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RESEARCH ARTICLE

Anther culture performance in selected high yielding *indica* (of Sri Lanka) and *japonica* rice varieties and their inter sub-specific hybrids

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Abstract: The ability to improve anther culture response of some high yielding commercially grown *indica* rice varieties by crossing them with a high anther culture responding *japonica* rice variety was studied. Performance of *japonica* rice variety Hu lo tao, *indica* rice varieties Bg 90-2, Bg 379-2, Bg 94-1 and their F1 hybrids and F2 plants were investigated for callus induction and plant regeneration. Panicles were stored at 8 ^{0}C for 14 days as the cold pre-treatment and anthers were cultured in Chu's N6 medium with 5% (w/v) sucrose, 2.0 mg L^{-1} 2,4 - dichloro phenoxy acetic acid (2,4 D) and 0.5 mg L^{-1} Kinetin. The cultures were kept in the dark at 28 $\pm$ 2 ^{o}C for callus induction. Induced calli of 1-2 mm diameter were transferred to half strength Murashige and Skoog (MS) medium supplemented with 2.0 mg L^{-1} Kinetin and 0.5 mg L^{-1} naphthalene acetic acid (NAA). Of the parental varieties, Hu lo tao is more responsive to anther culture. F2 plants, compared to F1 hybrids and parental rice varieties, had greater callus induction frequencies and plant regeneration. Calli from F1 hybrids and F2 plants of Bg 90-2 × Hu lo tao showed the highest green plant regeneration (63.3% and 69.7% respectively). The regeneration of F1hybrids and F2 plants were higher than in the parents. According to the results, it is possible to improve the anther culture performance of F1 hybrids and F2 plants by crossing high yielding *indica* rice varieties with high anther culture responding *japonica* varieties.

Keywords: Anther culture**,** callus induction, *indica*, *japonica*, plant regeneration, rice.

INTRODUCTION

Rice anther culture is an effective and time saving technology for obtaining homozygous lines in varietal improvement (Chu *et al*., 2002). In the recent past, anther culture in rice has been improved substantially (Roy & Mandal, 2005). Anther culture has become a powerful tool for the rapid production of haploid and inbred lines used for obtaining hybrid cultivars. Use of doubled haploids in breeding programmes can thus greatly reduce the time required for development of improved cultivars. However, the usefulness of this approach is limited because some genotypes respond poorly to anther culture (Sopory & Munshi, 1996; Reed, 2002). Practical application of androgenic haploids in the improvement of rice is still limited by the low number of doubled haploids recovered, particularly in *indica* type (Senadheera *et al*., 2002). The low percentage of callus induction and green plant regeneration has limited the application of anther culture techniques in *indica* rice breeding programmes (He *et al*., 2006). A genetic approach to improve plant regeneration would be the selection for accumulation of favourable alleles for regenarability into a single presumably superior genotype. Plant regeneration from cultured tissues has been shown to be under genetic control in a number of species (Bregitzer & Campbell, 2002). By understanding the inheritance patterns of anther culture response, breeders can overcome genotype differences by crossing highly responsive to non responsive genotypes and can, to some extent, predict the level of response of the hybrids (Hou *et al*., 1994). Significant genotypic differences have been observed for callus induction and plant regeneration among the different hybrids of *indica* and *japonica* (Narasimman & Rangasamy, 1993).

Anther culture has been identified as the most appropriate technique at present to improve rice cultivars in Sri Lanka (Mendis *et al*., 1993). High yielding commercially grown rice varieties with desirable characteristics in Sri Lanka are of *indica* type, which show poor response to anther culture. Transfer of anther

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culture response traits from *japonica* to *indica* rice varieties by crossing can be beneficial to improve *indica* rice varieties. Only few studies have been conducted on anther culture of F1 hybrids from crosses of cultivated *indica* and *japonica* rice varieties (Mendis *et al*.,1993; Herath *et al*., 2007). It is important to investigate whether good combinations with high callus induction frequency and green plant regeneration could be selected by hybridization of suitable parents. The aim of this work was to study the ability to improve anther culture response in the F1 hybrids and F2 plants by crossing some high yielding, commercially-grown *indica* rice varieties with a *japonica* rice variety, which is highly responsive to anther culture.

