Short Communication

Plant Regeneration from Isolated Pollen Grains in Indica Type Rice (Oryza sativa L.)

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Isolated immature pollen grains from the Indica type rice cv. IR24 were able to regenerate plants via calli without pre-anther culture. After low temperature pretreatment at 10°C, the pollen grains 42–50 μm in diameter were mechanically isolated and incubated in a medium containing one-fourth strength of R2 salts and B5 vitamins, 0.5 mg/l 2,4-D, 5 mM sucrose and 0.4 M mannitol (pH 5.8). A low temperature pretreatment of more than 20 days was required for the pollen grains to start dividing. The pollen grains that started to divide formed multicellular masses on day 7 after incubation which ruptured to develop to calli on day 10. The pollen grains that were pretreated for 25 days with a low temperature gave the highest colony formation frequency of 0.4%. For plant regeneration, the growing calli were transferred to a medium containing R2 salts, B5 vitamins, 30 g/l sucrose and 8 g/l agar (pH 5.8) 49 days after incubation. Out of 263 calli, one produced a green plant while five produced albino plants.

KEY WORDS: Oryza sativa, indica-japonica, pollen culture, plant regeneration

Introduction

Culture of isolated immature pollen grains is considered to produce doubled-haploid plants more effectively than anther culture. It was reported that the culture was successful in Japonica type rice (Chen 1986, Jia et al. 1987, Cho and Zapata 1988, Matsushima et al. 1988), but not in Indica and Javanica types rice so far.

To develop a culture system of isolated immature pollen grains that can be applied to any varietal groups, Japonica, Indica and Javanica types (Morinaga 1968, Chang 1976), we examined the effects of low temperature pretreatment on the division and colony formation of immature isolated pollen grains and the regenerating ability of the derived calli in the Indica type rice.

Materials and Methods

Plant materials: Indica type variety IR24 was used in this study. Plants were all grown in a green house. The air temperature of the green house was controlled at above 20°C. Panicles were collected at the booting stage (at which the length between the auricles of a flag leaf and of the previous one reached 7 to 10 cm) and were cold-treated in a low temperature room at 10°C until use.

Isolation and culture of pollen grains: Anthers were aseptically removed, transferred to a 0.4 M mannitol solution and crushed with a spatula on a stainless mesh. The pollen grains

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Table 1. Composition of the media used in the study

<table>
<thead>
<tr>
<th>Constituents</th>
<th>Medium</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
</tr>
<tr>
<td>Salts</td>
<td>1/4 R2(^1)</td>
</tr>
<tr>
<td>Vitamins</td>
<td>1/4 B5(^2)</td>
</tr>
<tr>
<td>2,4-D</td>
<td>0.5 mg/l</td>
</tr>
<tr>
<td>Sucrose</td>
<td>5.0 mM</td>
</tr>
<tr>
<td>Mannitol</td>
<td>0.4 M</td>
</tr>
<tr>
<td>Proline</td>
<td>—</td>
</tr>
<tr>
<td>Asparagine</td>
<td>—</td>
</tr>
<tr>
<td>Agar</td>
<td>—</td>
</tr>
<tr>
<td>pH</td>
<td>5.8</td>
</tr>
</tbody>
</table>

\(^1\) Ohira et al. 1973
\(^2\) Gamborg et al. 1968

Table 2. Effects of low temperature pretreatment on colony formation and plant regeneration in the culture of isolated immature pollen grains

<table>
<thead>
<tr>
<th>Duration at 10°C (day)</th>
<th>Colony formation</th>
<th>Plant regeneration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of colonies formed(^1) (%)</td>
<td>No. of calli tested(^2)</td>
</tr>
<tr>
<td>10</td>
<td>0 (0)</td>
<td>—</td>
</tr>
<tr>
<td>15</td>
<td>0 (0)</td>
<td>—</td>
</tr>
<tr>
<td>20</td>
<td>7.3 (0.02)</td>
<td>24</td>
</tr>
<tr>
<td>25</td>
<td>126.7 (0.42)</td>
<td>239</td>
</tr>
</tbody>
</table>

\(^1\) Figures show the mean number of colonies more than 200 μm in diameter in a dish 21 days after incubation in three replications.
\(^2\) The total number of calli from three replications were used for regeneration.

