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Anther culture response of indica rice hybrids

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ABSTRACT

The nutritional requirements of Rajalaxmi and Ajay, two elite indica hybrid rice cultivars, were investigated using three artificial media. The results indicate that the response of genotype to media were highly significant for all the three stages of anther culture examined. Among the media, the callus induction frequencies were higher on N6 medium (34.56%) followed by MO19 (28.29%) and SK-1 (25.58%) and the callus regeneration and green plnt regeneration efficiency was also high in N6 media as compared to MO19 and SK-1. The results suggest that both genotype and culture media play a critical part in the anther culture response in indica rice.

Key words : *indica rice, anther culture, callus induction, medium*

Since the first success of anther culture in japonica rice (Niizeki and Oono in 1968), doubled haploids breeding has been practiced in rice. Anther culture is being viewed as a promising target for genetic manipulation, increasing selection efficiency, widening genetic variability through the production of gametoclonal variants and for production of double haploid populations (Gosal *et al*. 1997).

Anther culture has been well integrated into japonica rice breeding programs to select for micropspore derived recombinants with improved traits such as high yield and disease resistance (Faruque *et al*. 1998). However, exploitation of anther culture technique in breeding and genetics research in indica cultivars is still limited (Balachandran *et al*. 1999) due to their poor response to culture. Research efforts on the enhancement of response to anther culture of indica cultivars have been confined mostly on manipulation of callus induction and plant regeneration protocols (Balachandran *et al*. 1999) and several efforts has been made to improve the efficiency of rice anther culture through improved methods of culture techniques (Trejo-Tapia *et al*. 2002), formulation of new media compositions (Cha-um *et al.* 2009), utilization of specialized chemicals for callus induction (Datta *et al* 2005) etc. However, among the various factors that influence the anther culturability in indica rice, genotype and nutrient composition of the culture medium are regarded to be the major factors of variation (Raina and Zapata, 1997; Bishnoi et *al*., 2000) as different rice species, subspecies and varieties behave differently in their response to anther culture.

Keeping in view the above points, the present study was undertaken to investigate the response of two indica hybrids employing different culture media so as to determine the suitable medium for these genotypes and production of doubled haploids.

Two popular indica hybrid rice varieties Rajalaxmi and Ajay developed at the Central Rice Research Institute, Cuttack were chosen for the study. Seeds of these hybrids were sown in dry seed beds and 25 days seedlings were transplanted in a well puddled field with 20x15cm spacing. Ideal field conditions were maintained through application of NPK as per recommendated dosage and undertaking need based plant protection measures. At booting stage, the panicles from the primary and secondary tillers were collected and were subjected to cold treatment at 8 ± 2^0C for 7-10 days. 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). Pretreated spikelets were surface sterilized using 20% commercial bleach (4% NaOCl) for 5 minutes and rinsed three to four times with sterile de-ionized water. Selection of the spikelets was based on cytological observation and

size and the position of the anther in the spikelets. Anthers were isolated and dusted uniformly over the surface of the medium. The inoculated anthers were incubated in dark at 25 ±1 ^{0}C and observations on the anther response to callus induction were recorded starting from 3-4 weeks after inoculation.

Three different nutritional compositions namely N_6 (Chu, 1978), MO19 (Raina and Zapata, 1997), and SK-1 (Raina *et al*. 1989) were employed for the study. All the three media were supplemented uniformly with sugar (maltose 30 gm l^{-1}) and two phytohormones (2, 4-D @ 2mg l^{-1} and kinetin @ 0.5 mg l^{-1}) and the pH of each medium was adjusted to 5.8 and agar (0.7 %) was used for solidification. The media was dispensed at the rate of 20-25ml per culture tube (25x150mm) and was sterilized through autoclaving at 15 psi for 18 minutes. Embryogenic calli of 2-3 mm size were transferred to MS medium (Murashige and Skoog 1962) supplemented with Kinetin 0.25 mgl^{-1}, BAP 0.75 mgl^{-1} and NAA 0.25 mgl^{-1} for shoot regeneration. Cultures were incubated with a 16/8h light/dark regime at 25 ±1^0 C under artificial light (2000 lux). Green shoots of 2-3 cm size were transferred to Murashige and Skoog medium supplemented with kinetin (0.25 mgl^{-1}) and NAA (1 mgl^{-1}) for vigorous root development.

