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In vitro Plant Regeneration Through Anther Culture of Five Rice Varieties

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Abstract: Anthers of five rice varieties viz. BR-5, BR-31, BR-34, BR-37 and BR-38 were cultured for callus induction and plant regeneration. Anthers were cultured on N6, Z2 and R2 media containing the same hormonal combination 2.5mg/l NAA, 0.5mg/l Kn and 0.5mg/l 2.4-D and incubated at 25±1°C in dark for callus induction. All the varieties in Z2 medium, two varieties in N6 medium and only one variety in R_2 medium produced callus. Out of all responding varieties BR-38 produced highest percentage of callus. Calli induced in different induction media were transferred to MS medium supplemented with 2.0 mg/l BAP + 0.5 mg/l Kn + 1.0 mg/l NAA and 1.0 mg/l Kn + 1.0 mg/l NAA and incubated at 25 ± 1°C in light for plantlet regeneration. Green plantlets appeared within 15-30 days of culture and highest number of regenerated green (33.32%) and albino (11.27%) plantlets were produced in BR-37.

Key words: Rice, Anther culture, Regeneration, and *In vitro*

Introduction

Haploid plant production has been reported in more than 200 species (Dunwell, 1986). Today, androgenic haploids have been developed in economically important plants including vegetable and cereals crops (Veillenx 1994; Cao ***et al.***, 1995). The production of haploid plants from anther culture technique offers a rapid achievement of homozygous lines for early release of new crop varieties. Besides, it allows the use of haploid cells or protoplasts for the induction and selection of recessive mutants.

In rice callus induction and subsequent plant regeneration from cultured anthers have rather been difficult. The factors affecting callus formation and plant regeneration from ***in vitro*** cultured anthers of rice have been studied by Niizeki and Oono (1968), Iyer and Raina (1972), Chen (1977) and Datta ***et al.*** (1990). The recalcitrance of certain rice cultivars to callusing may happen to genotypic differences (Guha-Mukerjee, 1973).

Under present initiative attempts have taken towards standardizing the condition necessary to stimulate callus induction and green plantlet regeneration from cultured anthers of different Bangladeshi rice varieties.

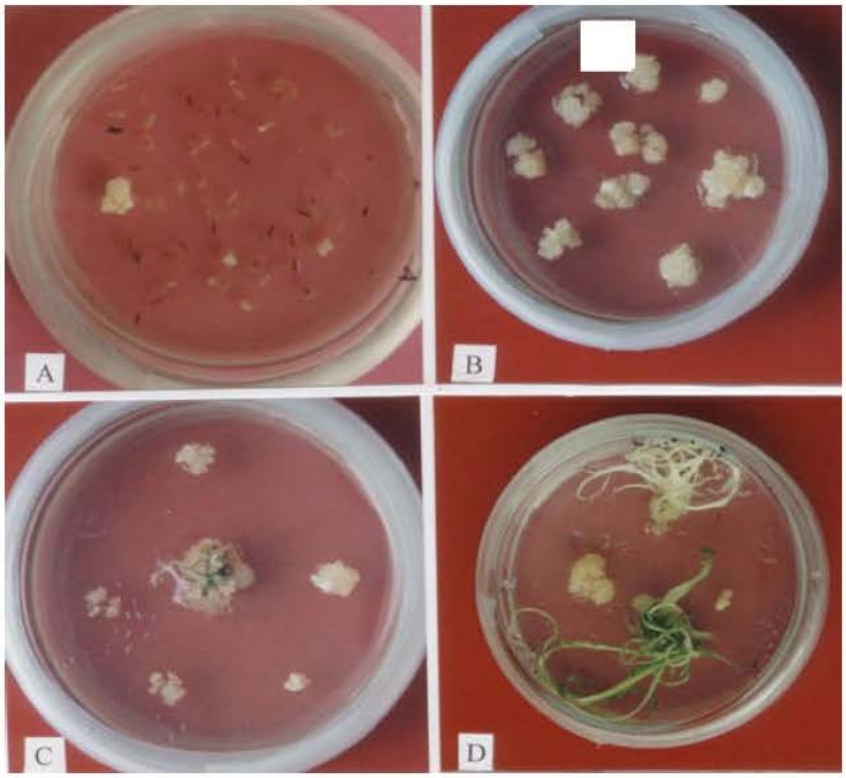
Materials and Methods

Anther donor plants of five rice varieties commonly cultivated in the country viz. BR-5, BR-31, BR-34, BR-37 and BR-38 were taken from the field of Regional Rice Research Institute, Rajshahi during the main crop season (June and November, 1999). Boots from primary tillers were collected when the anthers in panicle contained pollen in uninucleate stage (observed by 1:3 acetocarmine staining). Boots were wrapped in moist tissue paper with aluminum foil and were cold pretreated for 1-6 days at 6-10°C. Sterilization of panicles was carried out by dipping intact boots in 70% alcohol for 2 min just before the inoculation of anthers. The outer covering of the panicle was removed with a sharp scalpel and spikelets were cut at the base to excise the anthers. On an overage 50 anthers were inoculated in 7.5 ml callus induction medium. Three callus induction media viz. N6, Z2 and R2 supplemented with NAA, Kn and 2,4-D (Table 1) in 6 cm, petridishes were used for this purpose.

