppm), nitrous content.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sampling station 2</td>
<td>Sponges</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Medium water pH (8.10)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Soft corals</td>
<td>-</td>
<td>Sinularia sp.</td>
<td>Sinularia sp.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

This result of study was consistent with some previous study regarding spatial competition or sponge –coral interactions in survival processes within tropical reefs, as competition has often been hypothesized as one of the most important processes that influence these communities. Competition for space becomes important because it influences the acquisition of substrate for supporting this living benthic organism. The result of this study provided consistent information regarding substrates acquisition, influencing the spatial competition, which shown in Table 2.

Table 2. Sponges and soft corals in three sampling station with highest antimicrobial activity and the environment parameters at each station.
Sampling station 3 (the nearest from human population)

<table>
<thead>
<tr>
<th>Sampling station 3 (the nearest from human population)</th>
<th>Sponges</th>
<th>Soft corals</th>
<th>Sarcophyton sp. Sinularia sp.</th>
<th>Demospongia sp.</th>
<th>Dendronephthya sp.</th>
</tr>
</thead>
</table>

Whereas the station 1, the richest station with organic compounds such as phosphate, nitrous and with highest oxygen contents, produced seven species of sponges and soft corals with antimicrobial activity. Sampling station 2 provided only 4 active species of totally 22 species, and sampling station 3 had only 3 active species from totally 11 species. Likewise many studies of living benthic organism competition for organic substrate acquisition discovered having correlation with secondary metabolites production and accumulation within those benthic organisms especially sponges and soft corals.

Figure 2. Sponge and soft corals having broad spectrum antimicrobial activity against the tested pathogenic bacterial strain collected from station 2 (Left: Sinularia sp. right: Sarcophyton sp.)

The distance of sponge and coral habitat from human population area affected the antimicrobial activity produced by them. We hypothesized that the environment near human population have the highest and various exposure of pathogenic bacteria, influencing antimicrobial activities of some sponge and soft corals inhabiting those area. Result of the study demonstrated that sample specimens from the sampling station 2 and 3, the nearest station to human population, mostly demonstrated broad spectrum antimicrobial activity against both gram negative and positive bacterial tested. However, the amount of active species from sampling station 2 was relative lower compare to
sampling station 3. This result maybe influenced due to the lack of the organic substrate at sampling station 3 (lowest oxygen, phosphate, and nitrous content). Antibacterial activity from sampling station 1, the most distance station from human population, demonstrated narrow spectrum of antimicrobial activity (mostly against gram positive bacterial strains) indicating the uniformity in microbial exposure toward them. The lack of various microbial exposures tends to perform narrow spectrum of antimicrobial activities of secondary metabolites produced by sponges and soft corals in those area.

Figure 3: The antimicrobial halo surrounding paper disc containing the methanol extract [code5 (against Staphylococcus aureus); code3 (against Salmonella thyphosa)]

Figure 4: Histogram of antimicrobial activity of sponges and soft corals collected from three sampling stations from Kepulauan Seribu

The ecological role of sponge and soft coral secondary metabolites in antimicrobial defense against co-occurring and potentially pathogenic marine bacteria is unclear. Moreover, their role in regulating symbiotic relationship between bacteria and their marine invertebrates host also obscure. In the current study we have shown that sponge and coral reef organic extract and major secondary metabolites play an important role in this
chemical defense. Those chemical defenses are utilized by sponge and coral reefs to discourage predation, competition and bacterial attachment. Inhibition of bacterial growth is one of secondary metabolites roles for sponges and soft corals in order to act as their chemically defense system. This inhibition is one major reason for their abundance and persistence. The protection of the sponge surfaces against bacterial colonization has been suggested by the antimicrobial activities of some sponge compounds. If unprotected, bacterial colonization begins by the primary attachment of bacteria to sponges and soft corals surface. This type of attachment will continue with rapid growth and surface colonization where the matrixes finally develop consisting bacteria and organic molecules. At this point the matrix allows the surface to be conditioned that lead to the formation of complex organic material with great possibility contain the antimicrobial activities.

