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Callus Induction and Regeneration of Local Rice (*Oryza sativa* L.) Variety Topa

[1]T.A. Jubair, [2]U. Salma, [2]N. Haque, [1]F. Akter, [3]I.J. Mukti, [3]A.K.M.F. Haque and [1]M.R. Ali
[1]Biotechnology and Genetic Engineering Discipline, Khulna University, Khulna-9208, Bangladesh
[2]Department of RDDR, Modern Herbal Group, Dhaka-1217, Bangladesh
[3]Biotechnology and Genetic Engineering Discipline, University of Development Alternative, Dhaka-1207, Bangladesh

Abstract: The investigation was done to find out the tissue culture potentiality of the local rice (*Oryza sativa* L.) variety Topa, cultivated mainly in Kishoregonj, the district of Bangladesh. In this present study, callus induction, callus growth rate and indirect regeneration potentiality of the variety was examined. One hundred percent callus induction efficacy was noted when dehusked mature seeds were cultured on MS media supplemented with 2.0 mg L^{-1} 2, 4-D. After first successive subculture the highest callus growth rate (0.0791±0.017 g $week^{-1}$) was observed under the best callus induction media. The highest regeneration response was recorded at treatment of 3.0 mg L^{-1} BA+0.5 mg L^{-1} NAA+0.5 mg L^{-1} Kn, which regenerated 80% shoot with an average of 3 shoots per explant.

Key words: Explant, dehusked, regeneration, callus, somaclonal variation

INTRODUCTION

Rice (*Oryza sativa* L.) belongs to the family Gramineae is the most important food crop in the world and feeds over half of the global population (Sasaki, 2005). In Asia it covers half of the arable land used for agriculture in many countries (Cantrell and Hettel, 2004). During the past few decades techniques of tissue culture, like anther culture (Faruque *et al*., 1998; Asaduzzaman *et al*., 2003), protoplast culture (Li and Murai, 1990), leaf culture (Boissot *et al*., 1990), root culture (John and Parathapasenan, 1999) and dehusked grain culture (Ella and Zapata, 1991) are being employed in rice to exploit somaclonal variation for creation of novel rice varieties (Ram and Sing, 1998). About 84% of the production growth has been attributed to the use of modern technologies (Tariq *et al*., 2008).

Topa is a pure variety of *Oryza sativa* cultured mainly in Kishoregonj, the district of Bangladesh. The fine grain quality, fabulous look and delicious smell after boiling are the specialty of this variety. But the yield of this variety comparing with other hybrid variety is very low which has made it a threatened one. Positive genetic manipulation through biotechnological approaches can improve the existing cultivar into high yielding variety as well as save the precious variety which have been reported earlier for another rice cultivar (Yang *et al*., 1999; Kumar *et al*., 2005). But production of callus and its subsequent regeneration are the prime steps in crop plant to be manipulated by biotechnological means (Saharan *et al*., 2004).

However, the use of tissue culture in rice improvement is limited, since the regeneration can be obtained only in limited number of genotypes (Taguchi-Shiobara *et al*., 1997). Objective of the present research was to study the potentiality of the variety in tissue culture as well as to determine the most suitable concentration and combination of growth regulators for excellent callus induction and regeneration which is of great importance for gene transformation to create high yielding variety.

MATERIALS AND METHODS

Dehusked seeds of mature grain from the rice (*Oryza sativa*) cultivar Topa were used for callus induction. This research work was carried out at Plant Biotechnology Laboratory, Biotechnology and Genetic Engineering Discipline, Khulna University, Khulna, Bangladesh.

