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Effects of Genotype on Induction of Callus and Plant Regeneration Potential *in vitro* Anther Culture of Rice (*Oryza sativa* L.)

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Abstract: The present studies were carried out to evaluate the response of anthers of six rice genotypes to callus induction and plant regeneration. For callus induction Z_2 media supplemented with 2, 4-D 0.5 mg l^{-1}, NAA 2.5 mg l^{-1} and Kinetin 0.5 mg l^{-1} was used. Of the six genotypes, five gave good callusing response. Highest callus induction was observed in BRRI Dhan-29 (8.06%) and lowest in BR-10 (1.42%). Callus induction frequency varied from 1.42-8.06% depending on genotypes. Modified MS medium supplemented with Kinetin 0.5 mg l^{-1}, BAP 2 mg l^{-1} and NAA 1.0 mg l^{-1} was used for plant regeneration. The results showed a significant genotypic difference in callus induction and plant regeneration. The regeneration of plantlet from anther derived calli ranged from 57-75% conclusion.

Key words: Anther culture, callus induction, genotype

INTRODUCTION

Bangladesh is a developing country. About 150 million people of Bangladesh live on Rice. The population of Bangladesh is increasing at an alarming rate but the land is not increasing accordingly. With the increasing demand of rice and to meet the challenge of 21st century rice varieties are needed with higher yield potential and multiple resistance to diseases, insects and resistance to abiotic stresses. Application of anther culture will provide a new avenue us to develop and improve rice varieties for meeting the challenges of increase rice production.

Anther culture has been used for crop improvement and the development of cultivars through anther culture has advantages over the conventional breeding[1-3]. Anther culture has proved to be very useful tools in plant breeding, providing a rapid method for regeneration of desired genotypes. This technique opens new vistas of breeding for resistance and quality of crop plants, not only shortening the breeding cycle by immediate fixation of homozygosity but also increasing selection efficiency. Scientists working on rice in different countries in the word have successfully produced callus and plant from anther and from isolated pollen[4-8, 21]. Several rice varieties have been developed through anther culture[9] though in *indica* rice callus induction and subsequent plant regeneration from cultured anthers have rather difficult compared to japanica. Genotypic variation in anther culture response have been reported by Guha-Mukerjee[10]; Pandey *et al.*[11] and Drazebal[12]. Many other scientists[13-16] also reported that genotypes played an important role in callus induction and plant regeneration. The success of anther culture mostly depends on genotype of the donor material. Genotypic variability in callus induction and plant regeneration through anther culture have not been studied extensively in major rice varieties in Bangladesh. This study considers the variation in callus induction and plant regeneration potential among six genotypes of rice *in vitro* anther culture.

MATERIALS AND METHODS

Six cultivars of rice viz, BR-3, BR-7, BR-10, BR-14, BRRI Dhan-29 and BR-802-78-2-1-1 were used for conducting the experiment. Boots of these cultivars were collected from the experimental field of Rice Research Institute, Rajshahi, Bangladesh in boro season, 2002 at the stage when the distance between flag leaf and the penultimate leaf is between 5 to 10 cm. Cold treatment was performed by sealing the panicles in polyethylene bags and refrigerated at 6°C for 7 days. Cold treated panicles were removed from the refrigerator and were placed in the laminar flow bench. Leaf sheaths enclosing the panicle were sterilized by dipping intact in 70% ethyl alcohol for 1 min just before inoculation of anther.

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Anthers were separated from the panicle with the help of forceps and put in 6 cm petridishes containing callus induction medium. For callus induction Z_2 medium modified H_5[17] supplemented with 2,4-D 0.5 mg l^{-1}+NAA 2.5 mg l^{-1}+Kinetin 0.5 mg l^{-1} was used. The pH of the medium was adjusted to 5.8. Petridishes were sealed with parafilm and incubated at 26±1°C in the dark for callus induction for 4-6 weeks. Three petridishes were used for per replication. Calli were formed within 4-6 weeks. The culture was examined every week for upto 6 weeks. Data was recorded on percentage of anthers formed calli in 6 weeks (callus induction frequency). Anther derived calli were transferred to regeneration medium MS+Kinetin 0.5 mg l^{-1}+BAP 2 mg l^{-1}+NAA 1.0 mg l^{-1} for regeneration and was exposed to 12 h photoperiod maintained by fluorescent tube light. The response of cultured anthers was calculated by counting the number of anthers forming calli multiplied by hundred and divided by total number of anthers plated.

