Effects of Physiological and Morphological Characteristics of Root Tips Excised from Rice Seminal Roots on Subsequent Growth in vitro*

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Abstract: Tissue culture of excised root is a useful method with which genetic variation of plant root itself can be determined without the influence of shoot. We aimed to determine the effects of age and elongation rates of the seminal root axis of rice on subsequent growth in a culture medium. Taichung Native 1 (indica type) and Yukara (japonica type) were used in the experiments. The seminal root tips (1-cm-length) were sampled every day after bedding on agar and their morphological and physiological characteristics were monitored by recording the presence of lateral roots and primordia, dry weight and C·N content. Root tips with different ages or elongation rates were then cultured, and subsequent growth was observed after three week. Root tips which were older and had lower elongation rates showed inferior root growth in both cultivars. When a more than three-day-old seminal axis on which lateral roots started to emerge was excised, less L-type first order lateral roots was produced than that grown on the two-day-old axis, and this affected the total root number and length. The dry weight or C·N content of excised segments decreased as the excision day, and these characteristics showed a close correlation with the subsequent root growth. Thus, we concluded that the excision of root tip segments at an earlier stage ensures excellent development of seminal root system in vitro.

Key words: Age, C·N content, Elongation rate, Excised root culture, Lateral root, Oryza sativa L., Rice, Root tip segment.

Evaluation of crop root system morphology is important since it affects the growth and yield performance through root functions. However, it is generally difficult to characterize the root system morphology of a certain species or cultivar, especially that grown under field conditions. This is mainly because the root system morphology is known to be greatly affected by various environmental factors, which are difficult to control or evaluate. One of the most promising experimental techniques to overcome the problem would be the excised root culture method. Using this method, the environmental factors that would affect root growth can be considerably controlled. In addition, the excised roots can be grown without the influence of the shoot, or

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interaction with other roots\textsuperscript{2,11}. The method, however, has been shown to have some problems.

Excised-cultured roots develop in a unique way, i.e., the phenotype is expressed by the genomes, which control only root growth. Hence, the physiological and morphological conditions of the excised root tip would greatly affect subsequent root growth during the culture. However, their influences have not been sufficiently investigated so far.

Kono \textit{et al.}\textsuperscript{9} reported that nitrogen and phosphorus supply from the endosperm to the seminal root of paddy rice decreased from the third day after germination when nodal roots started to emerge. They also observed that the metabolic state of the seminal root changed during the same period, i.e., the root tip changed its growth characteristics from being meristematic to elongative. This fact strongly indicates that the physiological status of root tip to be cultured may have great impact on the subsequent growth \textit{in vitro}. Therefore, serious attention should be paid to this aspect to reasonably compare the morphologies of seminal root system (seminal root axis and lateral roots) among several rice cultivars by using the excised root culture method.

Thus, in this study, we aimed to determine the physiological and morphological characteristics of the seminal root tips at different ages and to assess the effects of these characteristics on their subsequent development \textit{in vitro} for two different rice cultivars.

\section*{Materials and Methods}

Two rice cultivars, cv. Taichung Native 1 (TN-1, indica type) and cv. Yukara (japonica type) were used in this study. We chose them because the preliminary survey showed that these two cultivars were very different in the root growth \textit{in vitro}.

Husked grains were soaked in 70\% Et-OH and 5\% sodium hypochlorite solution for 30 seconds and 30 minutes, respectively, for surface sterilization. The seeds were then washed with sterilized water three times. Subsequently, they were soaked in sterilized water for two hours and then placed over a well-sterilized germination bed made of 2\% agar in Petri-dish (9 cm in diameter).

A preliminary experiment using hundreds of seeds from the two cultivars was conducted to determine the average length of seminal root axis at 2, 3, 4, 5 and 6 days after seed bedding. Based on the data from the preliminary experiment, two experiments were conducted, in which the time and way of excision of 1-cm-length root tip segments for culture materials are different. Series 1 (focused on root age of seminal root axis) : from 2 to 6 days after bedding, root tips (culture materials) were excised every day from the seminal root axes (mother roots) which attained the length close to the average value in the preliminary experiment (Table 1). In other words, culture materials were sampled from mother roots which showed the average elongation rate at each sampling day. Series 2 (focused on elongation rate of seminal root axis) : in the same number of days, culture materials were excised from mother roots which attained the same length shown in Table 1 in different number of days. Namely, culture materials were sampled in several sets according to mother root length, within each set, the length of mother roots was same but their elongation rates (number of days required to attain the length) were different. This series of experiment was attempted because variation in the elongation rates of seminal root axis among plants was not negligible.

Surgical scissors were used for excision. Some of the excised root tip segments were

\begin{table}[h]
\centering
\begin{tabular}{lcccc}
\hline
Cultivars & Day after seed bedding & 2 & 3 & 4 & 5 & 6 \\
\hline
TN-1  & & 17 & 43 & 53 & 63 & 70 \\
Yukara  & & 15 & 24 & 40 & 56 & 62 \\
\hline
\end{tabular}
\caption{Changes in average seminal root axis length (mm) of two rice cultivars grown on sterilized agar with days after seed bedding.}
\end{table}

\*: Data of seeds that did not produce seminal root were excluded for calculation.