METHODS AND MATERIALS

***Plant material*:** Seeds of parental *japonica* (Hu lo tao) and *indica* (Bg 90-2, Bg 379-2, Bg 94-1) varieties were obtained from the Plant Genetic Resources Centre (PGRC), Gannoruwa (Table 1). They were grown in a greenhouse and crosses of *indica* × *japonica* (Table 2) were conducted to obtain F1 hybrids. The F1 hybrids were self-fertilized to produce F2 progeny. Seeds of parents, F1 hybrids and F2 plants were sown in pots and the plants were grown in a greenhouse following the standard agronomic practices.

Table 1: Genotype, variety and characteristics of parental rice varieties

Genotype and variety	Characteristics
Hu lo tao	*japonica* variety, highly responsive to anther culture
BG 90-2	*indica* variety, high yielding
BG 379-2	*indica* variety, high yielding, resistant to Brown Plant Hopper and bacterial blight
BG 94-1	*indica* variety, high yielding

***Anther pre-treatment*:** First two to three panicles from plants of each genotype were harvested between 9.00 to 10.00 a.m. on sunny days. Panicles were harvested when the distance between collar of flag leaf and penultimate leaf was about 5-7 cm (Croughan, 1998) and the anthers were at uninucleate stage (by microscopic observation), wrapped in aluminium foil with a moistened cotton plug at the base and sealed in polypropylene bags separately. Panicles were stored at 8 ^{0}C for 14 d (Herath *et al*., 2009) as the cold pre-treatment. The intact panicles were rinsed with 70% (v/v) ethanol for 20 s. Spikelets were then removed and surface sterilized with 30% (v/v) commercial bleach solution (Chlorox®) for 20 min and rinsed thoroughly with sterilized distilled water.

***Anther culture*:** Spikelets were cut at the base and the anthers were gently squeezed out with a needle. Hundred anthers were inoculated in 100 ‹ 15 mm petri dishes with agar solidified N6 medium (Chu, 1978) supplemented with 2.0 mg L^{-1} 2, 4 - dichlorophenoxyacetic acid (2, 4-D), 0.5 mg L^{-1} Kinetin and 5% (w/v) sucrose. One petri dish constituted one replicate and an average of 10 replicates were cultured for each genotype. The cultures were placed in the dark at 28$\pm$ 2 ^{0}C (Chen *et al*., 1991) for callus induction and examined at weekly intervals for six wks and the percentage of anthers forming calli (callus induction frequency) was recorded after six wks.

***Plant regeneration*:** When the calli were approximately 1-2 mm in diameter, they were transferred to 100 ‹ 15 mm petri dishes containing 25 mL of half strength Murashige and Skoog (MS) medium (Murashige & Skoog, 1962) supplemented with 2.0 mg L^{-1} Kinetin and 0.5 mg L^{-1} naphthalene acetrc acid (NAA) (Croughan & Chu, 1991). The cultures were subject to a 16 h photoperiod (50 µE m^{-2} s^{-1}). Observations were made at weekly intervals and the data on percentages of calli regenerating green and/ or albino plants were recorded after 6 wks of incubation. The experiment was repeated thrice and the means were taken for analyses.

The regenerated shoots were transferred to hormone-free, half strength MS medium for rooting. Rooted plants were acclimatized and grown to maturity on paddy soil in a greenhouse under standard agronomic practices. At maturity, spikelet fertility was measured as the ratio of number of grains per panicle to the total number of spikelets per panicle and expressed as a percentage.

***Data analyses*:** Data analyses was done using the Statistical Analyses System (Release 9.1), Analysis of Variance (ANOVA). Mean separation was done by Duncan's multiple range test (DMRT). All experiments were analyzed as a completely randomized design.

