Development of Callus Initiation and Regeneration System of Different Indigenous indica Rice Varieties

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Abstract

The aim of the present study was to develop an efficient protocol for best callus induction and complete plant regeneration for varieties of rice (Oryza sativa) i.e. Pakhi Biroin, Hati Baromashi, Kacha Biroin, Badal Boro, Porichok Amon, Khoiya Boro, Joria Aman and BRRI Dhan53. Pakhi Biroin, Hati Baromashi, Kacha Biroin and BRRI Dhan53, but 75% was the highest percentage of callus for Kacha Biroin. For plantlet regeneration of Pakhi Biroin, Hati Baromashi and Kacha Biroin, MS media with 0.5 mg/L NAA (1-Napthaleneacetic acid), 0.5 mg/L Kinetin and various concentrations of BA (6-benzyladenine) (1.0, 2.0, 3.0, 4.0 mg/L) were employed. On the other hand, MS media with 1.0 mg/L NAA, 1.0 mg/L Kinetin and various concentrations of BA (1.0, 2.0, 3.0, 4.0 mg/L) were used for BRRI Dhan53 regeneration. Different result was recorded for different varieties at various hormone concentrations.

Keywords: Mature Embryos, Genotypic Variability, Embryogenic Callus, Plant Regeneration, Indica Rice

1. Introduction

Rice (Oryza sativa L) is one of the most versatile and important cereal crops of Poaceae family cultivated for more than 10,000 years (Sasaki, 2001). Currently this crop supports more than 50% of the world population (Christou, 1997). In Asia it covers half of the arable land used for agriculture in many countries (Cantrell & Hettel, 2004). But the rate of growth in rice production has slowed down. A considerable improvement has already been made by exploiting the natural variation through conventional breeding. Recent advancement in biotechnology, such as transformation and In situ and In vitro hybridization has enhanced the introgression of new genes from different sources to the cultivated species (Sikder et al, 2006).

Efficient plant regeneration from cultured cells and tissues requires successful application of biotechnology in crop improvement. Therefore, the success of cell and tissue culture research depends upon reliable callus culture and plant regeneration procedures. The frequencies of callus induction and plant regeneration in tissue culture of rice are influenced by many factors: culture medium composition, explants source, genotype and environment (Torbert et al, 1998). Among them the genotype and nutrient composition are regarded to be the major sources of variation in in vitro culture (Khamna & Raina, 1998).

Pakhi Biroin, Hati Baromashi, Kacha Biroin, Badal Boro, Porichok Aman, Khoiya Boro and Joria Aman are pure varieties of Oryza sativa cultured mainly in Sylhet and Sunamgonj, district of Bangladesh. BRRI Dhan53 are hybrid rice variety from BRRI. It has moderate salt tolerance.

Objective of the present research was to study the
potentiality of the varieties in tissue culture as well as to
determine the most suitable concentration and combina-
tion of growth regulators for excellent callus induction and
regeneration which is of great impotence for gene
transformation to create high yielding varieties.

2. Material and Method

This research work was conducted at the plant genetic
engineering lab of Department of Genetic Engineering and
Biotechnology, Shahjalal University of Science &
Technology (SUST), Sylhet, Bangladesh.

Mature Dehusked rice seeds were taken as source of
explant. Dehusked seeds were first washed in distilled
water mixed with Tween 20 (one drop/30 ml of water) for
ten minutes, steeped in 70% (v/v) ethanol for two minutes
with gentle agitation followed by rinsing three times with
sterile distilled water. The seeds were surface sterilized in
0.1% (w/v) HgCl₂ for 15 minutes with gentle agitation and
rinsed five times with sterile distilled water. Surface
sterilized seeds were thoroughly washed six to ten times
with autoclaved sterile distilled water. The seeds were
finally placed on the sterilized petriplate having sterile
filter papers with the help of forceps to remove excess
water.

