Effects of Genotype on Induction of Callus and Plant Regeneration
Potential in vitro Anther Culture of Rice (Oryza sativa L.)

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Abstract: The present studies were carried out to evaluate the response of anthers of six rice genotypes to callus induction and plant regeneration. For callus induction Z_{5} media supplemented with 2, 4-D 0.5 mg 1^{-1}, NAA 2.5 mg 1^{-1} and Kinetin 0.5 mg 1^{-1} was used. Of the six genotypes, five gave good callusing response. Highest callus induction was observed in BRRI Dhan-29 (8.06%) and lowest in BR-10 (1.42%). Callus induction frequency varied from 1.42-8.06% depending on genotypes. Modified MS medium supplemented with Kinetin 0.5 mg 1^{-1}, BAP 2 mg 1^{-1} and NAA 1.0 mg 1^{-1} was used for plant regeneration. The results showed a significant genotypic difference in callus induction and plant regeneration. The regeneration of plantlet from anther derived calli ranged from 57-75% conclusion.

Keywords: Anther culture, callus induction, genotype

INTRODUCTION

Bangladesh is a developing country. About 150 million people of Bangladesh live on Rice. The population of Bangladesh is increasing at an alarming rate but the land is not increasing accordingly. With the increasing demand of rice and to meet the challenge of 21st century rice varieties are needed with higher yield potential and multiple resistance to diseases, insects and resistance to abiotic stresses. Application of anther culture will provide a new avenue us to develop and improve rice varieties for meeting the challenges of increase rice production.

Anther culture has been used for crop improvement and the development of cultivars through anther culture has advantages over the conventional breeding[1-3]. Anther culture has proved to be very useful tools in plant breeding, providing a rapid method for regeneration of desired genotypes. This technique opens new vistas of breeding for resistance and quality of crop plants, not only shortening the breeding cycle by immediate fixation of homozygosity but also increasing selection efficiency. Scientists working on rice in different countries in the word have successfully produced callus and plant from anther and from isolated pollen[4,5,6]. Several rice varieties have been developed through anther culture[7] though in indica rice callus induction and subsequent plant regeneration from cultured anthers have rather difficult compared to japonica. Genotypic variation in anther culture response have been reported by Guha-Mukerjee[8,9]; Pandey et al[10] and Drazdja[11]. Many other scientists[12-14] also reported that genotypes played an important role in callus induction and plant regeneration. The success of anther culture mostly depends on genotype of the donor material. Genotypic variability in callus induction and plant regeneration through anther culture have not been studied extensively in major rice varieties in Bangladesh. This study considers the variation in callus induction and plant regeneration potential among six genotypes of rice in vitro anther culture.

MATERIALS AND METHODS

Six cultivars of rice viz, BR-3, BR-7, BR-10, BR-14, BRRI Dhan-29 and BR-802-78-2-1-1 were used for conducting the experiment. Boots of these cultivars were collected from the experimental field of Rice Research Institute, Rajshahi, Bangladesh in boro season, 2002 at the stage when the distance between flag leaf and the penultimate leaf is between 5 to 10 cm. Cold treatment was performed by sealing the panicles in polyethylene bags and refrigerated at 6°C for 7 days. Cold treated panicles were removed from the refrigerator and were placed in the laminar flow bench. Leaf sheaths enclosing the panicle were sterilized by dipping intact in 70% ethyl alcohol for 1 min just before inoculation of anther.

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Anthers were separated from the panicle with the help of forceps and put in 6 cm petri dishes containing callus induction medium. For callus induction Z_2 medium modified II_2 supplemented with 2,4-D 0.5 mg l^{-1} + NAA 2.5 mg l^{-1} + Kinetin 0.5 mg l^{-1} was used. The pH of the medium was adjusted to 5.8. Petri dishes were sealed with parafilm and incubated at 26±1°C in the dark for callus induction for 4-6 weeks. Three petri dishes were used for per replication. Calli were formed within 4-6 weeks. The culture was examined every week for up to 6 weeks. Data was recorded on percentage of anthers formed calli in 6 weeks (callus induction frequency). Anther derived calli were transferred to regeneration medium MS+Kinetin 0.5 mg l^{-1} + BAP 2 mg l^{-1} + NAA 1.0 mg l^{-1} for regeneration and was exposed to 12 h photoperiod maintained by fluorescent tube light. The response of cultured anthers was calculated by counting the number of anthers forming calli multiplied by hundred and divided by total number of anthers plated.

RESULTS AND DISCUSSION

After performing cold treatment of panicles of six genotypes of rice followed by sterilization, anthers were separated from the panicle and cultured on Z_2 medium for callus induction. The culture was examined every week for up to 6 weeks. Callus was developed from the anthers within 4-6 weeks. The induction of multiple calli from a single anther was also observed. Calli were mostly creamy white in colour and of variable texture (Table 1).