Investigation of antimicrobial effect of secondary metabolites effect of secondary metabolites with disc-diffusion assays using agar media is limited, due to the variable diffusion rates of compounds in agar. Moreover, the simulation of natural concentration on a volumetric basis, assumes that the extract are equally distributed throughout the volume of organism being tested. In some corals and sponges it reasonable to assume that higher concentration of bioactive compounds accumulates on their surfaces, thus providing greater defense against bacterial colonization. However since sponge’s pass great quantity of water trough their surface and tissue, which contain potentially harmful bacteria, as well as encountering them to their external surfaces, it may be advantageous for sponges to distribute their antimicrobials throughout their tissues. Several evidence has suggested the localization of natural product within the tissues of sponges. Further investigation on the localization of antibiotic in tissues of marine sponges is therefore warranted.

In the current study, the assays were performed in liquid media, which ensures a uniform concentration of antibiotic and increase interaction between antimicrobial agents and the tested microorganism. In our view, such assay condition better stimulate the condition experienced by microbes in nature.

CONCLUSION

The result of this study suggested that there are defensive roles of some sponges and soft corals extract against microbial infection as well as protection against predators at the natural habitat. The sample collected from sampling station adjacent to settlement
area tends to have broad spectrum antimicrobial activity indicating that the chemical
defense performed by their secondary metabolites towards the microbial exposure
towards sponges and corals inhabitant those areas.

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granatum pericarp on verocytoxin production by enterohemorrhagic Escherichia coli
P-BM07

Toxoplasma gondii DETECTION BASED ON NON-RADIOACTIVE LABELLED PROBE DERIVED FROM Surface Antigen1 (SAG1) SEQUENCE

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ABSTRACT

Toxoplasma gondii is an obligate intracellular protozoan parasite that infects all warm-blooded animals, including humans, and is the pathogenic agent of toxoplasmosis. This disease is generally benign in immunocompetent individuals, but it can be fatal in immunosuppressed patients and congenitally infected individuals. Current disease diagnosis of felines is based on fecal examination for T. gondii oocyst, latex agglutination test (LAT), modified direct agglutination test (MAT) or enzyme linked immunosorbent assay (ELISA). Diagnostic kits of LAT and ELISA are commercially available but the use of antigens extracted from parasites makes them expensive. Diagnostic tools based on nucleic acid is an attractive alternative for parasite detection. The surface antigen1 (SAG1) of T. gondii is considered as an important candidate for the development of an effective diagnostic reagent. Surface antigen1 is the most predominant antigen of T. gondii, being detected only in the tachyzoite stage, but absent in the sporozoite and bradyzoite stages. The nucleotide sequence of the intronless SAG1 gene and its deduced amino-acid sequence has been established. The purpose of this study is to developed probe from SAG1 sequence for detection T. gondii and have high spectivity and sensitivity. SAG1 sequence was isolated from genome of T. Gondii. Amplified by PCR using sense and antisense to derived 210 bp. The sequence was labeled with anti-digoxigenin (non radioactive labeled) using PCR Dig Labeling Mix. Probe was able to detect sensititively tachizoid DNA of T.gondii.

Keywords: Toxoplasma gondii, probe, SAG1

INTRODUCTION

Toxoplasma gondii is an obligate intracellular protozoan parasite that caused systemic protozoan infection for human¹. Toksoplasmosis caused by T. gondii could be fatal for AIDS, emerged some problem abortus, mental disoder, blindness, decreasing productivity even life-threatenning for humans and animals². Toksplasmosis diagnostic able to did with three method that is clinical condition, blood examining, and serologis condition. Due to toxoplasmosis is not specific and usually asymptomatic, the diagnostic is hard to be accomplished clinically therefore there is a need to develop initial detection Toxoplasma gondii infection³. An approach genomic can used to improve acuration,
spesivity, and sensitivity a beginning detection of Toksoplasmosis. Diagnostic molecular method based on SAG1 nucleic acid form one or the other gene of T. gondii that can used to diagnostic developed, therefore SAG1 is a surface protein dominant that to be role playing for attached in nursemaid cell and infection process. Gene sequence a code SAG1 has been established so that can made primer to in vitro amplified and made as DNA probes T. gondii can be applicated for effective molecular diagnostic method.

