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CALLUS INDUCTION AND PLANT REGENERATION IN ANTHER CULTURE OF RICE (*Oryza sativa* L.)

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Abstract: The effect of genotypes and physical state (liquid and semisolid) of the N_6 medium on callus formation and subsequent plant regeneration from cultured anthers of six rice varieties (*Oryza sativa* L.) were examined. Significant differences were observed among the genotypes in regard to anther response to callus induction and subsequent plant regeneration. Average callus induction rate in liquid and in semisolid media did not differ significantly. Callus induction frequency of the genotypes varied from 1.2% to 64.0% and regeneration frequency varied from 0 to 56.5%. The traditional variety Chini Atab produced both callus and regenerated plants in maximum frequencies; 64% and 56.5% respectively.

Key words: Rice, Anther, Callus, Plant regeneration, Haploid.

Introduction

Rice (*Oryza sativa* L.) belongs to the family Gramineae. Rice is the major source of calories for 40% of the world's population (Datta, 1981). Worldwide rice ranks second to wheat in area harvested; but it ranks first as a food crop, providing more calories/ha. It is grown all the year round in Bangladesh. Its importance as staple food emphasizes its improvement. Undoubtedly, a considerable improvement was done through conventional rice breeding. Nevertheless, many countries are now employing different techniques of biotechnology including anther culture for varietal improvement of crops plants. Anther culture as a tool in plant breeding has several advantages:

i). It speeds up the breeding cycle by fixing homozygosity in one generation.

ii). It allows an increase in selection efficiency due to better discrimination between genotypes within any generation of desirable genes in later generations (Zapata *et al.;* 1995).

iii) It can be considered complimentary to mutation breeding because both dominant and recessive genes will be phenotypically expressed allowing easier isolation of desirable recessive mutations (Rownak *et al.*; 2000).

Since the first use of anther culture technique in rice (Niizeki and Oono, 1968), there has been a steady increase not only in efficiency of the technique but also in number of varieties and hybrids where androgenesis is possible. Although earlier studies indicate that only *Japonicas* were capable of regenerating sufficient number of doubled haploids, on which selection can be practiced, but it is now also possible to induce high regeneration efficiency in *Indicas* (Reddy *et al.*, 1985). Nevertheless, the anther culture technique is widely used for practical rice breeding; its application is still limited by many factors which influence culture efficiency, such as the genotype of the explant (Shen *et al.,* 1982 and Li, 1991*),* the growing conditions of donor plants (Chen, 1988), the developmental stage of the microspores (Chen, 1977 and Genovesi *et al.,,* 1979), pretreatment of panicles (Qu and Chen, 1983), the culture methods (Chen, 1977; Yang and Zhou, 1979), the media (Chen, 1977; Sun *et al.*, 1990) and the culture conditions (Qu and Chen, 1983; Wang *et al.*, 1977).

The present research work was therefore; undertaken with the following specific objectives:

i). to study the ability of six rice varieties on callus induction and plant regeneration from rice anther culture.
ii). to study the influence of liquid and semisolid states of culture medium on callus induction and subsequent plant regenerations.

Materials and Methods

The present experiment was conducted in 2000-2001 at the laboratory of Genetics and Plant Breeding of Agrotechnology Discipline, Khulna University. Six rice varieties comprising two traditional such as Chini atab and Ratna and four modern varieties- BR-11, BR-23, BR-28 and BR-30 were tested to determine their variability in callus induction from cultured anthers and subsequent plant regeneration.

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At the late uninucleate stage (when the distance between the base of the flag leaf and the auricle of the last leaf was 3-6 cm.), the panicles were collected between 8 and 10 am and treated 8 days at 4-8^{0}C in sealed polythene bags in order to enhance callus production. Cold treated panicles were thoroughly washed in tap water and were placed in the laminar flow cabinet and were surface sterilized by immersing in 70% ethanol for 20 seconds and then in 0.2% $HgCl_2$ solution for 10 minutes, washed 3 times with sterile distilled water. Only those panicles were selected in which maximum anthers had reached 50% length of the spikelets and were green in colour. Fifty to sixty spikelets were taken at a time on sterile petridish and when surface was dried, individual spikelets were cut at the base to free the anthers from the filaments. After that, with the help of sterile forceps and needle, the anthers were plated to conical flask containing N6 medium. The culture was incubated in the dark at 26±1^{0}C for one month and then under 16 hours' photoperiods at about 3000 lux.
Two different states of N_6 medium (Chu *et al*, 1975)) were used for callus induction, liquid and semi solid (with 4% bacto-agar), containing 60g L^{-1} sucrose, 2,4 D-2 mgL^{-1} and kinetin 0.05 mgL^{-1}, p^H was adjusted to 5.8.