42~50 μm in diameter were separated from the suspension with nylon mesh sieves. After centrifugation (100 g, 3 min.), the pellets were resuspended into medium A (Table 1) at a density of 1.5 × 10⁴/ml. Two ml of the suspension was poured into a plastic petri dish (3.5 cm in diameter) and placed at 25°C under a 16-hour photoperiod.

The colonies derived from the immature pollen grains were transferred to liquid medium B on day 21, to solid medium C on day 35 and lastly to medium D for plant regeneration 49 days after incubation.

**Results and Discussion**

A green plant could be successfully regenerated from isolated immature pollen grains of the Indica type rice cv. IR24. We found that the low temperature pretreatment was very effective in enhancing the response of the pollen grains. Isolated pollen grains needed to be pretreated at 10°C for more than 20 days to start dividing (Table 2). There was a relation-
ship between the frequency of colony formation and the time of the pretreatment: the pollen grains that had been pretreated for 25 days formed a larger number of colonies than those treated for 20 days. The pollen grains that were pretreated for 25 days gave the highest frequency of colony formation of 0.4%.

It had been reported that low temperature pretreatment was effective in the division of isolated immature pollen grains in Japonica type rice (Chen 1986, Jia et al. 1987, Cho and Zapata 1988, Matsushima et al. 1988). In these reports, the time of the pretreatment at 10°C was about 10 days in all the varieties used. The pollen grains of the Japonica type rice cv. Nipponbare required a pretreatment of 15 days at 10°C in our preliminary test (data not shown). However, the pollen grains of the Indica type cv. IR24 began to divide, when they were pretreated at 10°C for at least 20 days in our experiment. These results suggest that the duration of the period of low temperature pretreatment required is different between the two varietal groups, Japonica and Indica types, and that this difference partly limited the application of the culture of isolated immature pollen grains to Indica type rice. We will confirm this suggestion using many varieties of Japonica and Indica types in our further study.

Fig. 1. shows the development of the pollen grains that were pretreated for 25 days. They started to divide within 5 days after incubation and developed to multicellular masses around day 7. The exine of the masses ruptured around day 10, when the masses grew into
colonies.

Two-hundreds-sixty-three calli were obtained and their regenerating ability was examined. After they were transferred to media lacking hormones, only one callus regenerated a green plant while 5 calli produced albino plants (Table 2). The green plant normally grew in a green house and was fertile, when transferred onto pots with soil.

The pollen culture system should be improved, because only a small number of pollen grains started to divide and more albino plants regenerated than green plants in this experiment. The present study indicated that an extended period of low temperature pretreatment enhanced the division frequency of the pollen grains. However, CHEN (1986) reported that a prolonged pretreatment greatly increased the regeneration frequency of albino plants in anther culture. Attempts are currently being made to examine different kinds of treatment.

Acknowledgement

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Literature Cited

Isolated Pollen Culture in Indica Type Rice

インド型イネ単離花粉からの植物体再生

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単離花粉培養法は、培養法に比べより効率的な半数体培養法として期待されるが、イネにおいては、日本型イネでのみ成功例が報告されている。本研究では、比較的長い期間低温処理を施した未熟花粉を用いることによって、インド型イネの単離花粉からの植物体再生に成功した。

インド型品種IR 24の穂を業耳間長7〜10 cmに採取し材料とした。低温処理は、10℃低温室内に一定期間静置することによって行なった。低温処理後、42〜50 μmの未熟花粉を単離し、分裂誘導培地（1/4 R 2 無機培、1/4 B5ビタミン、0.5 mg/l 2,4-D、5 mMショ糖、0.4 Mマンニトール、pH 5.8）中に懸濁し培養した。以後、液体培養（培養開始21日目）、固体培養（培養開始35日目）を行ない、培養開始49日に再分化培地にカルスを移植し再分化を図った。

単離花粉の分裂を誘導するには、少なくとも20日間以上低温処理を施することが必要であった。最も高いコロニー形成率を示したのは25日間低温処理を施した花粉でその時の頻度は0.4%であった（Table 2）。分裂を開始した花粉は、培養開始7日に多細胞化し、10日目には花粉壁が壊れカルス化していった（Fig. 1）。得られた263個のカルスを再分化培地に移植したところ、6個のカルスから植物体が再生し、うち1個のカルスで緑色個体が得られ、5個のカルスではアルビノであった。

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