Research Paper

Comparative Study on Regeneration Potentiality of Aromatic Indica Rice (Oryza sativa L.) of Bangladesh

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Abstract

In this study, two varieties of aromatic rice (Oryza sativa L.) BRRI Dhan 50 (Bangla Moti) and BRRI Dhan 34 (Khaskhani) were used to establish a suitable system for callus initiation and regeneration. MS (Murashige & Skoog, 1962) media supplemented with different concentrations i.e. (1.0, 2.0, 3.0, 4.0 mg/L of 2,4-D (2,4-dichlorophenoxyacetic acid) and combination with BAP (6-benzylaminopurine), (2.0 mg/L) were used for callus induction from mature dehusked rice seeds. MS medium supplemented with only 3.0 mg/L of 2,4-D, produced maximum percentage of callus that is 90% for BRRI Dhan 50 and 80% for BRRI Dhan 34. On the other hand, MS media with 3.0 and 4.0 mg/L of 2,4-D in combination with 2.0 mg/L BAP produced highest percentage of callus (80%) for BRRI Dhan 50 and 70% for BRRI Dhan 34. For plantlet regeneration, MS media with 1.0 mg/L NAA (1-Naphthaleneacetic acid), 2.0 mg/L BA (6-benzyladenine) and various concentrations of Kinetin (0.0, 1.0, 2.0, 3.0, 4.0 mg/L) were employed. The maximum percentage of shoot regeneration was recorded at MS media supplemented with 4.0 mg/L of Kinetin +1.0 mg/L NAA and 2.0 mg/L BA for both varieties. These results will be very helpful to improve rice quality through somaclonal variation and genetic transformation.

Keywords: Auxin, BRRI Dhan, Cytokinin, Plantlet Regeneration, Aromatic Rice, Oryza sativa

1. Introduction

Rice, like wheat, corn, rye, oats and barley belongs to Gramineae or grass family. Fine rice, is a part of the rice family (Oryza sativa L.). In Bangladesh, there are about more than 7,000 varieties of rice are grown in various parts of the country. Nearly 70% of the land area of the country has been brought under rice cultivation. Out of this 70%, fine rice is cultivated on roughly 10% land. This lower coverage is primarily due to the emphasis of government policy and research on food grain production but with low input technology. Fine rice production reached 28 million tons in 1993 but has declined marginally over the last three years due to consecutive droughts and floods. It is observed that the fine rice is a profitable farming venture for the farmers, as its cultivation does not normally require additional expenditure on fertilizer, pesticides and irrigation, and a good source of livelihood. Rather using indiscriminate use of fertilizers increases the thickness of the rice and reduces aroma. The use of organic fertilizers and pesticides, through traditional practices, believes to help enhancement of aroma and preservation of fineness as mentioned by the farmers. The average yield of fine rice is about 2.6 tons/hectare and has increased at a rate of 2.65 tons/hectare per year from 1980 to 1992 (Islam et al, 1996).

Plant cells possess totipotency, i.e., whole plants can be regenerated from single cells by modulating culture conditions (Steward et al, 1958). The mechanisms of totipotency, however, are little understood so far, and are mainly discussed in relation to the concentration and ratio
of phytohormones (Toonen & De Vries, 1996). Plant tissue culture is a practice used to propagate plants under sterile conditions, often to produce clones of a plant. Callus formation can be induced by culture on media containing intermediate levels of an auxin and a cytokinin. Calli are made up of a mass of undifferentiated cells, which are usually rapidly dividing. The genetic material of these cells often changes in culture (‘somaclonal variation’) giving rise to variant plants. This process has been used to produce improved strains of plants e.g. potato plants resistant to certain fungal diseases. Recent advancement in biotechnology, such as transformation, and in situ hybridization enhanced the introgression of new genes from different sources to the cultivated species (Sikder et al, 2006).

