Evolution and future challenges of grasslands and grassland agriculture in the East Asia

Organized by the Japanese Society of Grassland Science in collaboration with the Chinese Grassland Society and the Korean Society of Grassland and Forage Science
Proceedings of the 4th Japan-China-Korea Grassland Conference
Centrair Hall, Aichi, Japan
30th March – 1st April 2012

Main theme:
Evolution and future challenges of grasslands and grassland agriculture in the East Asia

Sub themes:
(1) Sharing the international, national and regional issues and challenges in grassland systems in the East Asia
(2) Forage production and utilization in cropland-based systems
(3) Ecology and management of grassland-based systems
(4) Utilization of genetic resources and seed technology

Organized by the Japanese Society of Grassland Science in collaboration with the Chinese Grassland Society and the Korean Society of Grassland and Forage Science

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Website: https://jckgc.brc.miyazaki-u.ac.jp/index.html
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Welcome Address

Professor Masakazu Goto

On behalf of the organizing committee of the four Japan–China–Korea Grassland Conference hosted by the Japanese Society of Grassland Science in collaboration with the Chinese Society for Grassland Science and the Korean Society of Grassland and Forage Science, I would like to extend my heartfelt welcome to all of you attending the international scientific symposium on grassland agriculture and animal production.

This conference was initially organized on the 50th anniversary of the Japanese Society of Grassland Science in 2004, and is rounded back to Japan this year. We are grateful to all of you for the distinguished contributions for developing activities for this international conference which should let us move on to higher stages of scientific collaboration.

We are also grateful and proud of all the participants for your scientific and kind cooperation. We have over 340 registrations at this moment. This is a big compensation for our experience that the conference scheduled in Wiszniki in 2011 was regrettably cancelled due to the spread of foot and mouth disease. We also thank all invitees and voluntary contributors who have addressed to our main theme, ‘Evolution and Future Challenges of Grasslands and Grassland Agriculture in East Asia’. We wish that the grassland scientists in Asian countries will be able to exchange their valuable new findings and experiences, and also you will discuss our possibility of substantial contributions to the grassland science and agriculture all over the world.

Finally, we would like to express our sincere gratitude toward the numerous emails expressing your sympathies and encouragements for the victims of the big earthquake and tsunami that hit Northeast and Kanto Regions of Japan and the accidents at Fukushima Nuclear Power Plant.

Don't you enjoy your stay in Japan.

March 31, 2012

Professor Dr. M. Goto
President, Japanese Society of Grassland Science
Welcome Address

Professor Jinfeng Yun

Ladies and gentlemen, and all our friends:

This is a beautiful season of the year in Japan with the cherry trees in blooming and we are happy to be here and attend the fourth Japan-China-Korea grassland conference which successfully opens in Japan. It is the beginning position of the second round of joint conferences.

On behalf of the Chinese Grassland Society, I offer my heartfelt congratulations to the Japanese Society of Grassland Science and the organization committee for preparing this symposium and I anticipate a very successful meeting.

Japan, China and Korea contain the major portion of the grassland area in East Asia. We are facing many same issues. In order to promote international cooperation in the field of grassland science, the first meeting "The Japan–Korea–China symposium on grassland agriculture and animal production" was held at Hiroshima in 2004. A forum for the scientific discussion was expansively succeeded to the second symposium at Lanzhou in 2006 and the third symposium at Seoul in 2009. Our joint symposium promoted to exchange ideas, share experiences and enhance friendship.

On the occasion when the meeting is hold again in Japan, with the aim of evolution and future challenges of grasslands and grassland agriculture in Asia. Organizing committee overcame the difficulties of the big earthquake and tsunami that hit Northeast and Kanto districts and completed many works for this conference. On behalf of all the participants of the conference, I would like to give our sincere thanks to you.

This conference absorbs more participants than before and covers more scientific areas including forage production and utilization, ecology and management of grassland-based systems, utilization of genetic resources and seed technology and so on. There are various activities during the meeting, consisting of presentation, poster viewing and discussions etc. I believe it will be a fruitful and successful meeting.

With the global economic integration, many common issues are faced for us, such as shortage of resources, deterioration of ecological environment, food safety and many others. Therefore, in the future, I suggest that member countries could increase gradually so as to strengthen and improve the cooperation on the research of grassland among the Asia counties and its influence on the world.

