SHORT COMMUNICATION

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Effect of genotype on anther culture response in indica rice hybrids of maintainer lines

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ABSTRACT

The present study was carried out to evaluate the response of F1 hybrids of maintainer x maintainer crosses of rice anthers for high frequency callus induction in N6 medium and shoot and green plant regeneration in MS medium. The effect of genotype was significant ($p<0.001$) for callus induction, shoot and green plant regeneration. Out of 4 inter-varietal crosses evaluated, the cross CRMS31B x CRMS24B was highly responsive for callus induction (37.83 %) as well as calli responsive for shoot regeneration (41.09 %) and green plant regeneration (15.00 %) whereas the cross CRMS32B x CRMS28B was found to be poor in callus induction (18.36 %), callus responsive for shoot regeneration (20.59 %) and also in green plant regeneration (11.58 %). The results suggest that genotypes play a critical role in the anther culture response in indica rice.

Key words: *indica rice, anther culture, callus induction, maintainer line*

Rice anther culture is an important biotechnogical method of creating homozygous breeding lines from heterozygous breeding lines/F_1 hybrids and compressing the breeding cycle in rice. Traits like grain yield, duration, resistance to several biotic and abiotic stresses can be improved through anther culture (Roy and Mandal, 2005). Presently improved rice varieties and doubled haploid (DH) lines derived from anther culture are widely grown in China, India, Taiwan, South Korea, Japan and several other countries (Gupta, 1999; Niizeki, 1997). Novel androclones of rice developed through anther culture by Chen *et al* (2001) had been shown to have higher quality and more yield. Moreover, haploids and doubled haploids provide useful tools in plant breeding of agriculturally important crops.

In case of rice, haploids are first produced through anther culture by Niizeki and Oono (1968) and closely followed by Nishi and Mitsuoka (1969) in Japan, and Guha *et al*. (1970). In general, indica cultivars of rice exhibits poor androgenic response as compared to japonica and javanica cultivars (Raina, 1997). There are various factors associated with anther culturability; one of the important factor is culture medium, which strongly influence the anther culture response (Raina and Zapata, 1997). Besides, different studies have shown that callus induction and anther culture response to plant regeneration were highly dependent on the genotype of donor plants (Lee *et al*. 2004) and the medium used for the culture (Bishnoi *et al*. 2000). In this study, anthers of F_1 plants from four different inter-varietal/inter-maintainer crosses (of rice hybrids) were used to evaluate the culture efficiency of genotypes in different media and plant growth regulators to develop improved maintainers of rice hybrids.

Four inter-varietal crosses, CRMS31B x CRMS24B, CRMS32B x CRMS24B, CRMS32B x CRMS25B and CRMS32B x CRMS28B were taken for this study. The seeds of these crosses were grown on well puddled field with 20x15 cm spacing at Central Rice Research Institute, Cuttack. Ideal field conditions were maintained and plants were uniformly fertilized with NPK. As per need, various plant protection measures were adopted to maintain the plants as healthy as possible. At booting stage, the boots were harvested from primary and secondary tillers. The harvested boots were cleaned by trimming off flag leaves and extra basal nodes and incubated at 8±2 ^{0}C for 7-10 days for cold pre-treatment. The stage of the anthers in the