The frequency of callus induction was calculated as:

Callus induction frequency (%) = (number of anthers producing calli/number of anthers cultured) x 100.

Likewise, the frequency of plant regeneration was calculated as:

Total regeneration frequency (%) = (number of plants recovered/number of calli cultured) x 100.

The anther response (%) was calculated as: number of green plants generated/number of anthers plated) x 100.

The analysis of variance revealed significant differences between the media employed in the study for both the hybrids for all the parameters studied (Table 1). The N6 medium promoted higher levels of callus induction than the other two media, while M O19 was better than SK-1. The callus induced on N6 had shown higher level of callus regeneration than the callus generated on the other two media. The callus generated on M 019 also showed modrate level of regeneration while the callus from SK-1 showed moderate regeneration but green plant production was poor. Thus, the green plant regeneration frequency also followed the same pattern of callus induction N6>MO19>SK-1.

The present study reveals that genotypic differences exist for anther response as evidenced by the significant variation in different parameters (except for albino formation in Ajay due to media and genotype and the result is in conformity with earlier report of Bagheri and Jelodar (2008). Of the three media evaluated, the data show that a high level of callus induction (~ 34%) can be obtained in indica rices. N_6 medium was found to be the best media followed by MO19 for all the stages of anther culture i.e. callus induction, callus regeneration and green plant regeneration in indica rice genotypes used in the present study, a finding in agreement with the earlier reports of Raina and Zapata (1997). Although MO19 had moderate level of callus regeneration, it resulted in less number of green plants.

Both the hybrids showed high callus induction (34.56% and 30.14%) frequencies but low regeneration rates resulted in few regenerated plants (20.12 % and 19.53%). However, both positive (Shahnewaz *et al*. 2004) and negative relationships were reported between callusing ability and regeneration (Talebi *et al*. 2007). Similar results have also been reported by other workers with different media and genotypes (Niroula *et al*.

Table 1. Effect of media on anther culture response of Rajalaxmi and Ajay.

Media	% of callus induction		% of callus regeneration		% of green plant regeneration		% of albino plant regeneration	
	Rajalaxmi	Ajay	Rajalaxmi	Ajay	Rajalaxmi	Ajay	Rajalaxmi	Ajay
N_6	34.56±0.33	30.14±0.35	37.23±0.26	35.43±0.37	21.12±0.20	19.56±0.33	17.11±0.18	15.87±0.14
MO19	31.62±0.32	26.54±0.23	33.43±0.21	29.23±0.22	17.81±0.17	15.42±0.29	15.73±0.33	13.82±0.40
SK-1	25.58±0.18	27.44±0.22	27.31±0.22	21.11±0.38	15.36±0.29	13.23±0.27	12.03±0.21	15.78±0.37
LSD at P=0.05	7.64	3.56	2.47	4.37	3.29	3.90	3.60	3.66

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

2005). The higher rate of callus induction and low rate of regeneration observed in this study might be attributed to relatively higher doses of auxin source used in the callus induction media. Therefore, optimum level of auxins in the callus induction media requires a compromise between callus induction and regeneration frequency. Genotype and nutrient composition of the medium are the most important factors for efficient rice plant regeneration and appropriate media composition can increase regeneration efficiency (Khatun *et al*. 2003). Since generation of high number green plants is the prerequisite for use in the breeding programs, green plant regeneration needs more attention than callus induction. This can only be achieved by successful and reproducible protocols which also should address the frequency of albino plants. The recovery of albino plants from microspores derived calli has been a formidable obstacle to the utilization of rice anther culture for indica rice improvement (Chowdary and Mandal, 2001) which might be due to the long culture duration and the genotype. Shortening of the culture period can be one of the solutions to reduce albinism.

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