Finally, I hope you have a good time and see you in China next.

March 31, 2012

Professor Jinfeng Yun
Honorary President of Chinese Grassland Society
Welcome Address

Professor Byong-tae Jeon

Hello, members!

First of all, I welcome all of you and thank you for your participation and cooperation for the 4th Japan–China–Korea Grassland Conference.

When I was in Japan 30 years ago studying at Tohoku University, a Japanese entrepreneur told me that I should do my best to increase bilateral understanding and friendship between students from Korea and Japan if I become a professor in Korea. He also predicted that the world would become a conflicting design of “zone versus zone”; European countries would unite into a group in order to compete against the United States. He added that Asian countries should closely cooperate with each other to win competitions in the international arena.

I still remember the entrepreneur’s vision that the three countries of Korea, Japan and China of East Asia would become the leader of the world, while on the other hand, they could face tremendous losses in case of making excessive competitions with each other.

I am confident this Japan–China–Korea Grassland Conference will not only encourage private researches in grassland science but also play a great role to enhance friendship and cooperation between the three countries. Moreover, I hope this event will become a great place for international scientific exchanges among researchers.

Finally, my special thanks go to all the staff members of the Japanese Society of Grassland Science who have made all the efforts preparing for the 4th Conference.

Thank you.

March 31, 2012

Byong-tae Jeon, PhD
President, The Korean Society of Grassland and Forage Science
Professor, Konkuk University, Department of Animal Science
## Program

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General Information for Attendees

Registration and information desk

The registration desk is located on the Lobby (next door of the Centair Hall). The information desk will be available same to the registration desk since 31 March (Day 2). Staff on-duty will be glad to help all conference guests. Service hours of the registration desk and information desk are as bellow:

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Check-in

All attendees are required to check-in at the registration desk. Each registered participant will receive a name tag, a proceedings, and receipt for all payments made.

Badge distribution

Participants are kindly reminded to wear name badges at all times while in the conference area or at post conference tour. Access will be prohibited to the exhibit, coffee break, interactive areas, and technical sessions without presenting a name badge.

Message boards

Message boards will be set up at the registration desk so that participants can get useful information from the conference or other participants.
Establishment of micropropagation in dwarf napiergrass (Pennisetum purpureum Schum) and estimation of somaclonal variation using flow cytometry analysis

Nafiul Umami1,3, Takahiro Gondo2, Genki Ishigaki2 and Ryo Akashi2
1Interdisciplinary Graduate School of Agriculture and Engineering, University of Miyazaki, Japan.
2Frontier Science Research Center, University of Miyazaki, Japan.
3Faculty of Animal Science, Gadjah Mada University, Yogyakarta, Indonesia.
Correspondence: Ryo Akashi, rakashi@cc.miyazaki-u.ac.jp

Key words: dwarf napiergrass, flow cytometry, multiple-shoot clumps formation

Abstract: We have established in vitro propagation system of dwarf napiergrass (Pennisetum purpureum Schum) via multiple-shoot clumps formation. Shoot apices as initial explants were isolated aseptically from shoot-tillers and cultured in vitro on solid Murashige Skoog (MS) medium. The most effective phytohormone treatment for multiple-shoot clumps induction was 0.1 mg L\(^{-1}\) 2,4-dichlorophenoxyacetic acid (2,4-D) plus 2.0 mg L\(^{-1}\) 6-benzylaminopurine (BAP). The addition of 50 \(\mu\)M cupric sulfate (CuSO\(_4\)) could increase the percentage of clump proliferation. Plant regeneration frequency was achieved 84% by culturing the clumps on solid MS medium containing 0.1 mg L\(^{-1}\) \(a\)-naphthalene acetic acid (NAA) and 2.0 mg L\(^{-1}\) BAP. All regenerants were successfully grown up in soil. Comparison of result from morphological characteristics evaluation and DNA content using flow cytometry (FCM) analysis showed that in vitro regenerants did not reveal any significant difference compare with control plants.

Introduction
Napiergrass (Pennisetum purpureum Schum) is an important grass in the tropics and subtropics area, valued for its high biomass, pest resistant and perennial nature. The propagation of material plants in napiergrass is performed with cutting of the nod. The main advantage of micropropagation are the rapid multiplication of new varieties without depending on season, the improved plant health and the usefulness of germplas storage.