MATERIALS AND METHODS
Isolation and amplification of SAG1 gene from the Indonesian isolate IS-1 by PCR and cloned (pGEX-2T). Positive clones (white colonies) were cultured and the plasmid prepared using “High Pure Plasmid Isolation Kit” (Roche). Plasmid isolation product were analyzed by electrophoresis on a 1% agarose gel. Recombinant plasmid as a template for probe synthesis using sense and antisense to derived 210 bp. The sequence was labeled with anti-digoxigenin (non radioactive labeled) using PCR Dig Labeling Mix. Probe was to detect sensitivitelly takizoid DNA of T.gondii.

RESULTS
The nucleotide sequence of the intronless SAG1 gene and its deduced amino-acid sequence has been established. Surface antigen1 (SAG1) is the most predominant antigen of T. Gondii and expressed as a precursor which is then processed to mature protein. Amplification of the DNA encoding the mature SAG1 gave rise to a DNA fragment of the expected size i.e. 800 bp.

![Figure 1. Analysis by electrophoresis on agarose gel SAG1 gene T. gondii IS-1; M: Marker DNA (1000 bp), 1. Gen SAG1 mature (800 bp)](image)
Result of BLASTN query on SAG1 Indonesian isolate of *T. gondii* (IS-1) is conserved. Analysis by electrophoresis on agarose gel on pGEX-2T containing SAG1 gene. (Figure 3a) and PCR amplified SAG1 gene (Figure 3b)

Figure 4. Probe synthesis with PCR Dig Labelling Mix

REFERENCES
P-BM08

EXTRACTING OF SINGLE CELL CAROTENOID AND ASSESSMENT FOR ITS ANTIOXIDANT PROPERTY

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Abstract

Beta-carotene is a phytonutrients family that represents of the one most widespread groups of naturally occurring pigments. It is known as “provitamin A”, able to be converted in the body into retinol, an active form of vitamin A. This carotenoid, which may be extracted from culture of fungi, yeast and algae, has played an important role in health because it becomes an antioxidant in the body. Single cell carotenoid like most carotenoids, is also a powerful antioxidant – so it has been recommended to protect against a variety of diseases such as cancer, cataracts and heart disease. We have successfully synthesized single cell carotenoid using whole cells of Phaffia rhodozyma, Rhodotorula minuta, Neurospora sitophylla and Monascus purpureus on sucrose-containing coconut water as medium. As the results, we found that sucrose concentration of the media were decreased according to the incubation period, while the color of media were changing to becoming orange or reddish. P. rhodozyma performed the highest carotenoid-pigments after 7-days incubation as much of 46.12 µg/g compared to M. purpureus, R. minuta and N. sitophila.

We have studied also the activity of extracted single cell carotenoids as antioxidant against DPPH as oxidator. We found that the extract of N. sitophila exhibited higher IC_{50} value (249,740 µg/ml) rather than that of other extracts of single cell carotenoid, even though, its antioxidant activity was 22 times lower than that of vitamin C (43,408 µg/ml). We, however, suggested that this study clearly shows the potential of single cell carotenoids extracted using whole cells of microbial strains as promising antioxidant-provitamin A substance.

INTRODUCTION

Single cell carotenoid is similar to the other carotenoids, is a natural fat-soluble pigment found principally in micro-organisms (Phaffia rhodozyma, Rhodotorula minuta, Neurospora sitophylla and Monascus purpureus), where it serves as an accessory light-gathering pigment and to protect these organisms against the toxic effects of oxygen. Purification of carotenoid from natural sources comprises extraction of the carotenoid from the source with a suitable extractant, optionally followed by additional purification steps until the desired purity is obtained. The extraction is carried out with organic solvents, such as ethyl acetate, butyl acetate, hexane; vegetable oils, or supercritical fluids, such as
propane, ethylene, CO₂. Subsequently, the carotenoid can be directly purified from the extract obtained after solvent extraction of the natural source by evaporation of the solvent. The present study shows investigation on the influence of single cell carotenoids extracted those were extracted from several microbial culture growth media against reactive oxidator generated by DPPH (1,1-diphenyl-2-picrylhydrazyl).

