Effect of Cold Pre Treatment on Callogenesis of Selected *Indica* Rice Genotypes Via Anther Culture

P.L.P.S. Sandaruvini and Disna Ratnasekera

Department of Agricultural Biology, Faculty of Agriculture, University of Ruhuna, Kamburupitiya, Sri Lanka

Abstract

Indica rice varieties are highly recalcitrant to anther culture. Androgenesis results in homozygous progeny from a heterozygous parent in a single generation and provides excellent material for research, plant breeding and plant transformation. Physical, chemical treatments given to flower buds or anthers prior to culture can be highly inductive to the development of pollen into plants. The most significant is cold pre treatment and is genotypic dependent. The aim of this study was to determine the optimum cold pre treatment duration required for anther culture in selected Indica rice varieties. Genotypes of BG 379-2, BW 267-3, Kahata wee, and AT 362 varieties were selected for the study based on their yield and other desirable characters such as grain quality, tolerance to iron toxicity, resistance to pest and disease which are sufficient for rice breeding programmes. Panicles of these varieties were cold pre treated at 10°C for 6-12 days and anthers were cultured in agar solidified N6 medium supplemented with 2.0 mg / l 2-4 D, 0.5 mg/l kinetin and 2.5 mg/l NAA. The highest callus induction frequency (4.09) was observed in At 362 while the least was recorded in Kahata wee. The effect of variety and cold pre treatment was significant ($p<0.05$) for the callus induction frequency. Cold pre treatment for 7 days at 10 °C had the highest callus induction frequency in genotypes of Kahata wee, BG379-2, BW267-3 and AT 362 had highest callus development in 12 days was found to be most effective for anther culture in experimental genotypes used in the study.

Key words: Anther culture, Callus induction, Cold pre treatment

Introduction

Anther culture is an important biotechnological tool in rice breeding. Androgenesis results in homozygous progeny from a heterozygous parent in a single generation and provides excellent material for research, plant breeding and plant transformation. Production of haploids and dihaploid plants have been useful in providing access to recessive genes and for biotechnological manipulations. Using pollen parents from anther culture in rice breeding can reduce breeding time, increase selection efficiency and save space and labour in the field by allowing selection of fine strains in early generations.

Exploitation of anther culture technique in breeding and genetics research is limited due to very low regeneration frequency of anthers of rice in indica cultivars and their genotypic dependence (Balachandran *et al.*, 1999).

It was reported that certain physical and chemical treatments given to flower buds or anthers prior to culture, can be highly inductive to the development of pollen into plants (Maheshwari *et. al.*, 1980).The most significant is cold treatment. According to Genovesi and Magill (1979), cold treated anthers containing uni nucleate microspores form callus at a much higher rate in rice. The aim of this study was to determine the optimum cold pre treatment duration required for anther culture in selected Indica rice varieties.

Materials and Methods

Plant materials and growth conditions: Seeds of BG379-2, BW267-3, Kahata wee, and AT 362 varieties were obtained from the Regional Rice Research and Development Institute, Bombuwala and were grown in pots under green house conditions following the standard agronomic practices recommended by Department of Agriculture.

Anther pre-treatment and preparation for culture: Panicles were collected at the booting stage, before the heading when the distance between the flag leaf auricle and penultimate leaf collar was 7-12 cm by making clean cut below the node where panicle arose. After removing the leaf blades panicles were wrapped together in Aluminum foil, placed in a polythene bags and kept in an incubator at 10 °C for 6,7,8,9,10,11 and 12 days for the cold pre treatment. The intact panicles were rinsed with 70% (v/v) ethanol for 20s. Spikelets were then removed and surface sterilized with 30% (v/v) commercial bleach solution (Clorox) for 20 min and rinsed thoroughly with sterilized distilled water.

Spikelets were cut at the base and the anthers were gently squeezed out using a sterilized forcep and a pair of scissors. Approximately hundred anthers were inoculated in petri dishes with agar solidified N6 medium supplemented with 2.0 mg/l 2-4 D, 0.5 mg/l kinetin and 2.5 mg/l NAA. One petri dish constituted one replicate and an average of ten replicates were cultured for each genotype with one cold treatment. The cultures were kept in dark at 28 °C for callus induction and examined at weekly intervals for six weeks and the percentage of anthers forming calli (callus induction frequency) was recorded after six weeks.

Data analyses: The data were analyzed as a completely randomized design using the analysis of variance (ANOVA).

Results and Discussion

Effect of cold pre treatment on callus induction

Callus induction started three weeks after culture establishment. The highest callus induction frequency was observed in At 362 while lowest was recorded in traditional variety, Kahata wee (Table 1).The effect of variety and cold pre treatment was significant ($p<0.05$) for the callus induction frequency. Similarly, the interaction effect of the above two factors was significant at 5% significance level. Significant genotype × pre treatment interaction implies that the determination of suitable pre treatment condition has the ability to further increase the responsiveness in both genetically high and low responsive genotypes.