After removing the water from the seeds surface, these
seeds were inoculated into culture tubes containing MS
(Murashige & Skoog, 1962) basal media supplemented
with different concentrations of 2, 4-dichlorophenoxyac-
etic acid (2,4-D) culture tubes were transferred and
maintained in an environmentally controlled growth room
for 4 weeks for callus and subcultured in the same medium
for 3 weeks. Regeneration efficacy was observ-ed with MS
media supplemented with different combina- tion and
concentration of NAA, Kn (Kinetin) and BA. The pH of
the media was adjusted to 5-8 with acid and alkali. The
media was autoclaved at 15 pound square inch (psi) for 20
minutes at 121 °C. Inoculation was carried out under a
sterilized environment in a laminar air flow cabinet. All
cultures were incubated at 25±1 °C with a photoperiod of
12 hours at 2000 lux light intensity of cool white
fluorescent light.

The response of all varieties of rice was determined in
terms of callus induction, callus growth and regeneration
frequencies. The data of callus growth was subjected to
ANOVA (Analysis of Variance) testing and Standard error
(SE) for 5% of means was calculated by using standard
statistical MSTAT-C software.

3. Result

Results are shows in Figure 1-4 and Tables 1-3. Callus
induction of Dehusked rice seeds of eight varieties i.e.

Pakhi Biroin, Hati Bromashi, Kacha Biroin, Badal Boro,
Porichok Aman, Khoiya Boro, Joria Aman and BRRI
Dhan53, was carried out on MS medium forti-fied with
different concentrations of 2,4-D (for landraces varieties
1.5 mg/l, 2 mg/l, 2.5 mg/l, 3 mg/l and for BRRI Dhan53,
1mg/l, 2mg/l and 3mg/l) (Figure 1). Badal Boro, Porichok
Amon, Khoiya Boro and Joria Aman did not show any
callus after 4 weeks. Calli were developed within 10 days
of inoculation of Pakhi Biroin, Hati Bromashi, Kacha
Biroin and BRRI Dhan53. Both embryo-genic and non-
embryogenic calli were initiated. After four weeks, large
calli were formed from the scutellum.

The frequency of callus initiation were calculated as Pakhi
Biroin 75%, Hati Bromashi 100%, Kacha Biroin 43.75%
and BRRI Dhan53 79.16%. The first subculture was, then, carried out and calli were removed from seed endosperms and transferred onto fresh media (Figure 2).

The response of explants to different concentrations of 2, 4-D in terms of callus induction, degree of callusing including the callus growth rate is shown in Table 1 and Table 2.

Upon transferring the calli to regeneration media, green spots were visible on the calli within 7-10 (Figure 3) days and after 4-5 weeks fully regenerated roots and shoots were observed (Figure 4). At every treatment, regeneration of roots, shoots, and the frequency were noted as shown in Table 3.

4. Discussion

Mature Dehusked rice seeds were used as an explants because calli initiated from scutellum of mature seeds of all rice varieties have high embryogenic potential (Ge et al, 2006 and Khaleda & Al-Forkan, 2006) and was excellent material for transformation of rice by Agrobacterium (Rashid et al, 1996; Toki, 1997; Rashid et al, 2001; 2003; Cho et al, 2004 and Ge et al, 2006). In this study, by offering suitable growth regulators a great number of embryogenic calli from mature seeds of four indica varieties i.e. Pakhi Biroin, Hati Baromashi, Kacha Biroin and BRRI Dhan53, were successfully induced.

MS medium was used as basal media as MS and N6 (Cho’s N6 basal media) were the most commonly used basal media (Pandey et al, 1994; Rashid et al, 1996 and Toki, 1997). Mostly 2,4-D has been used as the only growth regulator in callus induction media (Katiyar et al, 1999 and Zhenyu et al, 1999). The present study showed that the MS medium supplemented with 2,4-D alone enables the production of calli from the seeds of Pakhi

Table 1. Effect of Callus Induction of Pakhi Biroin, Hati Bromashi and Kacha Biroin Varieties on MS Supplemented with Different Concentrations of 2, 4-D