There have been no researches revealing shoot multiplication via multiple-shoot clumps induction of napiergrass, especially dwarf napiergrass. Meanwhile, this dwarf napiergrass does not produce seeds. Therefore, the dwarf type requires another alternative of vegetative propagation. Here we describe in vitro propagation system of dwarf napiergrass via multiple-shoot clumps from shoot apices. In order to estimate somaclonal variation, regenerants were evaluated morphological characteristics and DNA content stability using FCM analysis compared with native plants.

Materials and methods
Shoot apices were excised from shoot-tiller and were cultured on MS media (Murashige and Skoog 1962) containing 3% sucrose, 0.3% phytage and 0.1% (v/v) preservative for plant tissue culture media supplemented with various concentration of hormone. These induction media containing 2,4-D: 0.0, 0.01, 0.1, and 0.5 mg L\(^{-1}\) and BAP: 0.0 and 2.0 mg L\(^{-1}\). In order to proliferate, multiple-shoot clumps were transferred to MS induction media (2.0 mg L\(^{-1}\) BAP and 0.1 mg L\(^{-1}\) 2,4-D) supplemented with several concentration of CuSO\(_4\) (0, 5 and 50 \(\mu\)M). The percentage of clump proliferation was calculated after 14 days culture.

For plant regeneration, multiple-shoot clumps were transferred into MS basal media supplemented with 0.0 and 2.0 mg L\(^{-1}\) BAP in combination with 0.0, 0.01, 0.1 and 0.5 mg L\(^{-1}\) NAA. Elongated shoots were transferred to fresh half-strength MS medium to induce root development. The regenerants were transferred to soil directly in nursery pot in the greenhouse. Estimation of DNA content using FCM analysis. Oryza sativa ssp japonica cv Nipponbare was use as internal standard (Ishigaki et al. 2010) by using Beckman Cell Lab QuantaSM SC Flowcytometer machine (Beckman Coulter, Inc., Tokyo, Japan). Seven regenerant line with 3 replications from multiple-shoot clumps grew well in the greenhouse. These tillers of the regenerants were transferred into small pots (1/5000 a size) placed in a greenhouse for 1 month. All plants were transplanted into larger pots (1/2000 a size) and were grown outside. The 10 control plants, which were obtained from tillers of dwarf napiergrass, were grown in the same conditions. The regenerants were compared to the control plants for six morphological characteristics. The measurements were taken for a) plant length (PL); b) plant height (PH); c) leaf blade length (LBL) and d) leaf blade width (LBW) taken from trifoliolate leaf of the longest stem; e) number of tiller (TN) and f) yield (gram). Each measurement except the yield, was repeated ten times. The mean of the ten measurements was used to define the six morphological characteristics.

Results and discussion
After 10 days, the size of the basal of shoot apices was enlarged. From the basal of the shoot apices produced multiple-shoot clumps. Multiple-shoot clumps were induced on MS medium containing 2.0 mg L\(^{-1}\) BAP and 0.1 or 0.5 mg L\(^{-1}\) 2,4-D (Table 1). The addition of 2.0 mg L\(^{-1}\) BAP and 0.1 mg L\(^{-1}\) 2,4-D to MS medium was the effective treatment with 29% of multiple-shoot clumps formed. Repeated subculture at 15 days intervals resulted in a lower frequency of shoot tip formation in along time. In order to promote proliferation of clumps, several concentration of CuSO\(_4\)(0.0, 0.5 and 50
μM) be added in MS media containing 2.0 mg L⁻¹ BAP and 0.1 mg L⁻¹ 2,4-D (Table 2). For proliferation experiments, a multiple-shoot clumps derived from a single shoot apex was visually selected, the criteria being prolific growth and has adventitious shoots structure. We found that high levels of CuSO₄ (50 μM) promoted proliferation capacity of multiple-shoots clumps and 84% could regenerate into plantlets. In previous report (Gondo et al. 2004) used a 50 μM CuSO₄ could produce highly regenerative callus, dense in compact polyembryogenic clusters. Table 3 shows the percentage of regenerant from multiple-shoot clumps on regeneration medium. After 15 days, multiple-shoot clumps germinate and regenerate. The efficiency of plant regeneration was ranged 22.2–84.1%. From each shoot apex, more than 100 green shoots approximately grew 14 weeks after culture initiation. All shoots could develope root in half-strength MS medium without hormone.