Each value is shown in average \pm standard deviation.
oven-dried at 80°C for 48 hours and then weighed, and their carbon and nitrogen contents were determined with the use of CHN corder (Yanaco MT-5). The rest of the root tip segments were cultured.

We followed the root culture method proposed by Kawata et al., Kim et al. and Lai & Lee. Two root tip segments were put into a 100 ml Erlenmeyer flask containing 15 ml of Lai & Lee's R2 culture medium sterilized at 120°C with autoclave for 5 minutes. A total of ten roots from five replicate flasks were used for each sampling. Then the flasks were incubated at 28°C in darkness. The R2 medium contained only nitrate ions as a nitrogen source.

The cultured seminal root systems were sampled at 3 weeks after transfer to the culture medium. The samples were fixed in FAA solution (70% Ethanol : Formalin : Acetic acid = 18 : 1 : 1). The lateral roots were classified into two categories; L-type lateral root, which is long, thick and able to branch high order lateral roots, and S-type lateral root, which is short, slender and has no branching capacity. The number of L-type and S-type lateral roots, the length of each lateral root of different branching orders and the seminal root axis length were determined manually.

The seminal root axes sampled through two timecourse series were observed under a light microscope to count the number of lateral roots and primordia formed on them.

**Results**

1. **Effect of age**

Fig. 1 shows the length of three-week-cultured seminal root axis. In TN-1, the later the excision day was, the smaller the final root axis length was. On the other hand, such a trend was not clearly observed for Yukara. Overall axis elongation was more vigorous in TN-1 than Yukara.

Such trends were also observed for both the number and length of all the root system components in TN-1 (Fig. 2). Comparing the value on day 2 with that on day 4, the total root number was fewer in the latter mainly due to the fewer number of second order lateral roots. This in turn was mainly caused by fewer emergence of L-type first order lateral roots, from which second order lateral roots originated. The differences in the number and length between day 4 and 5 were due to seminal root axis length, and number and length of S-type first order lateral roots. In the case of Yukara, when root tip was excised later than day 2, total root number and length were markedly smaller because only a few L-type first and their concomitant second order lateral roots emerged.

The formation of lateral roots and primordia on seminal root axes started on day 3 for both cultivars and increased in number with the elongation of the seminal root axis (Fig. 3). The increase rate from day 2 to 3 was faster in TN-1 than in Yukara.

Fig. 4 shows dry weight, and C • N content of the culture materials sampled at different days. Dry weight of TN-1 sharply decreased
with the delay in time of excision. In accordance with this, C • N content decreased gradually. Yukara also showed these trends, but more slowly than TN-1, as in its mother root length.

2. Effect of elongation rates

The elongation rate of seminal root axis in TN-1 was considerably uniform among the plants sampled each day. Therefore, it was impossible to collect a sufficient number of segments from seminal root axes on different days to evaluate the effect of elongation rates on subsequent growth, so data was not collected. However, in the comparison between groups that showed different elongation rates, the same tendency observed in Yukara were noted in all parameters.

For Yukara, growth of cultured root was very poor when culture materials were sampled from mother root which was longer than 30 mm regardless of excision day. Thus, the comparison was made only for the cultured seminal root systems developed from culture materials which were derived from the shorter

![Fig. 3. Changes in sum of the number of lateral roots and primordia recognized on seminal root axis of two rice cultivars sampled through the timecourse series 1 (See Materials and Methods). Error bars show standard errors.](image)

![Fig. 4. Differences in dry weight and C • N content per 1-cm root tip segment excised from seminal root axis of two rice cultivars. Root tips were sampled through the timecourse series 1 (See Materials and Methods). Error bars show standard errors.](image)

Table 2. The influence of average elongation rate of seminal root axis till excision on subsequent development in four characters of 3-week cultured seminal root system of Yukara.

<table>
<thead>
<tr>
<th>Axis length on excision</th>
<th>Excision day after bedding</th>
<th>Elongation rate (mm/day)</th>
<th>Seminal root axis length (mm)</th>
<th>Number of 1st-L***</th>
<th>Total root number</th>
<th>Total root length (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;20mm*</td>
<td>2</td>
<td>7.5</td>
<td>91±4</td>
<td>7±1</td>
<td>226±18</td>
<td>1541±103</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>5.4</td>
<td>67±4</td>
<td>1±1</td>
<td>94±11</td>
<td>900±112</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>4.5</td>
<td>68±4</td>
<td>0±0</td>
<td>99±8</td>
<td>708±61</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>3.6</td>
<td>50±6</td>
<td>not emerged</td>
<td>70±9</td>
<td>333±50</td>
</tr>
<tr>
<td>20-30mm**</td>
<td>3</td>
<td>8.1</td>
<td>88±4</td>
<td>not emerged</td>
<td>128±5</td>
<td>1028±105</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>6.4</td>
<td>83±4</td>
<td>not emerged</td>
<td>123±5</td>
<td>721±43</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>5.9</td>
<td>60±5</td>
<td>not emerged</td>
<td>90±7</td>
<td>702±122</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>5.0</td>
<td>45±3</td>
<td>not emerged</td>
<td>52±3</td>
<td>276±36</td>
</tr>
</tbody>
</table>

*: Root tips were sampled through the timecourse series 2 (See Materials and Methods), i.e., they were sampled from seminal root axes which attained the length close to the average value (Table 1) on day 2 and 3, respectively.