Table 1. Mean calli frequency of tested varieties

Variety	Mean calli frequency
Kahata wee	1.85^{b}
BG 379-2	3.33^{ab}
BW 267-3	1.95^{b}
AT 362	4.09^{a}

Overall, cold pre treatment at 10°C for 7 days showed higher callus induction frequencies in genotypes of Kahata wee, BG379-2, BW267-3 compared to other cold pre treatment durations. AT 362 produced highest callus development in 12 days at 10 ° C and kahata wee performed best in 7 days at 10 °C (figure 1, table 2). It implies that the application of cold pre treatment is genotype dependent.Datta (2005) reported that, the response to chilling or heating is also genotype dependent. Temperature is one of the most important factors that influences the induction of pollen embryo callus development. According to Reddy *et al.* (1985), pre treatment of anthers by low and elevated temperatures had stimulatory effect for callus induction and plantlet yield in rice, which supporting our results.

Table2. Duration of cold pre-treatment on callus induction in anther cultures of selected Indica rice varieties.

Variety	Treatment Duration (days)						
	6	7	8	9	10	11	12
Kahata wee	1.67^{bc}	3.67^{a}	2.67^{ab}	2.00^{bc}	1.67^{bc}	1.00^{dc}	0.34^{d}
BG 379-2	3.33^{b}	9.00^{a}	3.34^{b}	2.67^{b}	3.33^{b}	1.34^{b}	0.50^{b}
BW 267-3	2.67^{b}	6.00^{a}	1.67^{bc}	1.00^{bc}	1.33^{bc}	1.00^{bc}	0.00^{c}
AT 362	3.67^{bc}	3.00^{c}	1.67^{c}	1.67^{c}	6.67^{b}	1.34^{c}	10.67^{a}

Different letters in a column indicate a significant difference among the cold pre-treatment duration on callus induction in anther cultures of selected Indica rice varieties based on Duncan's Multiple Range Test at 5% level.

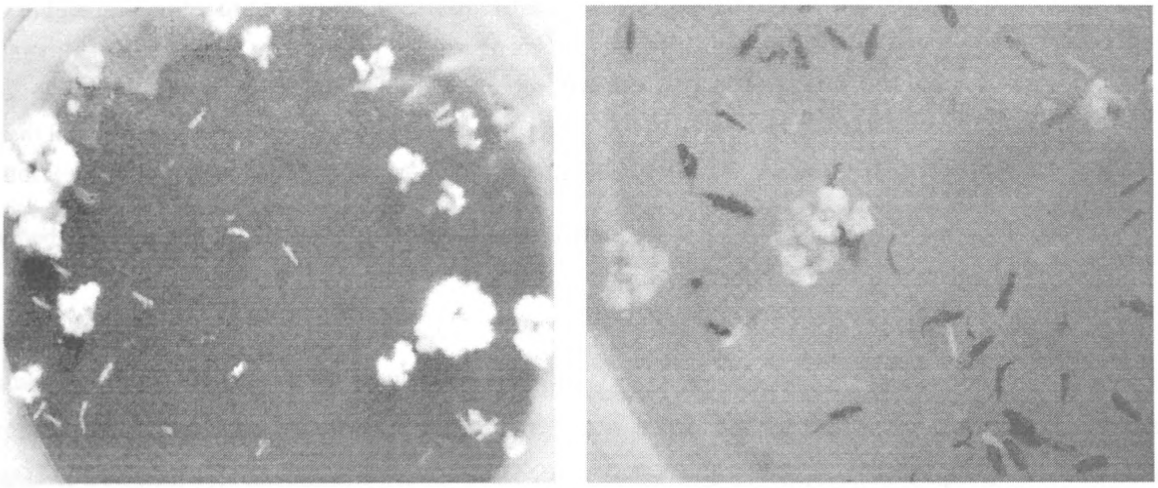

Figure 1. (a) Callogenesis of AT362 on twelve days cold pre treatment duration (b) Callus of Kahata wee on seven days cold pre treatment duration.

Reference

Balachandran, S.M., Sarma, N.P. and Siddique, E.A. 1999. Inheritance of anther culture response in rice. Current Science; 77 (7): 962-964.

Datta, S.K. 2005. Androgenic haploids: Factors controlling development and its application in crop improvement. Current Science. 89 (11): 1870-1878.

Genovesi, A.D. and Magill, C.W. 1979. Improved rate of callus and green plant production from rice anther culture following cold shck. Crop Sci. 19: 662–664.

Maheshwari, S.C. Tyagi, A.K. and Malhotra, K. and Sopory, S.K. 1980. Induction of haploid from pollen grains in angiosperms – the current status. Theor. Appl. Genet, 58: 193–206.

Reddy, V.S., Leelawathi, S. and Sen, S.K. 1985. Influence of genotype and culture medium on microspore callus induction and green plant regeneration in anthers of Oryza sativa. Physiol. Plant. 63: 309–314.