First the seeds were kept in the sun light for 30 min and cooled for 3-4 h at normal temperature. Then seeds were dehusked manually. The dehusked seeds were then washed with tap water and then steeped in 70% ethanol

Corresponding Author: Tamim Al Jubair, Biotechnology and Genetic Engineering Discipline, Khulna University, Khulna-9208, Bangladesh Tel: 880-411720171-3

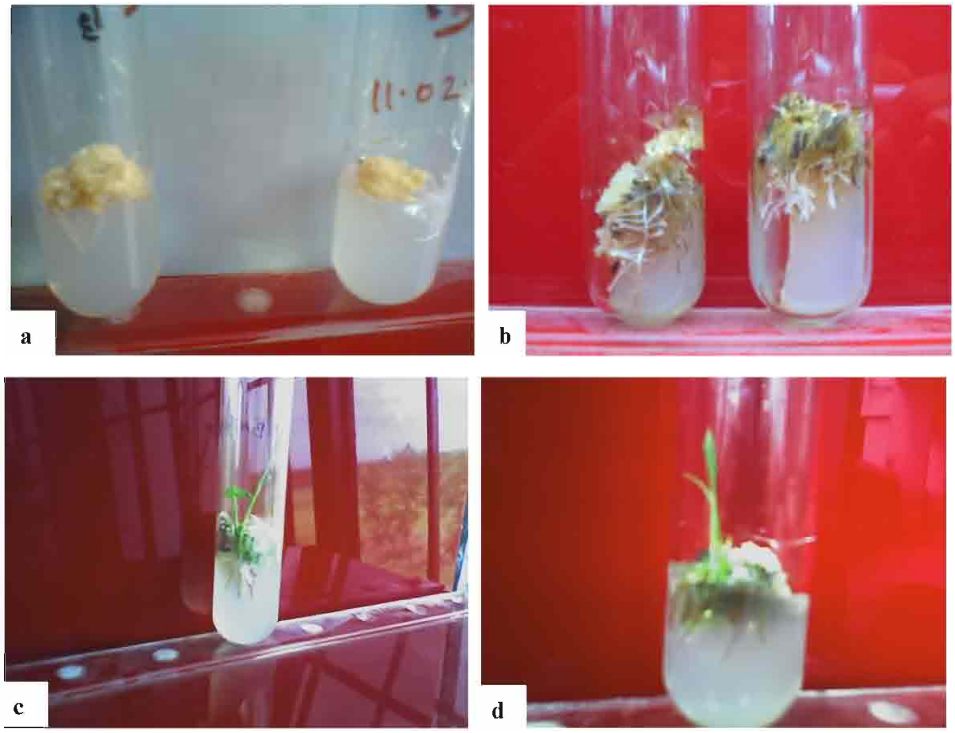

Fig. 1: Callus induction and regeneration of the local rice cultivar Topa. (a): Callus developed on MS medium supplemented with 2 mg L^{-1} 2, 4-D (left test tube) and 1 mg L^{-1} 2, 4-D (right test tube). (b): Root regenerated on MS medium supplemented with 2.05 mg L^{-1} NAA+1.0 mg L^{-1} Kn (right test tube) and 3.0 mg L^{-1} NAA+ 1.0 mg L^{-1} Kn (left test tube). (c and d): Plantlet regenerated on MS medium supplemented with 3.0 mg L^{-1} BA+ 0.5 mg L^{-1} NAA +0.5 mg L^{-1} Kn

for 1 min with gentle agitation followed by then washed three times with autoclaved double distilled water. After surface sterilization the dehusked seeds were kept on autoclaved filter paper in a Petri dish to remove the excess water. After removing the water from the seeds surface, these seeds were inoculated into culture tubes containing MS (Murashige and Skoog, 1962) basal media supplemented with different concentrations of 2, 4-dichlorophenoxyacetic acid (2, 4-D) for callus induction (Fig. 1).

Regeneration efficacy was observed with MS media supplemented with different combination and concentration of NAA, Kn (kinetin) and BA (Fig. 1). The pH of the media was adjusted to 5.8 with acid and alkali. The media was autoclaved at a temperature of 121°C and pressure of 15 lbs psi for 20 min. Inoculation was carried out under a sterilized environment in a laminar air flow cabinet. After inoculation, culture tubes for callus induction were incubated in dark for 4 weeks. The sub-cultured tubes of the callus were incubated in dark for first 1 week and rest of the 3 weeks in light for increasing the amount of callus and measuring the rate of callus growth.