The aromatic rice has demand in both domestic and foreign market for attractive flavour, good taste and fine grains. Bangladesh can be benefited by earning foreign exchange by the production and export of aromatic rice. But there are some limitations for the farmer, such as lack of high yielding variety, fine grain quality lack of disease or pest resistant, stress and salt tolerance variety and proper cultural management, to cultivate aromatic rice. The conventional breeding techniques are time consuming and self incompatibility act as barrier for distant hybridization and fertilization. The aromatic variety can be improved (disease and pest resistant variety, stress and salt tolerance variety) through tissue culture techniques viz. somaclonal variation or genetic manipulation like protoplast fusion (hybrid and cybrid) and through gene transfer. Tissue culture of rice may help to get somaclone and their performance can be observed in the field. Chemical and physical mutagenic agents treated organs produce mutant callus that also help to obtain somaclones, disease, pest or insect resistant, stress or salt tolerant mutant line of aromatic rice. Somaclonal variation could be regulated through changing explants, medium especially the phytohormones in medium, culture methods and length of time spent in vitro. The callus obtained from the mature dehusked seed of aromatic rice variety is amenable to multiple shoot formation and could be used for genetic transformation studies (Chawla, 2004).

Therefore, the experiment was undertaken, to find out 1) the potentiality of aromatic rice (Oryza sativa L.) variety for callus induction and plant regeneration from mature dehusked seeds and 2) comparative study of two related rice varieties for callus induction and shoot formation with different concentration of plant growth regulators.

2. Material and Methods

This experiment was conducted in the Plant genetic engineering laboratory of the Department of Genetic Engineering and Biotechnology, Shahjalal University of Science and Technology (SUST), Sylhet, Bangladesh. In this experiment field grown seeds of aromatic rice (Oryza sativa L.) variety were used for callus induction and plant regeneration. The rice (Oryza sativa L.) seeds of BRRI Dhan 50 (Bangla Moti) and BRRI Dhan 34 (Khaskhani) were collected from the Bangladesh Rice Research Institute (BRRI). Seeds were dehusked by hand. Dehusked seeds were then soaked in the 70% v/v ethanol for one minute, followed by washing with autoclaved distilled water for one to two minutes. Seeds were further sterilized by continuous shaking with 50% v/v sodium hypochlorite and 5.25% Clorox for 20 minutes. Surface sterilized seeds were thoroughly washed three to four times with autoclaved distilled water after a regular interval of 5 minutes. The seeds were then placed on the sterilized petriplate having sterile filter papers with the help of forceps to remove excess water.

Surface sterilized seeds of two varieties were inoculated on solid MS (Murashige & Skoog, 1962) basal media supplemented with different cytokinins (Kinetin, BA (6-benzyladenine), BAP (6-benzylaminopurine)) and auxins NAA (1-naphthaleneacetic acid), 2,4-D (2,4-dichlorophenoxyacetic acid) at varying concentrations and combination were prepared for callus induction and plantlet regeneration. The pH of the medium was adjusted to 5.8 and solidified with 0.7% agar.

Surface sterilized seeds of two varieties were inoculated on solid MS (Murashige & Skoog Medium, 1962) supplemented with different concentration of only growth hormone 2,4-D (1.0, 2.0, 3.0 and 4.0 mg/L) and different concentration of 2,4-D in combination with BAP (2.0 mg/L) in a laminar airflow cabinet. In each test tube one seed was planted. After inoculation, the surface sterilized seeds of two rice varieties were transferred and maintained in an environmentally controlled growth room for 3 weeks for callus induction and growth. The cultures were positioned away from continuous light provided by general electric white florescent tubes. Temperature was maintained at 25 ± 3 ºC throughout the growth period. Callus induction frequency for two varieties was recorded 7-9 days after inoculation. Callus quality was recorded at 2-3 weeks after inoculation in two rice varieties for all treatments. Subculture was carried out once in every two weeks with transfer of only the vigorously growing portions of calli. For plant regeneration, the embryogenic part of calli was cut into small pieces by removing non embryogenic part. Calli were then inoculated on regeneration media i.e. MS basal medium supplemented with 3% sucrose and Kinetin (0.0, 1.0, 2.0, 3.0, 4.0 mg/L) while maintaining 1.0 mg/L of NAA and 2.0 mg/L of BA as constant. The pH of media was adjusted to 5.8 ± 2 before autoclaving. The culture was performed at 25 ± 3 ºC under a cycle of 16 hours light/ 8 hours dark for 4 weeks, after which the frequencies of plant regeneration
were calculated, based on the appearance of shoots. The data obtained from above experiments were statistically analyzed by using Stata/SE version 11.1 software (Gould, 1985).