RESULTS

Callus induction

Callus induction commenced three weeks from culture and the frequency of the anthers forming calli varied between 4.3% and 78.3 % depending upon the genotype (Figure 1a, 1b, 1c; Table 2). There were strong genotypic effects on callus induction frequency among F1 hybrids, F2 plants and parental rice varieties. Of the parental varieties, Hu lo tao (*japonica*) is more responsive to anther culture with 34.3% callus induction frequency. Out of *indica* varieties, Bg 90-2 had a significantly higher callus induction frequency than the other two tested

Table 2: Callus induction frequency and plant regeneration in anther cultures of selected *indica*, *japonica* rice varieties, their F1 hybrids and F2 plants. (Significance level 5 %)

Genotype	Callus induction frequency (%)	Percentage of calli regenerating plants		
		Green (G)	Albino (A)	G/A
Parents				
Bg 90-2	10.5^{g}	2.2^{g}	5.1^{f}	0.43
Bg 379-2	6.7^{h}	0.0	0.0	0.0
Bg 94-1	4.3^{h}	0.0	0.0	0.0
Hu lo tao	34.3^{e}	29.3^{c}	26.3^{c}	1.11
F1 hybrids				
Bg 90-2 × Hu lo tao	69.8^{b}	63.3^{b}	57.8^{a}	1.09
Bg 379-2 × Hu lo tao	48.2^{d}	13.8^{e}	15.1^{d}	0.91
Bg 94-1 × Hu lo tao	23.5^{f}	7.2^{f}	11.3^{e}	0.63
F2 plants				
Bg 90-2 × Hu lo tao	78.3^{a}	69.7^{a}	48.2^{b}	1.44
Bg 379-2 × Hu lo tao	57.6^{c}	28.4^{c}	29.7^{c}	0.95
Bg 94-1 × Hu lo tao	34.7^{e}	20.9^{d}	23.8^{d}	0.87

* In each column the means with same superscript letters are not significantly different ($p< 0.05$)

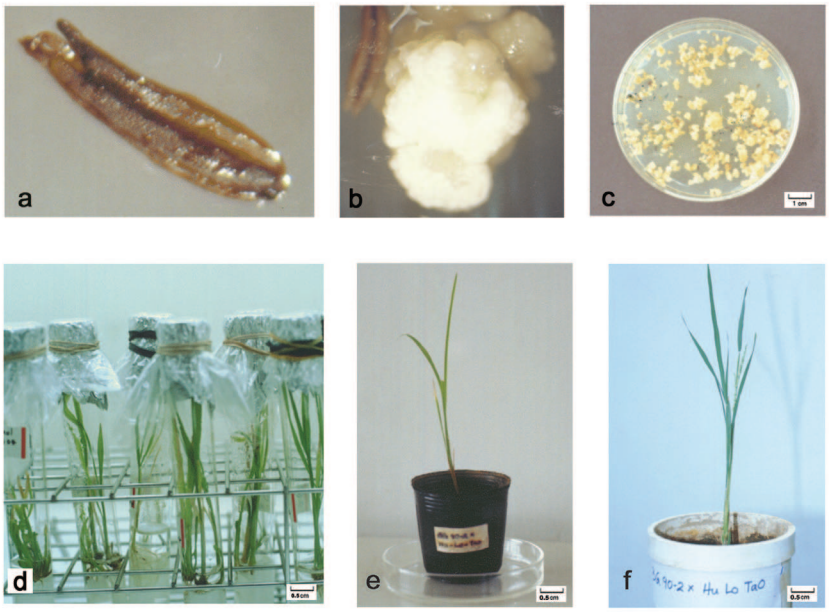

Figure 1: Anther culture response of *indica* × *japonica* crosses
(a) A magnified rice anther after 4 weeks of culture (b) A magnified callus developed after 6 weeks from culture establishment, (c) Callus development after 6 weeks from culture establishment, (d) Green plants regenerated from anther derived calli, (e) Acclimatized anther culture derived rice plantlet and (f) Transplanted rice plantlet of Bg 90-2 × Hu lo tao F1 hybrid [magnification of (a): 1×100, (b): 1×40. Bar in panel (c) = 1.0 cm, bars in panels (d), (e), (f) = 0.5 cm]

indica types. The F2 plants, compared to F1 hybrids and parental rice varieties, had greater callus induction frequencies. All crosses except Bg 94-1 × Hu lo tao had significantly higher callus induction frequencies than the *japonica* parent (Hu lo tao). The highest callus induction frequency in F1 hybrids and F2 plants (69.8% and 78.3% respectively) was observed in Bg 90-2 × Hu lo tao cross.