RESULTS AND DISCUSSION

After performing cold treatment of panicles of six genotypes of rice followed by sterilization, anthers were separated from the panicle and cultured on Z_2 medium for callus induction. The culture was examined every week for upto 6 weeks. Callus was developed from the anthers within 4-6 weeks. The induction of multiple calli from a single anther was also observed. Calli were mostly creamy white in colour and of variable texture (Table 1).

From the results (Table 1), it was evident that callus was induced in all the genotypes but the frequency of callus induction varied from 1.42 to 8.06% depending on the genotype. BRRI Dhan-29 responded the highest callus induction frequency (8.06%) followed by BR-7 (4.60%), BR-3 (3.43%) and BR-802-78-2-1-1 (3.20%). Induction was found to be poor in BR-10 (1.42%). BRRI Dhan-29 was the highest callus inducer (Fig. 1). Variations in callus induction ability among the tested varieties indicated that the difference in response was due to difference of genotype. Our results are higher than that of reported by results of Hakim *et al.*[18], Abe and Futsufare[13], Guo and Cao[14], Wu and Chen[16]. They reported that genotypes played an important role in callus induction and plant regeneration. Chen[19] reported that pre-treatment of rice panicles at 10°C for 48 h greatly increased the frequency of callus induction. Sharmin and Bari[20] studied and reported that cold shock of rice panicles at 6°C for 7 days increased the frequency of callus induction and plant regeneration. Increased rate of callus induction was found in all the genotypes by applying cold treatment reported by Sharmin and Bari[20] in our Present study compared to our previous published results.

Table 1: Callus induction frequency of anthers of different rice genotypes

Genotypes	No. of anthers inoculated	No. of anthers formed calli	(%) callus induction[1]	Texture of callus	Colour
BR-3	320	11	3.43	Compact	Creamy
BR-7	260	12	4.60	Compact	White
BR-10	350	5	1.42	Compact	White
BR-14	270	7	2.60	Compact	White
BRRI Dhan-29	310	25	8.06	Compact	White
BR-802-78-2-1-1	280	9	3.20	Compact	White

Table 2: Frequency of plantlet regeneration from anther derived calli of different genotypes of rice

Treatment	Genotypes	No. of anther derived calli	No. of calli regenerated	% of calli regenerated plantlet[1]
MS+0.5 mg l^{-1}	BR-3	11	7	63.6
Kinetin+2mg l^{-1}	BR-7	11	8	72.7
BAP+1.0 mg l^{-1}	BR-10	5	-	-
NAA	BR-14	7	4	57.0
	BRRI Dhan-29	24	18	75.0
	BR-802-2-1-1	8	4	62.5

[1]Average results of three replications

Fig. 1: Initiation of callus from cultured rice anther

Fig. 2: Regenerating plantlets from rice callus

For regeneration of plantlets anther derived calli obtained from different genotypes of rice were transferred to MS media supplemented with Kinetin 0.5 mg l^{-1}+BAP 2 mg l^{-1}+NAA 1 mg l^{-1} (Table 2).

The regeneration efficiency of BRRI Dhan-29 was found to be highest (75%) among the genotypes tested (Fig. 2). A highly significant difference was observed among genotypes for both callus induction and plant regeneration. Maximum regeneration efficiency was recorded in BRRI Dhan-29 (75%) and lowest in BR-14 (57%) (Table 2). However, low plant generation frequency is one of the most serious limitation in anther culture

technique in *indica* variety, BR-10 did not regenerate any plantlet. Anther culture technique is to be more applicable in breeding it is necessary to undertake further research on anther culture to establish dependable regeneration system in rice improvement.