MATERIALS AND METHODS
Microbial Strains and Growth.

P. rhodozyma and R. minuta strains were maintained on slants of 10 g/L glucose, 3 g/L yeast extract, 3 g/L malt extract with 20 g/L agar (Yeast Malt Agar, YMA) and refrigerated at 4°C. Yeast strains were also stored in 40% glycerol/60% YM broth at -70°C. N. sitophila and M. purpureus strains were maintained on slants of 10 g/L glucose, 5 g/L peptone, 3 g/L yeast extract, 3 g/L malt extract with 20 g/L agar (Malt Extract Agar, MEA) and refrigerated at 4°C. Fungal strains were also stored in 40% glycerol/60% ME broth at -70°C.

Single Cell Carotenoid Production.

P. rhodozyma, N. sitophila, R. minuta, and M. purpureus were grown on 250 mL erlenmeyer flask containing 150 ml coconut water media supplemented with 1.0% saccharose and small amount of micro nutrients at pH 5.5-7.0. The respective media were incubated on rotary shaker at 120rpm, 22-37°C for for 4-5days.

Single Cell Carotenoid Extraction

The pellets were obtained through the procedure were then collected and added with 1.0 mL of 0.1 M Dimetil sulfoxide (DMSO) that was preliminary warmed to 55°C, and then homogenized for 10 min. The liquid aliquots were added with 1.0 mL sodium phosphate (0.1 M) pH 7.0 and 2.0 mL petroleum ether, and homogenized again using glass beads for 5 min and finally centrifuged again at 4000 rpm for 15 min to obtain bilayer supernatant. The ether layer containing carotenoid was transferred into evaporation-flask and evaporated. The carotenoid pigment was measured using spectrophotometer at 474 nm after addition with 5.0 mL methanol.

Assay on Antioksidant Activity

The reaction mixture was prepared by pipetting 1.0 mL of 0.05 mM DPPH in methanol into tube containing 1.0 mL of 100 ppm of respective carotenoid extracts and
then added with methanol until a final volume was upto 5.0 mL. A negative control was prepared by dilution of 1.0 mL DPPH with methanol until the final volume was upto 5.0 mL, while positive control was prepared by dilution of 1.0 mL DPPH and 1.0 mL vitamin C with methanol until a final volume was upto 5.0 mL. The decreasing in absorbance of DPPH at interval 0 to 30 min was measured at λ 515 nm using UV-VIS spectrophotometer.

RESULTS AND DISCUSSION

The highest yield of fungal biomass were exhibited by N. sitophila strain as much of 29.5 g/L at 120 h, and M. purpureus strain as much of 18.5 g/L at 144 h, while the highest yield of yeast biomass were exhibited by R. minuta as much of 10.7 g/L at 120 h and P. rhodozyma as much of 14.6 g/L at 96 h. The optimal production of yeast and fungal SCC was varied based on microbial source. The highest yield of microbial SCC was exhibited by P. rhodozyma strain as much of 46.12 µg/g, at 168 h, and M. purpureus strain as much of 25.30 µg/g at 120 h and R. minuta as much of 14.72 µg/g at 120 h, while the lowest yield of microbial SCC was exhibited by N.sitophila as much of 12.50 µg/g at 72 h.

In the present study, both of the concentration of SCC extracts and vitamin C employed to assay were at 100 ppm. Figure 6 showed that the SCC extract of N. sitophila performed the highest antioxidant activity rather than the other SCC extract where the obtaining value of its IC50 was 249.740 µg/ml. It was indicated by decreasing of absorbance value towards DPPH at every 5 min interval for 30 min. This result indicated that antioxidant activity of the SCC extract of N. sitophila was 22 times lower rather than antioxidant activity of vitamin C where the obtaining value of its IC50 was 43.408 µg/ml.