spikelets was determined through a cytological test using ethanol acetic acid as fixative, iron as mordant and haematoxylin as the staining agent (Chang *et al*. 1978). The spikelets were surface sterilized using 20% commercial bleach [bleach contains NaOCl 4% (W/V)] for 5 minutes and rinsed three times with sterile de-ionized water. Spikelet selection was made on the basis of cytological observation and size and position of the anthers in the spikelet. Selected anthers were isolated and dusted uniformly over the surface of the medium. The position of the anthers at just above the centre of the spikelet was considered the proper stage when the anthers were inoculated in N6 medium (Chu *et al*, 1975). The medium supplemented with maltose 30 gm l^{-1}, 2,4D 2mg l^{-1}, kinetin 0.5mg l^{-1} and pH of the medium was adjusted to 5.8. Medium was solidified with 0.8% w/v agar. The media was dispensed 20-25ml per tube (25x150mm) and was sterilized through autoclaving at 15 psi for 18 minutes. The culture tubes were incubated in the dark at 26±10C for callus induction. Observations on the anther response to callus induction were recorded starting from 3-4 weeks after inoculation. Embryogenic calli of 2-3 mm size were transferred to MS medium (Murashige and Skoog 1962) supplemented with Kinetin 0.3 mg l^{-1}, BAP 0.9 mg l^{-1} and NAA 0.3 mg l^{-1} for shoot regeneration. The transferred calli were incubated with 16/8 light/dark regime at 25±10 C under artificial light intensity of 2000 Lux. After regeneration, 2-3 cm size of green shoots were transferred to MS medium supplemented with kinetin 0.25 mg l^{-1} and NAA 1 mg l^{-1} for root development.

The frequencies of callus induction and regeneration were estimated as follows: callus induction frequency (%) = number of anthers producing calli/ number of anthers plated x 100, green or albino plant frequency (%) = number of green or albino plant regenerating calli/number of calli transferred x 100.

The analysis of variance revealed significant differences between the inter-varietal crosses employed in this study. The cross CRMS31B x CRMS24B showed high callus induction frequency and also high green plant regeneration frequency as compared to other crosses. In case of CRMS32B x CRMS28B, lowest callus induction and green plant regeneration frequency was observed. The callus induction frequency and also the green plant regeneration frequency followed the same pattern of development (CRMS31B x CRMS24B > CRMS32B x CRMS24B > CRMS31B x CRMS25B > CRMS31B x CRMS28B) (Table-1).

The present study reveals that depending on the genotype, the callus induction frequency of all the F_1 hybrids varied. CRMS31B x CRMS24B responded the highest for callus induction frequency (37.83%) followed by CRMS32B x CRMS24B, CRMS32B x CRMS25B. However, the callus induction frequency was found lowest (18.36%) in CRMS31B x CRMS28B. Variations in callus induction ability among the tested crosses indicated that the difference in response was due to difference of genotypes used for the culture. A number of factors such as culture medium, growth regulators, culture environment, explant and genotype of donor plants are known to influence regeneration of plants. Most of the calli did not respond in the MS medium containing Kinetin 0.3 mg l^{-1}, BAP 0.9 mg l^{-1} and NAA 0.3 mg l^{-1} and simultaneously significant genotypic differences of green plant regeneration was observed among the genotypes used for this study. Highest regeneration frequency of 26.10% was observed in CRMS31B x CRMS24B while the cross, CRMS32B x CRMS28B showed the lowest frequency (11.58%) of shoot regeneration. Callus induction

Table 1. Anther culture response of inter-varietal/inter-maintainer crosses of rice.

Genotype	% of callus induction	% of callus regeneration	%of green plant regeneration	% of albino plant regeneration
CRMS31B X CRMS24B	37.83±0.43	41.09±0.30	26.10±0.35	15.00±0.25
CRMS32B X CRMS24B	34.86±0.23	38.26±0.29	23.59±0.41	14.67±0.20
CRMS32B X CRMS25B	25.52±0.31	26.36±0.26	14.07±0.28	12.29±0.36
CRMS32B X CRMS28B	18.36±0.32	20.59±0.32	11.58±0.39	09.00±0.11
HSD at 1%	0.37	0.12	0.52	0.32

(Figures in each cell represent mean values and standard error in that order)

followed by shoot regeneration frequency varied in four genotypes used for this study. This finding is consistent with the previous reports (Lentini *et al*., 1995; Sah, 2008) as it was observed that a strong relationship exist between genotypes and media for in-vitro anther culture in rice.

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