<table>
<thead>
<tr>
<th>Concentration of 2,4-D (mg/l)</th>
<th>Varieties</th>
<th>Frequency of Callus Initiation (%)</th>
<th>Degree of Callus</th>
<th>Callus Growth Rate on 1st Subculture in Size ± SE (mm/week)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.5</td>
<td>Pakhi Biroin</td>
<td>50</td>
<td>+</td>
<td>1.61±0.11</td>
</tr>
<tr>
<td></td>
<td>Hati Bromashi</td>
<td>100</td>
<td>+</td>
<td>1.61±0.35</td>
</tr>
<tr>
<td></td>
<td>Kacha Biroin</td>
<td>0</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>Pakhi Biroin</td>
<td>75</td>
<td>+++</td>
<td>1.97±0.23</td>
</tr>
<tr>
<td></td>
<td>Hati Bromashi</td>
<td>100</td>
<td>+++</td>
<td>2.13±0.16</td>
</tr>
<tr>
<td></td>
<td>Kacha Biroin</td>
<td>62.5</td>
<td>+++</td>
<td>1.56±0.13</td>
</tr>
<tr>
<td>2.5</td>
<td>Pakhi Biroin</td>
<td>100</td>
<td>+++</td>
<td>2.18±0.13</td>
</tr>
<tr>
<td></td>
<td>Hati Bromashi</td>
<td>100</td>
<td>+++</td>
<td>1.63±0.62</td>
</tr>
<tr>
<td></td>
<td>Kacha Biroin</td>
<td>62.5</td>
<td>++</td>
<td>1.50±0.83</td>
</tr>
<tr>
<td>3</td>
<td>Pakhi Biroin</td>
<td>75</td>
<td>++</td>
<td>1.83±0.35</td>
</tr>
<tr>
<td></td>
<td>Hati Bromashi</td>
<td>100</td>
<td>+</td>
<td>1.58±0.18</td>
</tr>
<tr>
<td></td>
<td>Kacha Biroin</td>
<td>52</td>
<td>+</td>
<td>1.41±0.14</td>
</tr>
</tbody>
</table>

Size of Callus = (width + length)/2

Table 2. Effect of Callus Induction of BRRI dhan53 Variety on MS Supplemented with Different Concentrations of 2,4-D

<table>
<thead>
<tr>
<th>Concentration of 2,4-D (mg/l)</th>
<th>Frequency of Callus Initiation (%)</th>
<th>Degree of Callus</th>
<th>Callus Growth Rate on 1st Subculture in Size ± SE (mm/week)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>66.66</td>
<td>++</td>
<td>1.90±0.28</td>
</tr>
<tr>
<td>2</td>
<td>100</td>
<td>+++</td>
<td>2.36±0.12</td>
</tr>
<tr>
<td>3</td>
<td>100</td>
<td>+++</td>
<td>2.14±0.26</td>
</tr>
<tr>
<td>4</td>
<td>50</td>
<td>+</td>
<td>2.13±0.28</td>
</tr>
</tbody>
</table>

Size of Callus = (width + length)/2

The response of explants to different concentrations of 2, 4-D in terms of callus induction, degree of callusing including the callus growth rate is shown in Table 1 and Table 2.
Biroin, Hati Baromashi, Kacha Biroin & BRRI Dhan53. Similar results were reported by Ge et al (2006) for the varieties of Zhenshan 97, Minghui 63, and 93-11. These findings are as per the reports of several researchers (Jubair et al, 2008; Summart et al, 2008 and Tariq et al, 2008).

Table 3. Regeneration Frequency of Pakhi Biroin, Hati Bromashi, Kacha Biroin, and BRRI dhan53 Varieties on MS Supplemented with Different Hormonal Combinations and Concentrations

<table>
<thead>
<tr>
<th>Pakhi Biroin, Hati Bromashi and Kacha Biroin</th>
<th>BRRI dhan53</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration of Growth Regulator (BA+NAA+Kn) (mg/l)</td>
<td>Varieties</td>
</tr>
<tr>
<td>1 + 0.5 + 0.5</td>
<td>Pakhi Biroin</td>
</tr>
<tr>
<td></td>
<td>Hati Bromashi</td>
</tr>
<tr>
<td></td>
<td>Kacha Biroin</td>
</tr>
<tr>
<td>2 + 0.5 + 0.5</td>
<td>Pakhi Biroin</td>
</tr>
<tr>
<td></td>
<td>Hati Bromashi</td>
</tr>
<tr>
<td></td>
<td>Kacha Biroin</td>
</tr>
<tr>
<td>3 + 0.5 + 0.5</td>
<td>Pakhi Biroin</td>
</tr>
<tr>
<td></td>
<td>Hati Bromashi</td>
</tr>
<tr>
<td></td>
<td>Kacha Biroin</td>
</tr>
<tr>
<td>4 + 0.5 + 0.5</td>
<td>Pakhi Biroin</td>
</tr>
<tr>
<td></td>
<td>Hati Bromashi</td>
</tr>
<tr>
<td></td>
<td>Kacha Biroin</td>
</tr>
</tbody>
</table>

