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ANTHER CULTURE RESPONSE OF SOME SALT RESISTANT AND SALT SUSCEPTIBLE INDICA RICE VARIETIES

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ABSTRACT

In view of utilizing anther culture in rice improvement programs for developing varieties, anther derived embryos and green plant regeneration ability of rice varieties showing different reactions to salt stress, were studied in different culture media. The rice varieties used in the study were Pokkali, Nonabokra, At 354 and At 401 that were tolerant, Bw 400 that was moderately tolerant and Bg 94-1, Bg 300 and Bg 750 that were susceptible in salt stress. Six different callus induction media (N6) with different combinations and concentrations of 2-4D, Kinetic (KN) and Napthalic acetic acid (NAA) and four different plant regeneration media (MS) with different concentrations of KN, NAA, and BAP were used in the study. Different varieties responded differently to different callus induction and regeneration media. Nonabokra and At 354 did not respond at all for any of the media used. Bg 750 showed the highest response (9%) for callus induction. Pokkali and Bw 400 also showed callus induction responses at acceptable levels (over 3.5%). When KN level was increased from 0.5 to 1.0 mg/l, callus induction response of Pokkali and Bw 400 was improved while that of Bg 750 was ceased. In regeneration Bg 750 and Bg 300 did not respond at all for any of the media while Pokkali responded in all the regeneration media used. Rest of the varieties namely Bw 400, Bg 94-1 and At 401 attained acceptable levels of plant regeneration frequencies ranging from 20.8 to 46.7 in the MS medium with 2 mg/l BAP, 1mg/l NAA and 0.5 mg/l KN for which Pokkali showed the highest response (82.7%). Thus, response to anther culture appeared independent of salt tolerance in rice. However, calli derived plantlets could be successfully established in the field only in Pokkali and Bw 400, which are having improved characters that can be used as breeding lines.

KEYWORDS: Anther culture, Callus formation, Plant regeneration, Rice.

INTRODUCTION

Anther culture is an important biotechnological tool in rice breeding since it offers the possibility of obtaining genetically diverse haploid and homozygous diploid plants within a short time. It may reduce the time needed to reach homozygosity by spontaneous or induced doubling of the haploids chromosome number (Oono, 1975 and Shen *et al.*, 1983).

It also allows early expression of recessive genes and increased selection efficiency as the number of plants required to obtain the desired recombinants is less than the conventional breeding.

The first successful haploid production using anther culture technique was achieved in *Datura innoxia* (Guha and Maheshwai, 1966). Shortly after this success, the technique was applied to rice (Niizeko and

Oono, 1968). The first rice varieties from haploid breeding were released for commercial production in 1976 (Pinghe *et al.,* 1998). At present, the most promising approach for haploid production of rice is microspore culture within or in isolation from the anther. Even though successful application of anther culture in the production of new rice varieties has been reported in China, Taiwan, South Korea, Japan, USA, India and several other countries (Raina and Ifran, 1998), convincing evidence of a general, simple, reproducible method applicable to wide range of varieties or subspecies in indica rice, is still lacking. Therefore, the evaluation of new factors and their manipulation for efficient callusing and green plant regeneration in anther culture of indica rice is still a challenging field. Mondal and Gupta (1997) reported that cold treatment of anthers induced calli formation, because it delayed the senescence of anther wall and facilitated the entry of nutrients for the growth and development of microspores.

Various combinations of auxins and cytokinins in the culture medium have been tested for their effect on both callus induction and plant regeneration in rice. Plant regeneration of calli is enhanced by water stress (Chen, 1986).

In anther culture, the promotive effect of proline suspension cultures has also been reported (Zapata and Abrigo, 1988). The superiority of maltose over sucrose as the carbohydrate source for callus induction in anther culture has been demonstrated (Lentini *et al.*, 1995 and Raina and Ifran, 1998). Spontaneous chromosome doubling was observed in plants obtained from the same callus mass in variety tetep and tulsi (Ranjan *et al.*, 1998). The reasons for spontaneous doubling of chromosome were suggested as endomitosis, irregular meiosis, spindle fusion and nuclear fusion (Oono, 1975 and Shen *et al.,* 1983).

Japonica cultivars are easier to culture than indica cultivars. In addition, genotypic specificity to anther response and green plant regeneration is a more serious constraint in indica than japonica cultivars. Scientists suggested that callus induction ability was inherited as a recessive trait conditioned by a single block of genes. Pinghe *et al.,* (1998) detected 8 independent QTLS involved in anther culturability of rice.

In Sri Lanka there has been tremendous improvement in rice sector through conventional breeding, but the creation of genetic variability and application of advanced technologies to develop varietal resistance for biotic and abiotic stresses had been limited. Utilization of potentially important anther culture technology in rice improvement has not yet been tried. Thus the present study was conducted with the main objective of investigating the anther culture response of selected rice varieties, showing varying response in salt stress (Sirisena and Abeysiriwardhana, 2005). This

technique will be applicable to further research activities of salt tolerant line development in rice.