![Figure](image)

**Figure.** Production curve of yeast and fungal single cell carotenoids (left) and antioxidant activity of yeast and fungal single cell carotenoids (right) of N. sitophila, R. minuta, P. Rhodozyma, M. Purpureus against DPPH (left to right).
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P-BM09

PHARMACEUTICAL BIOPROSPECTING OF ENDOPHYTIC FUNGI ISOLATED FROM Curcuma longa

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Abstract

Endophytic microorganisms, reside in the living tissues of the host plant, may either make the identical plant medicinal or mimic the plant product with one or more other compounds that are structurally related, or similar to the medicinal. Novel antibiotics, antimycotics, immunosuppressants, and anticancer compounds are only a few examples of what has been found after the isolation and culturing of individual endophytes and their natural products. Many reports revealed that Endophytic microorganisms could degrade many compound became their derivatives with higher biological activity than the source of substrate. For many centuries, Curcuma longa have been used throughout Indonesia as food additive and traditional herbal medicine. It has been reported that curcumin, the yellow pigment extracted from rhizomes of Curcuma longa has several biological activities as anti-inflammatory, anticancer and antiseptic. In this study, a total of 45 endophytic fungi were isolated from rhizomes of Curcuma longa collected from various parts of Java (Parung, Cibinong, Bogor, Serang Central Java area and Yogyakarta). The isolates were then tested for their ability to biotransform curcumin into its analog. The result indicated that four of them have the ability however only one showed stability. The biotransformation product (BP) were evaluated for its hepatoprotective, antibacterial and anticancer activity. The results suggested that BP had antibacterial activity twofold better than curcumin against Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Ralstonia solanacearum, and most active against Salmonella typhi. BP has observed as anticancer, and showed activity against T47D/breast cancer cell lines (IC50 4.8 µg/µL), but this activity still lower than curcumin (IC50 3.2 µg/µL). Bp has not shown activity for HCT116/colon cancer cell lines. As for the hepatoprotector test using BP to protect against CCL4-induced hepatotoxicity in Rat, pretreatment with BP at a dose of 12 mg/kg partially prevented the elevation of AST and ALT levels. Using the same of dose, curcumin have shown better as hepatoprotector than BP. Beside BP, these endophytic fungi yielded secondary metabolites that have antibacterial and antiviral activity. For antibacterial, we have assayed against Staphylococcus aureus, Escherichia coli, Salmonella typhi and Ralstonia solanacearum. Secondary metabolites from these fungi have shown inhibitor activity for RNA helicase of HCV 1,26 –75.97%.

Keywords: Bioprospecting; endophytic; Curcuma longa; Biotransformation
INTRODUCTION

Endophytic microorganisms, reside in the living tissues of the host plants, may either make identical medicinal products or mimic the products with one or more other compounds that are structurally related, or similar to the medicinal products(1). Many reports revealed that endophytic microorganisms could also degrade many compounds to become their derivatives with higher biological activity than the source of substrate (2). It has been reported that curcumin, extracted from rhizomes of Curcuma longa has several biological activities such as anti-inflammatory, anticancer and antiseptic. In this study, a total of 45 entophytic fungi were isolated from rhizomes of Curcuma longa collected from various parts of Java (Parung, Cibinong, Bogor, Serang, Central Java area and Yogyakarta). The isolates were then tested for their ability to biotransform curcumin into its analog. The biotransformation product (BP) was evaluated for its antibacterial and anticancer activity as well as hepatoprotector activities. Secondary metabolites (SM) from these fungi had also studied for their inhibitory effect on RNA helicase activity of hepatitis C virus (HCV).