The cultures were incubated at 25±1°C for 4-5 weeks in the dark for callus induction. Anther derived calli subcultured on regeneration medium were exposed to 16 h photoperiod for shoot regeneration. The pH of all media was adjusted at 5.8 and all the media were solidified with Difco-bacto-agar. Anther response and green plantlet regeneration were observed in each experiment by counting the No. of calli/anther and No. of green plants/callus respectively.

Table 1: Frequency of callus induction from anthers of different rice varieties in three induction media (supplemented by 2.5 mg/l NAA + 0.5 mg/l Kn + 0.5 mg/l 2,4-D

Media	Genotype	Callus formation (%)	Embryogenic response (%)
N6	BR-5	15.25	28.79
	BR-31	-	-
	BR-34	-	-
	BR-37	-	-
	BR-38	22.15	35.85
Z2	BR-5	35.45	83.30
	BR-31	27.79	77.29
	BR-34	36.35	79.89
	BR-37	50.20	91.00
	BR-38	53.23	93.39
R2	BR-5	-	-
	BR-31	-	-
	BR-34	-	-
	BR-37	-	-
	BR-38	16.38	28.57



Figs. A-D: Plant regeneration from anther culture of rice.
A-B: Anther derived callus, C-D: Plantlets from anther derived callus.

Results and Discussion

Callus induction

Five varieties of rice viz. BR-5, BR-31, BR-34, BR-37 and BR-38 were tested for callus induction in three basal media (N6, Z2 and R2) supplemented with 2.5 mg/l NAA, 0.5 mg/l Kn and 0.5 mg/l 2,4-D. Callus was induced in all the varieties in Z2 medium; however, the induction frequency was varied. In N6 medium BR-5 and BR-38 in R2 medium and only BR-38 produced lower percentage of callus. Highest percentage (53.23%) of callus and highest embryogenic structures (93.39) was observed in BR-38 with Z2 medium (Table 1). Out of the three media tested Z2 was found to be most effective medium than N6 (Chu *et al*., 1975) and R2. Nilufer *et al*. (1991) showed that callus induction efficiency in Z2 was higher than that of either B5 or Z2, respectively. They suggested that Z2 and Z3 media could be used for efficient callus induction of indicia varieties of rice. It was also observed that only BR-38 induced calli in all three basal media used for this experiment. The result indicated that

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

Treatment (mg/l)	Varieties	% of green plants	% of albino plants
MS+BAP2.0mg/l +Kn0.5mg/l+NAA1.0mg/l	BR-5	25.44	8.72
	BR-31	10.20	4.15
	BR-34	11.18	5.30
	BR-37	33.32	11.27
	BR-38	28.25	10.25
MS+Kn 1.0mg/l + NAA1.0mg/l	BR-5	7.15	3.72
	BR-31	8.72	6.38
	BR-34	25.86	11.72
	BR-37	23.32	9.27
	BR-38	30.15	10.12

different varieties hold different degrees of potentiality of anther induction in different media. The genotype of the pollen plant has the greatest influence on the frequency of pollen callus formation (Chu, 1982).

Plantlet regeneration

For plantlet regeneration calli were transferred to regeneration MS media (Murasinge and Skoog,1962) supplemented with 2 mg/l BAP + 0.5 mg/l Kn + 1.0 mg/l NAA and 1.0 mg/l NAA + 1.0 mg/l Kn. Among five genotypes upon transfer to MS medium with 2.0 mg/l BAP + 0.5 mg/l Kn 210 + 1.0 mg/l NAA highest percentage (33.32) of green plants and albino plants (11.27%) were produced in BR-37 followed by 28.25% green and 10.25% albino plants in BR-38 (Table 2). In MS + 1.0 mg/l Kn 210 + 1.0 mg/l NAA highest percentage of green (30.15) and of albino plants (10.12%) in BR-38 followed by 23.32% green and 9.27% albino plants in BR-37. Oono (1975) reported the differentiation of primarily green plants from micro spore derived callus of the japonica variety Minehikari. In regeneration medium some calli lost their ability to produce plants and died while the others differentiated into green and albino plants. Occurrence of albino pollen plants seems to be a common phenomenon in pollen plants of Gramineae. The recover of primarily albino plants from micro spore derived calli has been a formidable obstacle to the utilization of rice anther culture. Results from several laboratories have indicated that the proportion of regenerated albino plants were genetically controlled (Oono, 1975; Chen and Lin, 1976). This study indicates that ability of callus formation from anthers of rice varieties are largely dependent on culture medium in addition choice of variety would be the critical factor to ensure the recovery of a high frequency of green plants.

In rice anther culture, the frequency of albino plants has been high. As long as we have to culture the pollen to obtain homozygous diploid plants, besides genotypes we must seek ways to improve the cultural conditions and the media to resolve the albino problem.

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