MATERIALS AND METHODS

Selection of explants

Eight popular rice varieties which show varying response to salt stress were selected (Table 1) and grown under natural conditions with regular supply of water and recommended cultural practices during two seasons. Panicles were collected from all these varieties, when the distance between the flag leaf auricle to the penultimate leaf was in between 7-11 cm and microspore stage (late uninucleate) was cytologically detected using heamatoxyline staining.

Table 1. Varietal ranking or grouping for tolerance to salinity based on green house screening test (Sirisena and Abeysiriwardhana, 2005).

Variety		*Reaction to salinity*
Traditional	Pokkali	Tolerant
	Nonabokra	Tolerant
Improved	At 354	Tolerant
	At 401	Tolerant
	Bw 400	Moderately tolerant
	Bg 94-1	Susceptible
	Bg 300	Susceptible
	Bg 750	

Pretreatments and sterilization of plant material

Cold treatment was given to the panicles for 7 to 12 days at 10°C. Panicles were sterilized with wiping 70% ethanol and taken to clean air bench. Then open panicles were sterilized with 20% Clorox for 15-20 minutes, followed by sterilization with 70% ethanol for 2 minutes and then washed thoroughly with sterilized distilled water.

Culture media for callus initiation and plant regeneration

Callus initiation from anthers was tested on N6 medium supplemented with different combinations and concentrations of growth regulators (Table 2). The spikeletes were cut at the base and anthers. About one hundred anthers were plated on each petridish containing 15ml of culture medium. For each medium 25 petridishes were used. Inoculated anthers were incubated under dark at 25°C.

Partial desiccation of calli and plantlet regeneration

Primary calli of 1-5 mm diameter were subjected to 24 hr water stress condition using oven-sterilized petridishes laid with two layers of Watmann No. 1 filter papers for 24 hours. Then partially desiccated calli were transferred to modified Murashige and skoog (MS) 1962 medium (Table 2) for regeneration and were incubated at 25°C with 16 h light/8 h dark photoperiod.

Table 2. Different concentrations and combinations of sugar types and growth regulators in callus induction (N6) and plant regeneration (MS) medium.

Treatment	*Sugar type*		*Growth regulators*			
	Sucrose %-	*Maltose %*	*2,4 D mg/l*	*KN mg/l*	*NAA mg/l*	*BAP mg/*
Callus induction						
N6-1		6	2	0.5	-	-
N6-2		6	-	0.5	2	-
N6-3		6	2	1	-	-
N6-4		6	-	1	2	-
N6-5		6	2	0.5	2	-
N6-6		6	2	1	2	-
Plant regeneration						
MS1	3		-	0.5	1	1
MS2	3		-	0.5	1	2
MS3	3		-	2	1	0.5
MS4	3		-	2	1	2

Identification of ploidy level and plantlet establishment in soil

Root tops of the regenerated green plants were examined for their ploidy status using aceto carmine stain. Developed plantlets were transferred to sterilized soil and kept in the greenhouse. Seeds collected from tissue cultured plants were screened under field conditions for uniform plant growth and seed setting over them and selected lines were advanced to screen against diseases and salt stress condition.

RESULTS AND DISCUSSION

Differential response with respect to callus induction and green plant regeneration was noticed among the eight cultivars (Table 3). Callus induction frequencies were variable and dependent upon the genotypes used.

Callus induction

Anthers of cold treatment of 7 days showed the highest response to callusing and green plant regeneration. Anther viability observed

Table3. Callus induction and plant regeneration potential of different rice varieties.

Variety	*N6-1*		*N6-2*		*N6-3*		*N6-4*		*N6-5*		*N6-6*	
	No. of plated anthers	*No. of calli produced*	*No. of anthers plated*	*No. of calli formed*	*No. of anthers plated*	*No. of calli formed*	*No. of anthers plated*	*No. of calli formed*	*No. of anthers plated*	*No. of calli formed*	*No. of anthers plated*	*No. of calli formed*
Pokkali	484	3	465	6	430	7	494	27	295	-	312	-
BW 400	352	10	314	5	374	9	387	15	325	-	316	-
Bg 300	267	1	275	1	284	-	304	2	245	-	302	-
Bg 750	306	17	244	22	269	18	202	-	224	-	220	-
Bg 94-1	310	3	291	-	285	4	215	-	205	-	196	-
At 354	281	-	290	-	206	-	260	-	254	-	276	-
At 401	395	6	282	11	397	2	123	-	208	8	156	27
Nonabokra	294	40	175	45	244	40	106	44	170	-	165	-

up to 12 days with the cold treatment and calli formation favored 7 days treatment than others. Anthers pretreated for 7 days were used for this media test.

The use of different modifications of N6 medium incited different responses (Table 3) but variety x medium interaction was not significant. It ranged from 0% to 17% with respect to the number of anthers cultured. The time required for callus initiation was different in different varieties; callus initiation started as early as 30 days after anther plating. Varieties Pokkali, Bw 400, Bg 750 and Bg 94-1 reached maximum callus formation in between 42 to 50 days. In varieties such as Bg 300 and Nonabokra it was prolonged up to 60 days after anther plating. Microspore derived calli formation was confirmed using microscopic studies. Two types of calli initiated from anther of rice varieties, some were friable and shiny white and others were hard and cream in color. Most of the calli showed fast proliferating types which were eventually turned necrotic with or without regeneration.