MATERIAL AND METHODS

Isolation of endophytic fungi was carried out applying the method described by Tanaka et al(3). Microbial biotransformation was done using the following technique: Aliquots of 3-day-old pre-cultures was inoculated into the shake cultures in PDB, 0.01% final concentration of curcumin was added to the cultures 3 day after the inoculation. After incubation with curcumin for 10 days on a rotary shaker at 25°C and 130 rpm, the cultures were extracted with EtOAc. The organic extracts were evaporated and then purified via silica gel column chromatography followed by preparatif TLC. The well diffusion method was used to test the antibacterial activities of BP (4) against Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Ralstonia solanacearum, and Salmonella typhi. Anticancer activities conducted using T47D for breast cancer and HCT116 cell lines for colon cancer. SRB assayed were used for measuring these activities The protective effect of BP against liver damage were evaluated in carbon tetrachloride (CCL4)-induced chronic hepatotoxicity in rats. Wistar rat were orally fed with BP (6;8;12 mg/kg bw) along for 7 days with administration single dose of CCL4 (1,5 ml/kg bw). Serum biological parameter (GPT and GOT) were assayed by IFCC Single Reagent Kinetic test kit (Reagensia, Jakarta). The general liver morphological changes were evidenced by histopathological examination. The samples were stained with hematoxylin and eosin Helicase gen of HCV were cloned on plasmid pET-21b and expressed on Escherichia coli BL21(DE3)pLysS,
The enzyme was purified by afinitas chromatography and then was used for screening of SM inhibitory activity(7).

RESULTS AND DISCUSSION

The result indicated that four of 45 tested isolates have biotransformation ability, however, only one showed stability, i.e isolate no. CL.Bel.5F of C. longa collected from Serang, Banten province. BP had antibacterial activity twofold better than curcumin against all of bacterial tested and was most active against Salmonella typhi. BP also showed activity against T47D/breast cancer cell lines (IC50 4.8 μg/μL), but this activity still lower than curcumin (IC50 3.2 μg/μL). Meanwhile on HCT116/colon cancer cell lines BP did not show any activity. As for the hepatoprotector test against CCL4-induced hepatotoxicity in rat, treatment with BP at a dose of 12 mg/kg prevented the elevation of AST and ALT levels, but at the same dose curcumin showed better as hepatoprotector than BP. Secondary metabolites from these fungi showed inhibitory activity for RNA helicase of HCV. Activity value varied among the isolates ranging from 1.26 up to 75.97%.

REFERENCES


P-BM10

ANTIBACTERIAL ACTIVITY OF METHANOL EXTRACT OF SIX VARIETIES OF GUAVA (Psidium sp.)

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Abstract

The objective of this study was to find out the antibacterial capacity of six varieties of Psidium members to enteropathogenic bacteria, Escherichia coli. Leaves from six varieties of guava (dark purple, stripped leaf, Bangkok, red, and small) were extracted by using 80% methanol under maceration method. The concentrations used in inhibition assay were 400, 200, 100, 50 and 25 mg/ml and the assay was carried out using disk diffusion method against E. coli ATCC 25922. The inhibition zones showed that inhibition capacities of the guava leaf extracts were significantly different among those varieties in the concentration range of 25-200 mg/ml, but it did not show significant difference among those varieties in the concentration of 400 mg/ml. It was also found that the antibacterial capacity of the guava leaf extract (400 mg/ml) comparable with chloramphenicol (30 µg/ml) From these results, those six varieties of guava could have the same healing property as antidiarrhea drugs.

Key words: Psidium, antibacterial activity, E. coli

INTRODUCTION

Ethanolic and methanolic extract of Psidium guajava leaf had similar inhibitory activities with Tetracycline, Chloramphenicol, Erythromycin, Gentamycin and Cotrimoxazole against Escherichia coli, Staphylococcus aureus, Proteus mirabilis and S. albus ¹). The aqueous guava leaf extract possessed broad spectrum antibacterial properties and showed a good activity against E. coli ²). Three types in guava leaves showed different characteristics of morphology and extract ³). The objective of this study was to find out the antibacterial activity of six varieties of Psidium members to enteropathogenic bacteria, E. coli.

MATERIALS AND METHODS

Leaves from six varieties of guava (dark purple, stripped leaf, Bangkok, red, and small) were extracted by using 80% methanol under maceration method. The dried
extract dissolved in 80% methanol and divided into five extract concentrations: 400, 200, 100, 50 and 25 mg/ml. The concentrations were used in inhibition assay which was carried out using disk diffusion method against *E. coli* ATCC 25922. Disk containing 30 μg Chloramphenicol applied as positive control. Both disk extract and antibiotic had 0.6 cm of diameter.