The healthy plantlets were transferred in the greenhouse. The FCM result shows that the mean 2C DNA content of control dwarf napiergrass is 4.5 ± 0.36 pg 2C⁻¹, while no differences in 2C DNA content are found (P > 0.05) between regenerated plants from multiple-shoot clumps (4.7 ± 0.33 pg 2C⁻¹) and control plants by FCM analysis. Statistical analysis of morphological characteristics shows no significant different in morphological traits between regenerants and control plants (Table 4).

In conclusion, we have established multiple-shoot clumps formation system in dwarf napiergrass (Pennisetum purpureum Schum). The high number of regenerants could produce from shoot-apices of shoot tillers and the regenerants have same morphological characteristics and DNA content with the control plants. Accordingly, this system is stable and suitable for production of nursery plants on dwarf napiergrass in the future.

Table 1. Effect of hormone concentration on the formation of multiple-shoot clumps derived from shoot apices in tiller of dwarf napiergrass.

<table>
<thead>
<tr>
<th>Hormone concentration (mg L⁻¹)</th>
<th>No. of inoculated shoots apices</th>
<th>No. of multiple-shoot clumps formation</th>
<th>% of multiple shoot-clumps formation</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,4-D BAP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>60</td>
<td>0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>0</td>
<td>2</td>
<td>60</td>
<td>0&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.01</td>
<td>2</td>
<td>60</td>
<td>0&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.1</td>
<td>2</td>
<td>60</td>
<td>17&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.5</td>
<td>2</td>
<td>60</td>
<td>8&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a,b,c</sup>: different letters following each value within a column indicate a significant difference by Tukey’s Test (P < 0.05) on experiment of multiple-shoot clumps formation.

Table 2. Effect of hormone and CuSO₄ on proliferation multiple-shoot clumps from shoot apices of dwarf napiergrass

<table>
<thead>
<tr>
<th>Hormone concentration (mg L⁻¹)</th>
<th>No. of inoculated clumps</th>
<th>No. of proliferated clumps</th>
<th>% of proliferated clumps</th>
<th>Proliferation rate&lt;sup&gt;1&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,4-D BAP CuSO₄ (μM)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>2</td>
<td>0</td>
<td>63</td>
<td>4&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.1</td>
<td>2</td>
<td>5</td>
<td>63</td>
<td>6&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.1</td>
<td>2</td>
<td>50</td>
<td>63</td>
<td>22&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a,b</sup>: different letters following each value within a column indicate significant difference by Tukey’s Test (P < 0.05).

Table 3. Effect of hormone concentration on plant regeneration from multiple-shoot clumps derived from shoot apices in tiller of dwarf napiergrass.

<table>
<thead>
<tr>
<th>Hormone concentration (mg L⁻¹)</th>
<th>No. of inoculated clumps</th>
<th>No. of regenerated clumps (%)</th>
<th>No. of shoot formation</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAA BAP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>63</td>
<td>27 (47.8)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>0</td>
<td>2</td>
<td>63</td>
<td>14 (22.2)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.01</td>
<td>2</td>
<td>63</td>
<td>33 (52.3)&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.1</td>
<td>2</td>
<td>63</td>
<td>53 (84.1)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a,b</sup>: different letters following each value within a column indicate significant difference by Tukey’s Test (P < 0.05).

Table 4. Morphological characteristics of regenerants from multiple-shoot clumps of dwarf napiergrass and control plants

<table>
<thead>
<tr>
<th>Plant</th>
<th>PH (cm)</th>
<th>PL (cm)</th>
<th>LBL (cm)</th>
<th>LBW (cm)</th>
<th>TN (cm)</th>
<th>Yield (gram)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regenerant&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>128.8</td>
<td>131.9</td>
<td>70.5</td>
<td>2.8</td>
<td>23.6</td>
<td>735.4</td>
</tr>
<tr>
<td>Control&lt;sup&gt;a&lt;/sup&gt;</td>
<td>128.6</td>
<td>134.0</td>
<td>77.4</td>
<td>2.9</td>
<td>21.6</td>
<td>731.6</td>
</tr>
</tbody>
</table>

<sup>a,b</sup>: Non significants.

**References**