3. Results & Discussion

Callus initiation was started at third to fifth days after transfer of the caryopses to culture tubes and their incubation. The final data on callus induction was recorded after two weeks of inoculation. It was noticed that MS media supplemented with only 3.0 mg/L of 2,4-D produced highest percentage of callus that is 90% and 80% for BRRI Dhan 50 and BRRI Dhan 34 respectively (Table 1, Figure 1 and Figure 2). On the other hand, MS media supplemented with 1.0 mg/L 2, 4-D produced lowest percentage of callus that is 40% and 30% for BRRI Dhan 50 and BRRI Dhan 34 respectively (Table 1).

Figure 1. Callus Initiation of BRRI Dhan 50 (MS+3.0 mg/L 2,4-D)

The recorded data was statistically analyzed by Stata/SE software. The analysis showed that the response of 2,4-D on callus induction on BRRI Dhan 50 (Bangla Moti) and BRRI Dhan 34 (Khaskhani) are significant at 5% level of probability (Table 1). The colours of the callus were yellow to white and the textures of them were friable. The response of callus induction using MS media supplemented with different concentrations of 2,4-D in combination with 2.0 mg/L BAP is also showed similar results. But the callus initiation percentages for both varieties decrease and produced 80% and 70% callus for BRRI Dhan 50 and BRRI Dhan 34 particularly (Table 2, Figure 3 and Figure 4). The statistical analysis of data for this experiment is significant in 5% level of probability (Table 2).

Figure 2. Callus Initiation of BRRI Dhan 34 (MS+3.0 mg/L 2,4-D)

Figure 3. Callus Initiation of BRRI Dhan 50 (MS+3.0 mg/L 2,4-D+2.0 mg/L BAP)

The effects of only 2,4-D on callus induction is more effective than using 2,4-D in combination with other growth regulators. Pandey et al (1994) cultured the
explants of 10 rice genotypes on MS medium with 5 different concentrations of 2,4-D and found that MS medium supplemented with 2.0 mg/L of 2,4-D produced the most desired calli i.e. our expected and good quality calli.

For plantlet regeneration, the produced calli of convenient size were transferred on MS medium supplemented with different combination of growth regulators auxins and cytokinins. The derived calli were cultured on MS medium supplemented with 1.0 mg/L NAA, 2.0 mg/L BA and various concentrations of Kinetin (0.0, 1.0, 2.0, 3.0, 4.0 mg/L). The calli showed highest percentage of shoot regeneration (80%) on MS medium containing 4.0 mg/L Kinetin + 1.0 mg/L NAA + 2.0 mg/L BA for both varieties (Table 3, Figure 5 and Figure 6). MS medium containing 0.0 mg/L Kinetin + 1.0 mg/L NAA + 2.0 mg/L BA showed least response on percentage of shoot regeneration (20%) for both varieties (Table 3). The result is significant in 5% level of probability (Table 3). Plant growth regulators play a central role in plant tissue culture, in which a high auxin/cytokinin ratio usually is used for initiation of the embryogenic callus, while a low ratio is used for the regeneration of plantlets. Although the exact functional mechanism of plant growth regulators in tissue culture remains unclear, it is suggested that they function by mediating the signal transduction cascade that leads to reprogramming of the expression of embryogenic genes (Dudits et al, 1995).

Table 1. Effect of Different Concentration of 2,4-D in MS Medium on Callus Induction from Mature Embryos of BRRI Dhan 50 and BRRI Dhan 34

<table>
<thead>
<tr>
<th>Variety</th>
<th>Treatment (mg/L) 2,4-D</th>
<th>Callus Initiation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRRI Dhan 50</td>
<td>1.0</td>
<td>40</td>
</tr>
<tr>
<td>BRRI Dhan 34</td>
<td>1.0</td>
<td>30</td>
</tr>
<tr>
<td>BRRI Dhan 50</td>
<td>2.0</td>
<td>70</td>
</tr>
<tr>
<td>BRRI Dhan 34</td>
<td>2.0</td>
<td>40</td>
</tr>
<tr>
<td>BRRI Dhan 50</td>
<td>3.0</td>
<td>90</td>
</tr>
<tr>
<td>BRRI Dhan 34</td>
<td>3.0</td>
<td>80</td>
</tr>
<tr>
<td>BRRI Dhan 50</td>
<td>4.0</td>
<td>80</td>
</tr>
<tr>
<td>BRRI Dhan 34</td>
<td>4.0</td>
<td>60</td>
</tr>
</tbody>
</table>