RESULTS AND DISCUSSION

The inhibition zones showed that inhibition capacity of the guava leaf extracts were significantly different among those varieties in the concentration range of 25-200 mg/ml, but it did not show significant difference among those varieties in the concentration of 400 mg/ml. It was also found that the antibacterial capacity of the guava leaf extract (400 mg/ml) could not be compared with chloramphenicol 30 μg. From these results, those six varieties of guava could have healing property as antidiarrhea drugs, but they were less effective than antibiotic.

REFERENCES


P-BM11

STUDY ON THE INTERACTION BETWEEN Gardenia tubifera EXTRACT WITH Salmonella typhi CHROMOSOMAL DNA
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Abstract
The high level typhoid fever caused by Salmonella typhi bacteria in Indonesia and the scarcity of antisalmonella drugs have encouraged many scientists to do a series of drug discovery research. Novel drug could be explored from natural product resources such as plants usually being used for traditional medicine. Since the plant is the chemical library which consist of various chemical compounds, the interaction between the molecules that are derived from natural product resources and cell component of pathogenic bacteria should be known before novel drugs being designed. The method to investigate the mechanism has been developed on molecular level based on High Throughput Screening method. DNA is an essential macromolecule and potential receptor for drug interaction. In order to study the mechanism, a dot blotting method was carried out to observe the interaction between Gardenia tubifera extract with the chromosomal DNA of Salmonella typhi. The isolated DNA was dropped onto membrane then was mixed into plant extract for overnight incubation. The interaction result was analyzed with TLC and HPLC methods. The TLC result showed that two Rt’s spot of 0.46 and 0.25 from G. tubifera extract was disappeared, whereas the HPLC of the extract illustrated the reduction of two peaks with retention time 2.0 and 3.2 minutes.

Keywords : DNA interaction, molecular interaction level, High Throughput Screening method, DNA-extract interaction.

INTRODUCTION
Typhoid fever is an endemic disease caused by Salmonella typhi, and it has induced the high level mortality, especially in developing countries such as Indonesia (DirJen PPM, 2007). The high level of this disease is associated with the inadequacy of antisalmonella drugs and the limited knowledge of the molecular mechanism of this fever (Ritiasa, 2007).

DNA as a carrier of genetic information is a major target for drug interaction because of its ability to interfere the DNA transcription (gene expression and protein
synthesis) and replication, a major step in cell growth and division. The later is central for tumorigenesis and pathogenesis (Drug-DNA Interaction, 2008).

The purpose of this experiment is to study the interaction of the Gardenia tubifera extract with Salmonella typhi chromosomal DNA.

MATERIALS AND METHODS

Salmonella typhi strain NCTC786 was used as source of chromosomal DNA for the molecular interaction studies. L-Broth (10 g bacto trypton, 5 g yeast extract, 5 g NaCl in 1000 mL, pH 7.2) was used to grow S. typhi. Barks of Gardenis tubifera was collected from Bulungan Research Forest, Malinau, East Kalimantan, and identified at Herbarium Bogoriense, LIPI, Bogor

To isolate the chromosomal S. typhi DNA, cells from a 10 mL overnight culture were centrifugation at 5000 rpm for 20 minutes, and suspended in 1 mL SET buffer (pH 8). After incubation the cells with lysozyme for 25 minutes at 37°C, 2% SDS and isoamylalcohol were added, and incubation was continued at 37°C for overnight. The clear solution was diluted with distilled water, and chrolofrom-isoamylalcohol (24:1) was added. The solution was then centrifugation at 5000 rpm for 15 minutes. After phenol extraction, the DNA was purified in ether as described by Maniatis. The powdered bark of G. tubifera (3.3 kg) was extracted with ethanol (3x5 L) at room temperature, filtered, and concentrated under vacuum, giving a dried extract. From other experiment, it was known that G. tubifera ethanol extract had an antisalmonella activity. For TLC and HPLC methods, the mixture solution of CH3OH-H2O (8:2, 7:3, 6:4 and 5:5) was used. The TLC apparatus using the RP18 TLC plates and the HPLC apparatus consists of Hitachi L-6200 Intelligent pump, Hitachi L-400 UV detector, Rheodyne 7125 injector, Graphic 450 Lloyd Instruments Recorder.