Plant regeneration

Frequency of green plant regeneration initiated from calli varied between 2.2 to 69.7 % depending upon the parental genotypes, F1 hybrids and F2 plants (Figure 1d; Table 2). Among the parental rice varieties Hu lo tao variety was relatively more responsive to green shoot regeneration. Calli from F1 hybrids and F2 plants of Bg 90-2 × Hu lo tao cross responded better with regard to green plant regeneration when compared to the parental varieties and other crosses. Similarly, plant regeneration of F1 hybrids and F2 plants were higher than in both parents. The highest green plant regeneration frequency (69.7 %) was observed in F2 plants of Bg 90-2 × Hu lo tao cross. Higher regeneration frequencies of green plants, compared to albino plants, were observed with *japonica* parent and F1/ F2 plants of Bg 90-2 × Hu lo tao cross. Although high frequency of green plants could be obtained from some of the F1 hybrids and F2 plants, green/ albino ratio for most of the genotypes remained low due to the high level of albino plant regeneration.

Transplanting and growth of anther derived plants

Anther derived green plants obtained from parents, F1 hybrids and F2 plants were acclimatized with approximately a mean percentage survival rate of 83 (Figure 1e, 1f). Spikelet fertility varied considerably with the genotype (Table 3). Out of the 278 plants that survived, 110 plants were completely sterile, 51 plants had 1-5 % spikelet fertility and 117 plants had more than 5 % spikelet fertility.

DISCUSSION

The poor response of *indica* types to anther culture has been clearly established. Even among different genotypes of a particular ecotype, *indica* or *japonica,* considerable variation in pollen callusing and green plant regeneration has been observed with the genotypic effect being greater among the *indica* types (Silva, 2010). Results of this study agree with the above observations. *Indica* varieties had significantly low callus induction response and plant regeneration than the *japonica* variety. *indica* variety Bg 90-2 performed better than the other two *indica* varieties. This improved variety had *indica* type parentage. However, one or more parents of this variety might be of better anther culture responding *indica* type than the other parents.

Crossing of *indica* varieties with a *japonica* variety

Table 3: Transplantation survival and spikelet fertility of the anther derived plants from *indica*, *japonic*a rice varieties, and their F1 hybrids and F2 plants

Genotype	Transplantation survival			Plants with spikelet fertility					
				0 %		1-5 %		> 5 %	
	Number of plants transferred	Number of plants survived	Percentage survival	No.	%	No.	%	No.	%
Parents									
Bg 90-2	11	9	81.8	1	11.1	2	22.2	6	66.6
Bg 379-2	0	0	0	0	0	0	0	0	0
Bg 94-1	0	0	0	0	0	0	0	0	0
Hu lo tao	25	21	84.0	3	14.2	5	23.8	13	61.9
F1 hybrids									
Bg 90-2 × Hu lo tao	81	73	90.1	32	43.8	12	16.4	29	39.7
Bg 379-2 × Hu lo tao	61	54	88.5	26	48.1	9	16.6	19	35.1
Bg 94-1 × Hu lo tao	47	32	68.0	18	56.2	6	18.7	8	25.0
F2 plants									
Bg 90-2 × Hu lo tao	41	35	85.3	12	34.2	5	14.2	18	51.4
Bg 379-2 × Hu lo tao	32	28	87.5	10	35.7	7	25.0	11	39.2
Bg 94-1 × Hu lo tao	30	26	86.6	8	30.7	5	19.2	13	50.0
Total	328	278		110		51		117	
Mean percentage			83.9		39.5		18.3		42.0