ANOVA (BRRI Dhan 50) Std. Err. =0.0624921  t = 2.24  P>|t| = 0.031
ANOVA (BRRI Dhan 34) Std. Err. =0.06932  t = 1.99  P>|t| = 0.050

Table 2. Effect of Various Concentration of 2, 4-D in Combination with BAP (2.0 mg/L) in MS Medium on Callus Induction from Mature Embryos of BRRI Dhan 50 and BRRI Dhan 34

<table>
<thead>
<tr>
<th>Variety</th>
<th>Treatment (mg/L) 2.0 mg/L BAP + 2,4-D</th>
<th>Callus Initiation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRRI Dhan 50</td>
<td>1.0</td>
<td>40</td>
</tr>
<tr>
<td>BRRI Dhan 34</td>
<td>1.0</td>
<td>30</td>
</tr>
<tr>
<td>BRRI Dhan 50</td>
<td>2.0</td>
<td>70</td>
</tr>
<tr>
<td>BRRI Dhan 34</td>
<td>2.0</td>
<td>40</td>
</tr>
<tr>
<td>BRRI Dhan 50</td>
<td>3.0</td>
<td>80</td>
</tr>
<tr>
<td>BRRI Dhan 34</td>
<td>3.0</td>
<td>70</td>
</tr>
<tr>
<td>BRRI Dhan 50</td>
<td>4.0</td>
<td>80</td>
</tr>
<tr>
<td>BRRI Dhan 34</td>
<td>4.0</td>
<td>70</td>
</tr>
</tbody>
</table>

ANOVA (BRRI Dhan 50) Std. Err. =0.0646041  t = 2.01  P>|t| = 0.051
ANOVA (BRRI Dhan 34) Std. Err. =0.0682488  t = 2.20  P>|t| = 0.034
The present study showed that the MS medium supplemented with 2,4-D alone enables the production of calli from the seeds of BRRI Dhan 50 and BRRI Dhan 34. However, the optimum concentration of 2,4-D varied depending on the explants source and genotype of rice (Raina, 1989).

### Table 3. Effect of Various Concentration of Kinetin in Combination with NAA (1.0 mg/L) and BA (2.0 mg/L) in MS Medium on Plantlet Regeneration from calli of BRRI Dhan 50 and BRRI Dhan 34

<table>
<thead>
<tr>
<th>Variety</th>
<th>Treatment (mg/L)</th>
<th>Callus initiation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRRI Dhan 50</td>
<td>1.0 mg/L NAA +2.0 mg/L BA + Kinetin</td>
<td>20</td>
</tr>
<tr>
<td>BRRI Dhan 34</td>
<td>0.0</td>
<td>20</td>
</tr>
<tr>
<td>BRRI Dhan 50</td>
<td>1.0</td>
<td>40</td>
</tr>
<tr>
<td>BRRI Dhan 34</td>
<td>1.0</td>
<td>20</td>
</tr>
<tr>
<td>BRRI Dhan 50</td>
<td>2.0</td>
<td>40</td>
</tr>
<tr>
<td>BRRI Dhan 34</td>
<td>2.0</td>
<td>40</td>
</tr>
<tr>
<td>BRRI Dhan 50</td>
<td>3.0</td>
<td>40</td>
</tr>
<tr>
<td>BRRI Dhan 34</td>
<td>3.0</td>
<td>40</td>
</tr>
<tr>
<td>BRRI Dhan 50</td>
<td>4.0</td>
<td>80</td>
</tr>
<tr>
<td>BRRI Dhan 34</td>
<td>4.0</td>
<td>80</td>
</tr>
</tbody>
</table>

ANOVA (BRRI Dhan 50)  Std. Err. = 0.0671144  t = 2.09       P>|t| = 0.048
ANOVA (BRRI Dhan 34)  Std. Err. = 0.0660698  t = 2.12          P>|t| = 0.045

### 4. Conclusion

It can be concluded that 2.0 mg/L to 3.0 mg/L 2, 4- D is suitable for callus induction for aromatic rice BRRI Dhan 50 and BRRI Dhan 34. These two varieties showed optimum shoot regeneration on 4.0 mg/L Kinetin in
combination with different auxins. These findings can be
used in future for improvement of these two varieties
through somaclonal variation and in transgenic rice
production.

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