For regeneration the cultured tubes were incubated on shelves of culture room. All cultures were incubated at 25±1°C with a photoperiod of 12 h at 2000 lux light intensity of cool white fluorescent light.

Weekly visual observation of culture was made and frequency of culture proliferation and regeneration were recorded. All experiments were repeated twice with at least 20 cultures per treatment and data were taken after 4-5 weeks of culture. For measuring growth rate of callus, the initial weights of the fresh callus was taken at the time of inoculation and the final weights of the fresh callus were taken after 4 weeks.

RESULTS AND DISCUSSION

Experiment for callus induction and regeneration was conducted on local rice cultivar Topa. Callus was invariably developed from dehusked seed under different concentration of 2, 4-D (1.0, 2.0, 3.0, 4.0 and 5.0 mg L^{-1}) and was visible within 10-12 days. It was observed that four treatments of 2, 4-D (1.0, 3.0, 4.0, and 5.0 mg L^{-1}) induced 80% callus, while treatment with 2.0 mg L^{-1} 2, 4-D induced 100% callus.

Table 1: Effects of different concentrations of 2, 4-D on callus induction and callus development of local variety Topa

Concentration of 2,4-D (mg L^{-1})	Percentage of callus induction	Degree of callus	Callus growth rate on first subculture (g $week^{-1}$)
1.0	80	+++	0.079±0.0174
2.0	100	+++	0.085±0.0016
3.0	80	++	0.048±0.0034
4.0	80	++	0.046±0.0040
5.0	80	++	0.056±0.0032

++ = good, +++ = very good

Table 2: Regeneration frequency and average number of shoots per explant of rice cultivar Topa on MS supplemented with different hormonal combination and concentration

Growth regulators	(Concentration mg L^{-1})	Regeneration frequency (%)	Average No. of shoots/explant
Kn+NAA	1.0+0.5	10	1±0
	1.0+1.0	10	3±0
	1.0+2.0	0	0±0
	1.0+3.0	0	0±0
BA+NAA	0.5+0.5	10	1±0
	1.0+0.5	10	1±0
	2.0+0.5	10	1±0
	3.0+0.5	20	2±0
BA+Kn+NAA	0.5+0.5+0.5	10	1±0
	1.0+0.5+0.5	30	1.6±0.33
	2.0+ 0.5+0.5	50	1.7±0.25
	3.0+0.5+0.5	80	3.1±0.13

The response of the explants to different concentration of 2, 4-D in terms of callus induction, degree of callusing including the callus growth rate is shown in Table 1. Callus can be induced and grown on both MS and N_6 media (Rashid *et al*., 2004) and the difference in the composition of culture medium can result in variation in callus induction (Torbet *et al*., 1998). MS media was found to be more effective in callus induction among the rice cultivars (Niroula *et al*., 2005).

Previous studies also showed that callus can be induced in rice using 2,4-D singly (Katiyar *et al*., 1999). Therefore, in the present study this growth regulator was used singly at different concentration in MS media.

Upon transferring the calli to regeneration media, green spots were visible on the calli within 7-10 days and after 4-5 weeks fully regenerated roots and shoots were observed. At every treatment, regeneration of roots was noted first and then the shoots. In case of Kn and NAA combination, two treatments regenerated only roots. The combination of 3.0 mg L^{-1} BA, 0.5 mg L^{-1} Kn and 0.5 mg L^{-1} NAA supplemented with MS showed the most efficient regeneration with 80% efficacy and an average of 3 shoots per callus (Table 2).

Dehusked rice seed culture is a valuable technique to exploit somaclonal variation. But its application is limited by many factors e.g., plant genotype (Li, 1991), hormonal composition of medium (Jain, 1997).

CONCLUSION

This present study was done to find out the tissue culture potentiality of the local rice cultivar Topa as well as to determine the most suitable media composition for callus formation and regeneration which will help to Improve the variety into high yielding one by genetic manipulation through any innovative approach.

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