REFERENCES

1. Hu Han, 1985. Use of haploid in crop improvement. In Biotechnology in International at Agric. Research, IRRI, Phillippines, pp: 75-84.
2. Shih-wei, L. and X. Zhi-Hong, 1991. Anther culture for rice improvement in China. In : YPS Bajaj (Eds.) Rice Biotechnology in Agriculture and Forestry, 14: 151-179, Springer, New York..
3. Meifang, L., 1992. In : Kangle Z and Murashige T (Eds.) Anther culture for rice breeders, CNNRI, Hangzhou, China, pp: 75-86.
4. Niizeki, H. and K. Oono, 1968 Induction of haploid rice plants from anthers culture, Proc. Japan, Acad., 44: 554-557.
5. Bishnoi, U., R.K. Jain, J.S. Rohilla, U.K. Chowdhury, K.R. Gupta and J.B. Chowdhury, 2000. Anther culture of recalcitrant indica × Basmati rice hybrids, Euphytica 114: 93-101.
6. Sharmin, S. and M.A. Bari, N.A. Siddique, N. Khatun, M.H. Rahman and M.E. Haque, 2003. Induction of Haploid Rich Plants Through in *vitro* Anther Culture. Pakistan J. Biol. Sci., 6: 1250-1252.
7. Sandhu, J.S., M.S. Gill and S.S. Gosal, 1993. Callus induction and plant regeneration from cultured anthers of indica rice varieties Plant Tissue Culture, 3: 17-21.
8. Hu Han, 1978. Advances in anther culture investigations in China, Proceeding of the Symp. on plant tissue culture 25-30 May, Peking, pp: 3-10.
9. Ying, C., 1983. Anther and Pollen culture of rice in China, Proc. workshop on cell and tissue culture techniques for cereal crop improvement Science Press, Beijing, pp: 27-46.
10. Guha-Mukerjee, S., 1973. Genotype differences *in vitro* formation and embryoids from rice pollen J. Exp. Bot., 24: 139-144.
11. Pandey, S.K., B. Ramesh and P.K. Gupta, 1994. Study of effect of genotype and culture medium on callus formation and plant regeneration in rice (*Oryza sativa* L), Indian J. Genetic and Plant Breeding, 54: 293-299.
12. Draz, A.E., J. Zapataj and G.S. Kush, 1991. Development of dihaploid lines through anther culture (1) IRRI News Letter, 16: 6.
13. Abe, T. and Y. Futsufara, 1984. Varietal differences of plant regeneration from root callus tissue in rice Japan. J. Breed., 34: 147-155.
14. Guo, C.Y. and Z.Y. Cao, 1982. Effect of different genotypes on induction frequency in anther and scutellum, culture of maize *in vitro* Hereditas, China 4: 8-10.
15. Kucherenko, L.A. and G.G. Mamaeva, 1980. Callus froamtion adn organogenesis in the tissue culture of various rice varieties Sel Skohozyaist Vennaya Biolosiya, 15: 384-386.
16. Wu, C.Y. and Y. Chen, 1987. Study of differences between genotypes in anther culture of Japonica Rice Act. Genetica Sinica, 14: 168-179.
17. Huang, H.S., T.H. Ling, P.L. Teseng, Y.L. Shin and P. Shi, 1978. Studies on composition of culture medium for (*Oryza sativa* L.) Subsp hsienby methods of mathematical analysis. In Proc. 1977. Syrmp on Plant Tissue Culture, pp: 244-246.
18. Hakim, L., A.J. Miah and M.A. Mansur, 1991. *In vitro* plant regeneration in rice through anther culture, Plant Tissue Culture, 1: 85-89.
19. Chen, C.C., 1976. Studies on anther culture of rice, Pollen stage and low temperature treatment. Natl. Sci. Coun. Mon. (Taiwan), 4: 49-52.
20. Sharmin, S. and M.A. Bari, 2003 Studies on the effect of cold shock on the induction of callus and plantlet regeneration frequency in anther culture of rice. Journal of Genetic and Bio-technol.
21. Sharmin, S. and M.A. Bari, 2002. Influence of Phytohormones on callus induction and plant regeneration through *in vitro* anther culture of rice. J. Bio Sci., 9: 111-115.