RESULTS AND DISCUSSION

The S. typhi DNA which was analyzes using agarose gel electrophoresis showed a thick band near the sample loading. It was indicated that the isolated DNA was not degradation. After dilution 30-folds, the A_{260:280} ratio of DNA solution was value of 1.92, and its concentration at A260 = 0.121 was 181 μg /mL. It confirms that the isolated DNA was apparently pure.

The Thin Layer Chromatography (TLC) results showed that there are were interaction between the DNA molecule and the components of the plant extract, which is
indicated as a reduced or moving spots. It was pointed out two Rf’s spots of 0.46 and 0.25 from the *G. tubifera* plant extract were disappeared (Table 1).

Table 1. TLC analysis of the interaction between *G. tubifera* ethanol extract with *S. typhi* DNA molecule

<table>
<thead>
<tr>
<th>No of spot</th>
<th>Rf value (x100) of <em>G. tubifera</em> ethanol extract</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mobile phase of CH$_2$OH-H$_2$O</td>
</tr>
<tr>
<td></td>
<td>CH$_2$OH (3 parts) – H$_2$O (2 parts)</td>
</tr>
<tr>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>I</td>
<td>46</td>
</tr>
<tr>
<td>II</td>
<td>35</td>
</tr>
<tr>
<td>III</td>
<td>29</td>
</tr>
<tr>
<td>IV</td>
<td>25</td>
</tr>
<tr>
<td>V</td>
<td>22</td>
</tr>
<tr>
<td>VI</td>
<td>15</td>
</tr>
<tr>
<td>VII</td>
<td></td>
</tr>
<tr>
<td>VIII</td>
<td>8</td>
</tr>
</tbody>
</table>

Note: A. Ethanol plant extract as a control, B. Ethanol plant extract reacted with Hybond membrane only, C. Ethanol plant extract reacted with Hybond membrane carrying of the DNA, T = spot disappeared

The molecular interactions analyze by High Performance Liquid Chromatography (HPLC) were shown in Table 2. The data observed that there were several peaks disappeared after the compounds of the plant extract reacted with the DNA molecule. The HPLC data exhibited the decreasing of two peaks with the retention time (t$_R$) 2.0 and 3.2 minutes (Table 2, coloumn C). From the TLC and HPLC analysis, it was known that the data of the plant extract and the data of plant extract reacted with the membrane only gave a similarity in pattern. There was no peaks disappear from both of these data, but more than one peak will vanish from the data if the plant extract reacted with the membrane carrying the DNA molecule. It predicted that more than one compound from plant extract interacted with the DNA molecule only.
Table 2. HPLC analysis of the interaction between G. tubifera ethanol extract with S. typhi DNA molecule

<table>
<thead>
<tr>
<th>No of spot</th>
<th>Retention time (tr, Minutes) of G. tubifera ethanol extract</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mobile phase of CH$_3$OH-H$_2$O</td>
</tr>
<tr>
<td></td>
<td>CH$_3$OH (3 parts) – H$_2$O (2 parts)</td>
</tr>
<tr>
<td></td>
<td>CH$_3$OH (1 part) – H$_2$O (1 part)</td>
</tr>
<tr>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>I</td>
<td>2.0</td>
</tr>
<tr>
<td>II</td>
<td>2.8</td>
</tr>
<tr>
<td>III</td>
<td>3.0</td>
</tr>
<tr>
<td>IV</td>
<td>3.2</td>
</tr>
<tr>
<td>V</td>
<td>4.4</td>
</tr>
<tr>
<td>VI</td>
<td>6.8</td>
</tr>
<tr>
<td>VII</td>
<td></td>
</tr>
<tr>
<td>VIII</td>
<td>11.4</td>
</tr>
</tbody>
</table>

Note: see on Table 1. A = Plant ethanol extract as a control, B = Plant ethanol extract reacted with membrane, C = Plant ethanol extract reacted with membrane carrying of DNA molecule, T = spot disappeared

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