improves the callus induction frequency in anther culture. Significant positive heterosis was observed in callus induction in rice. It has been suggested that in rice, callus induction was mainly controlled by additive genetic effects (Yan *et al*., 1996). That the quality and frequency of callus induction and subsequent plant regeneration could be improved by selecting better responsive rice genotypes has been suggested (Niroula & Bimb, 2009). Callus induction in rice is a quantitative trait controlled by multiple genes with additive effect. The callus induction process is under gametophytic control and the positive alleles would be strongly selected during the anther culture of F1 hybrids (Ping *et al*., 1997).

The plant regeneration frequency of F1 hybrids and F2 plants were better than both parents. In a similar study of androgenesis in *indica* × Basmati rice hybrids, high plant regeneration frequencies were observed from microspore derived calli of some of F1 hybrids and F2 plants as compared to their actual parents, supporting the above observations (Bishnoi *et al*., 2000). Although green plant regeneration from anther culture of *indica* cultivars was much lower than from *japonica* cultivars a considerable frequency of green plants from different crosses of *indica* cultivars have been obtained (Thuan *et al*., 2001).

The highest green plant regeneration frequency was observed in F2 plants of Bg 90-2 × Hu lo tao cross. This could be due to the fact that the segregating progeny has a range of favourable gene combinations that govern anther culture responses. According to Chu and Croughan (1989), from a practical stand point for rice anther culture, higher regeneration rates can be obtained from crosses involving a high and low parent by utilizing the F2 rather than the F1 generation for anther culture. A higher green plant regeneration than albino plants was observed with *japonica* parent and F1/ F2 plants of Bg 90-2 × Hu lo tao cross. Although high frequency of green plants could be obtained from some of the F1 hybrids and F2 plants, green/ albino ratio for most of the genotypes remained low due to the high level of albino plant regeneration. The genotype appears to be the most important factor in androgenic albinism (Caredda & Clement, 1999). According to Immonen *et al*. (1999), the use of doubled haploids is a production method that can be applied to a large number of genotypes, and a large number of green regenerants that are of high quality, displaying genetic variability of the cross can be produced. Although inheritance of anther culture response seems complex, crosses between high and low response types of barley has primarily shown additive effects (Hou *et al*., 1994). This agrees with the above results obtained. There have been several previous studies on the genetic control of anther culture in rice. Genetic additive effect was reported to be significant for callus induction, green plant regeneration and culture efficiency (Yan *et al*., 1996). Similarly, it has been found that the potential to introduce high responding ability into low lines was moderate, suggesting that the frequency of anther culture response might be improved by suitable hybridization and selection strategies (He *et al*., 2006). A genetic approach to improving plant regeneration from anther-derived callus in *indica* rice through sexual hybridization and selection has been suggested. (Silva, 2010). The higher heritability estimates for callus induction and culture efficiency suggest that relatively rapid genetic gain can be reached by transferring this trait from high culture ability to low culture ability germplasm (Yan *et al*., 1996). According to Silva (2010), irrespective of the method used, it was vitally important that such incorporation of genes should be recoverable in commercially advantageous rice varieties. The performance of F2 populations confirmed F1 additive effects. The F2 generation demonstrated that combining genes from different populations can sometimes have unexpected positive effects (Fenster & Galloway, 2000). In general, F2 hybrids respond better to anther culture than F1 hybrids in *indica,* Basmati rice breeding (Rohilla *et al*., 1997). Better than expected performance of recombinant hybrids may be attributed to the chance bringing together of groups of alleles in combination. According to Fenster and Galloway (2000), the presence of epistasis or gene interaction may cause the positive effects.

High spikelet sterility was observed in anther culture derived F1 hybrids and F2 plants. According to Roy and Mandal (2005), spikelet sterility occurs in androclones under *in vitro* culture conditions, due to physiological factors and changes in gene distribution.

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