We have found that the Hati Bromashi produced 100% callus, BRRI Dhan53 produced 79.16% callus and Pakhi Biroin produced 75% callus. Similar result was also reported by Kabir et al (2008) for BRRI Dhan32 variety. They also reported that BRRI Dhan29 produced 69.45% callus which is relevant to Kacha Biroin variety produced 43.75% callus. Saharan et al (2004) observed callus induction frequency was 60.5% in cv. HKR-46, whereas in HKR-126 it was 83.5 per cent. This finding was also similar with our results.

Figure 3. Plantlet Regenerate Initiation A) Pakhi Biroin on MS Medium with 2 mg/l BA + 0.5 mg/l NAA + 0.5 mg/l Kn B) Hati Bromashi on MS Medium with 3 mg/l BA + 0.5 mg/l NAA + 0.5 mg/l Kn C) Kacha Biroin on MS Medium with 3 mg/l BA + 0.5 mg/l NAA + 0.5 mg/l Kn D) BRRI dhan53 on MS Medium with 2 mg/l BA + 1 mg/l NAA + 1 mg/l Kn

We have found that the Hati Bromashi produced 100% callus, BRRI Dhan53 produced 79.16% callus and Pakhi Biroin produced 75% callus. Similar result was also reported by Kabir et al (2008) for BRRI Dhan32 variety. They also reported that BRRI Dhan29 produced 69.45% callus which is relevant to Kacha Biroin variety produced 43.75% callus. Saharan et al (2004) observed callus induction frequency was 60.5% in cv. HKR-46, whereas in HKR-126 it was 83.5 per cent. This finding was also similar with our results.

Figure 4. Plantlet Regenerated A) Pakhi Biroin on MS Medium with 2 mg/l BA + 0.5 mg/l NAA + 0.5 mg/l Kn B) Hati Bromashi on MS Medium with 3 mg/l BA + 0.5 mg/l NAA + 0.5 mg/l Kn C) Kacha Biroin on MS Medium with 3 mg/l BA + 0.5 mg/l NAA + 0.5 mg/l Kn D) BRRI dhan53 on MS Medium with 2 mg/l BA + 1 mg/l NAA + 1 mg/l Kn

Three varieties i.e. Hati Bromashi, Kacha Biroin, BRRI
Dhan53 showed better callus induction response on MS medium supplemented with 2mg/l 2,4-D similar to Pandey et al (1994). They found that MS medium, supplemented with 2.0 mg/l 2, 4-D, produced the most desired calli for 10 rice genotypes. Other researchers (Islam et al, 2005 and Khalequzzaman et al, 2005) found better callussing frequency at a concentration of 2.5 mg/L 2,4-D. It coincides with our findings as we found that Pakhi Biroin shows better callussing frequency at a concentration of 2.5 mg/l 2,4-D and embryogenic calli obtained from mature seed explant have high regeneration capacity (Khalequzzaman et al, 2005). Combinations of auxin and cytokinin along with the effect of basal salts played an important role for plant regeneration (Prodhan et al, 2001 and Lee et al, 2002). In this study we found Pakhibiroin shows best regeneration frequency at the similar to Jubair et al (2008) study, in which they found best regeneration frequency for local variety Topa at MS + NAA 1 mg/l + BA 2 mg/l + Kinetin 3 mg/l which are similar to Jubair et al (2008) study, in which they found best regeneration frequency for local variety Topa at MS + NAA 0.5 mg/l + BA 3 mg/l + Kinetin 0.5 mg/l.

5. Conclusion

The investigation was conducted for establishment of callus initiation and regeneration system for these local rice varieties using mature seed embryo as explants. This highly efficient, reproducible system will be used to develop genetic transformation techniques for these important rice cultivars.

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References