Anthers of eight varieties showed differences in response in each media. Pokkali and Bw 400 showed the highest response to medium N6-4 but for Bg 750 and At 401 the highest response could be observed with medium N6-2. Variety At 401 produced the highest number of calli when cultured on 6 (N6-6) medium while variety At 354 did not produce calli on any of the media tested. Bg 750 showed the highest response in callus formation with media N6-1, N6-2 and N6-3 when compared with all the other varieties. The culture medium supplemented with 2mg/l 2-4D combined with 0.5 mg/l KN (N6-1) and 2mg/l 2-4 D combined with 1mg/lKN appeared to be suitable for four varieties (BW 400, Bg 750, At 401 and Nonabokra). However, the medium supplemented with 2mg/l NAA and 0.5 mg/l KN showed high frequency of callus formation with the variety Bg 750. In contrast to the result reported, the results of the present study showed that 2-4D can be replaced by NAA. Furthermore, Bg 750, improved variety, having different genetic constituents from Japonica lines, responded well to three culture media than other varieties and none of the varieties responded to media supplemented with 2mg/l 2-4 D and 2mg/l NAA. It may be possible that supply of high auxin concentration may suppress the callus initiation.

Plant regeneration

Regeneration frequencies of different varieties vary with media used. Interaction effect of variety x medium was not significant with respect to plantlet regeneration. Out of eight varieties tested, four varieties viz, Pokkali, Bg 94-1, At 401 and Bw 400 produced green plants on MS2 and the frequency of regeneration was 82.7%, 20.8%, 46.7% and 26.7% respectively. In agreement with this result Ranjan *et al.* (1998) reported that genotype

Table 4. Plant regeneration efficiency in different MS media.

Variety	*MS1*		*MS2*		*MS3*		*MS4*	
	No. of calli cultured	Regenerated plants	No. of calli cultured	Regenerated plants	No. of calli cultured	Regenerated plants	No. of calli cultured	Regenerated plants
Pokkali	17	2(11.7%)	29	24(82.7%)	16	2(12.5%)	41	16(39%)
Bg 750	39	-	26	-	41	-	10	-
Bg 300	7	-	5	-	3	-	7	-
Bg 94-1	7	-	24	5(20.8%)	17	-	10	-
At 401	17	-	15	7(46.7%)	36	-	33	-
Bw 400	10	-	15	4(26.7%)	15	-	8	2(25%)
Nonabokra	12	-	11	-	9	-	9	-

variation of the rice varieties has an effect mostly on callus induction and plant regeneration. The regeneration potential of variety varied with different modifications of MS medium and MS2 (1mg/l NAA, 2mg/l BAP, 0.5mg/l KN, 3% Sugar, 0.8%Agar) was found to be the best among all modifications (Table 4). High frequency of green (82.7%) and albino (15%) plant regeneration was observed in Pokkali. Direct green and albino shoots were observed on the same callus induction medium from the calli still attached to the anthers.

Calli derived plantlets produced in the varieties (16 plants from Pokkali and 2 plants from Bw 400) were successfully established in the soil. Somatic metaphase analysis from root tips of regenerated green plants of two varieties showed haploid and diploid number of chromosomes. Fertile plants showed doubled number of chromosomes. In first generation (G0) 50% of the plants were sterile and others produced seeds with 85% fertility. In second (G2) generation more then 200 plants were screened in the field and third (G3) generations 3000 plants screened. All the Pokkali plants showed homogeneity, uniformity, seed setting and shorter plant height (73 cm) and smaller seed type compared to that of the mother plant (Table 5). Bw 400 showed homogeneity, uniformity and 100% fertility but plant morphology was the same as mother plant. Results of Pokkali reveal retention of desirable recessive genes and improved protocol developed from this study can be extended to other indica varieties and F1 to improve plant with desirable genetic constituents.

Table 5. Performance of anther derived lines of Pokkali and Bw 400.

Line derived from anthers	*No. of plants*	*Seed setting*	*Plant height*	*Seed type*
Pokkali G0	16	85%	65 cm	White, Small,Long
Pokkali G1	200	100%	73 cm	White, Small,Long
Pokkali G2	3000	100%	73 cm	White, Small,Long
Bw 400 G0	2	50%	70 cm	Red,large,Round
Bw 400 G1	200	100%	95 cm	Red,Large,Round
Bw 400 G2	3000	100%	95 cm	Red,Large,Round

CONCLUSIONS

Variety into medium interaction was not significant in calli induction and plant regeneration of selected indica rice varieties. Even though calli obtained from Pokkali, Bg 94-1, At 401 and Bw 400 could be regenerated on the medium containing 2mg/l BAP with 1mg/l NAA and 0.5m/l KN, only plants derived from varieties of Pokkali and Bw 400 were successfully established in soil. Anther derived breeding lines were obtained from Pokkali with improved characters. Thus anther culture technology can be used in rice improvement program via creating genetic variants and producing double

haploids with early expression of recessive genes and shortened breeding cycle.

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