**: Number of L-type first order lateral roots.

Each value is shown in average ± standard error.
Fig. 5. Changes in the number of lateral roots and primordia recognized on seminal root axis of Yukara sampled through the time-course series 2 (See Materials and Methods). Error bars show standard errors. The average length of seminal root axis was 15.1 mm on day 2, 17.2 mm on day 3, 18.9 mm on day 4, and 17.0 mm on day 5.

Fig. 6. Differences in dry weight and C • N content per 1-cm root tip segment of Yukara excised from seminal root axes which attained similar length on each excision day. Root tips were sampled through the timecourse series 2 (See Materials and Methods). Error bars show standard errors. The average length of seminal root axis on excision are shown in the note of Fig. 5.

seminal root axes (Table 2). Overall, the cultured root axis length became smaller when the root tip excision delayed. More drastic changes were observed in development of L-type first order lateral roots. When the root tip was excised later than day 2, few L-type first order lateral roots were produced. This difference in turn affected both the total number and length of the root system. Further, no L-type lateral roots were produced on the cultured roots when the mother root was longer than 20 mm. Therefore, the difference in total root number and length were not as drastic as in the former group (less than 20 mm axis length).

The morphological changes of seminal root axis which were distinguished by the presence or absence of lateral roots and primordia (Fig. 5) were observed from day 3 to 4. The results of analysis of 1-cm-length root tip segments of Yukara, whose mother seminal root axis length was almost the same (~20 mm) is shown in Fig. 6. It is clear that even though the lengths were similar, the dry weight and C • N content decreased as the time of excision delayed.

Discussion

From the results of the two experiments we found that younger and faster-growing root tips which were greater in dry weight, and richer in C and N contents grew more vigorously in vitro.

Kawata et al.61 reported that growth of excised root was improved when culture material was sampled as soon as possible after bedding and sampled from mother root with lower elongation rates. The latter result seems to disagree with ours. Kawata et al., however, focused on the seasonal variation of seminal root axis elongation in relation to seed dormancy. In our case, the target is the individual variation of elongation. Therefore, it appears that there is then no conflict between these two results.

Brown11 reported that the metabolic activity of excised root tip segment, which is closely related with cell age, dominated subsequent cell division rate in culture system. Our results are firmly supported by this result. From visual observation on the seminal axis, it was also confirmed that the state of the axis changed with time. Although no morphological change was observed in series 2 between day 2 to 3 (Fig. 5), a crucial physiological change might have occurred.

The seminal root system development and C • N content of the culture materials were positively correlated (Table 3). The N content showed closer correlation with root characters than C content. It is noteworthy that N content, which accounts for a lesser portion of dry
weight than C, had greater impact on root growth. Therefore, N content seemed to express the physiological state of root tip accurately.

Correlation coefficients between dry weight and C • N content of the culture materials and the cultured root axis length were relatively similar for the two cultivars, but the coefficients for total root length were considerably different. This indicates that the difference between cultivars was mainly in their lateral root development.

Drew et al.\(^3\) reported that nitrate concentration in an external medium affected the growth of lateral roots but not the seminal axis of barley in hydroponic culture. \(R_2\) medium contains only nitrate ions as an inorganic nitrogen. Hence, roots need to assimilate inorganic nitrogen for growth except for organic nitrogen available from excised segments themselves. Consequently, it was assumed that the different correlation coefficient in total root length would reflect nitrate metabolic activity. Kim et al.\(^3\) suggested that the root of japonica rice cultivars has less nitrogen assimilation capacity than that of indica rice. In this respect, it was suggested that the root growth of Yukara would depend on the organic nitrogen originally contained in the culture material more than TN-1.

In our experiment, very few L-type first order lateral roots were produced on the root tips when excised after day 2, which considerably affected total root growth (Fig. 2 and Table 2). Previously, we reported that the emergence and the development of L-type first order lateral roots required organic nitrogen\(^8\). On this point, further detailed study of nitrogen metabolism in the excised culture system is needed.

In conclusion, we found that the aging of culture material greatly affects the development of seminal root system in vitro in both rice cultivars examined. Hence, it is very important to excise root tip segments from seminal root axes at an early developmental stage as possible. To ensure excellent development of cultured root, a careful seed preparation and a suitable germination method is necessary.

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**References**


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* In Japanese.
** In Japanese with English summary.
*** In Chinese with English summary.