From the results (Table 1), it was evident that callus was induced in all the genotypes but the frequency of callus induction varied from 1.42 to 8.06% depending on the genotype. BRRI Dhan-29 responded the highest callus induction frequency (8.06%) followed by BR-7 (4.60%), BR-3 (3.43%) and BR-802-78-2-1-1 (3.20%). Induction was found to be poor in BR-10 (1.42%). BRRI Dhan-29 was the highest callus inducer (Fig. 1). Variations in callus induction ability among the tested varieties indicated that the difference in response was due to difference of genotype. Our results are higher than that of reported by results of Hakim et al. [8], Abe and Fukusake [9], Guo and Cao [10], Wu and Chen [11]. They reported that genotypes played an important role in callus induction and plant regeneration. Chen [12] reported that pre-treatment of rice panicles at 10°C for 48 h greatly increased the frequency of callus induction. Sharmen and Bari [13] studied and reported that cold shock of rice panicles at 6°C for 7 days increased the frequency of callus induction and plant regeneration. Increased rate of callus induction was found in all the genotypes by applying cold treatment reported by Sharmen and Bari [13] in our present study compared to our previous published results.

For regeneration of plantlets anther derived calli obtained from different genotypes of rice were transferred to MS media supplemented with Kinetin 0.5 mg l^{-1} + BAP 2 mg l^{-1} + NAA 1 mg l^{-1} (Table 2).

The regeneration efficiency of BRRI Dhan-29 was found to be highest (75%) among the genotypes tested (Fig. 2). A highly significant difference was observed among genotypes for both callus induction and plant regeneration. Maximum regeneration efficiency was recorded in BRRI Dhan-29 (75%) and lowest in BR-14 (57%) (Table 2). However, low plant regeneration frequency is one of the most serious limitation in anther culture

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**Table 1:** Callus induction frequency of anthers of different rice genotypes

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>No. of anthers inoculated</th>
<th>No. of anthers formed</th>
<th>(%) callus induction</th>
<th>Texture</th>
<th>Colour</th>
</tr>
</thead>
<tbody>
<tr>
<td>BR-3</td>
<td>320</td>
<td>11</td>
<td>3.42</td>
<td>Compact Cream</td>
<td>Compact White</td>
</tr>
<tr>
<td>BR-7</td>
<td>280</td>
<td>12</td>
<td>4.60</td>
<td>Compact White</td>
<td>Compact White</td>
</tr>
<tr>
<td>BR-10</td>
<td>330</td>
<td>5</td>
<td>1.48</td>
<td>Compact White</td>
<td>Compact White</td>
</tr>
<tr>
<td>BR-14</td>
<td>270</td>
<td>7</td>
<td>2.60</td>
<td>Compact White</td>
<td>Compact White</td>
</tr>
<tr>
<td>BRRI Dhan-29</td>
<td>310</td>
<td>25</td>
<td>10.60</td>
<td>Compact White</td>
<td>Compact White</td>
</tr>
<tr>
<td>BR-802-78-2-1-1</td>
<td>280</td>
<td>9</td>
<td>3.28</td>
<td>Compact White</td>
<td>Compact White</td>
</tr>
</tbody>
</table>

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**Table 2:** Frequency of plantlet regeneration from anther derived calli of different genotypes of rice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Genotype</th>
<th>No. of anther derived calli</th>
<th>% of calli regenerated</th>
<th>plantlet (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MS + 0.5 mg l^{-1}</td>
<td>BR-3</td>
<td>11</td>
<td>7</td>
<td>83.6</td>
</tr>
<tr>
<td>Kinetin + 2 mg l^{-1}</td>
<td>BR-7</td>
<td>11</td>
<td>8</td>
<td>72.7</td>
</tr>
<tr>
<td>BAP + 10 mg l^{-1}</td>
<td>BR-10</td>
<td>5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>NAA</td>
<td>BR-14</td>
<td>7</td>
<td>4</td>
<td>57.0</td>
</tr>
<tr>
<td>BRRI Dhan-29</td>
<td></td>
<td>24</td>
<td>18</td>
<td>75.0</td>
</tr>
<tr>
<td>BR-802-78-2-1-1</td>
<td></td>
<td>3</td>
<td>4</td>
<td>62.5</td>
</tr>
</tbody>
</table>

1 Average results of three replications
technique in indica variety, BR-10 did not regenerate any plantlet. Anther culture technique is to be more applicable in breeding it is necessary to undertake further research on anther culture to establish dependable regeneration system in rice improvement.

REFERENCES