The induced calli were transplanted into test tubes containing MS medium (Murashige and Skoog, 1962) supplemented with 0.5 mgL^{-1} α-NAA, 3 mgL^{-1}kinetin, 50 gL^{-1}sucrose, 8 gL^{-1} agar and adjusted to p^H 6.5. The calli were incubated in a growth chamber at 26±1^{0}C with 16 hours of light, at a light intensity of about 2000 lux.

In the 7th week after inoculation of anthers, callus induction frequency was calculated on the basis of the number of anthers producing callus. Regenerated plants were counted on the basis of the number of callus producing plantlets. The frequency of callus induction and plant regeneration were calculated as below:

$$\text{Callus induction frequency (\%)} = \frac{\text{No. of anthers producing callus}}{\text{No. of anthers plated}} \times 100$$

$$\text{Plant regeneration frequency (\%)} = \frac{\text{No. of calli regenerated plantlets}}{\text{No. of calli plated for regeneration}} \times 100$$

Analysis of Variance (ANOVA) was done to determine the effect of genotypes and media on callus induction.

Results

Callus induction: The results of callus formation from plated anthers of six rice varieties are presented in Table 1. Results of callus induction on liquid medium indicated that calli were induced from all genotypes except BR-23. However, induction frequencies varied with the genotypes studied and ranged from 2%-59.5%. The variety Chini atab produced callus with higher frequency (59.5%) while variety BR-11 and Ratna showed poor response in callus induction; 2% and 9% respectively. The mean frequency of callus induction on liquid medium was 19.8%.

Table 1. Callus induction from cultured anthers of six rice varieties:

Varieties	*Liquid N_6 medium*			*Semisolid N_6 medium*			*Mean frequency per variety (%)*
	Numbers of anthers plated	Numbers of anthers producing callus	Frequency of callus induction (%)	Number of anthers plated	Number of anthers producing callus	Frequency of callus induction (%)	
BR-11	150	3	2	200	6	3	2.5a
BR-23	100	0	0	250	6	2.4	1.2a
BR-28	250	69	27.6	100	31	31	29.3b
BR-30	250	52	20.8	100	9	9	14.9c
Chini atab	200	119	59.5	200	137	68.5	64.0d
Ratna	200	18	9	250	47	18.8	13.9c
Mean frequency per medium			19.8			22.1	

Mean frequency of callus induction followed by the same letter is not statistically different

Results of callus formation on semisolid medium (Table 1) showed that all the six rice varieties responded to callus formation, which varied from 2.4% to 68.5%, with a mean value of 22.1%. Chini atab produced maximum (68.5%) and BR-23 produced minimum (2.4%) callus. It was observed that the mean induction

frequencies on liquid and semisolid media did not differ significantly. However, comparison among the mean induction frequencies of the six rice genotypes showed that callus induction frequency of BR-11 and BR-23 significantly lower than other varieties. Similarly, induction frequency of Chini atab was significantly higher than the rest rice varieties studied (Table 1.).

Regeneration of plants: Calli obtained from the cultured rice anthers were subcultured on agarified MS medium. The results of the plant regeneration from plated calli of four rice varieties are presented in Table 2. A total of 274 calli, derived from the anther of four different rice varieties were plated on regeneration MS medium and 42.7% calli formed regenerated plants. It was noticed that calli derived from the variety BR-28 lost their ability to produce plants and died, while others differentiated into green and albino plants.

Table 2. Plant regeneration efficiency from plated calli of four rice varieties:

Varieties	*Number of calli plated*	*Number of regenerating plant*			*Frequency of plant regeneration (%)*		
		Total	Green	Albino	Total	Green	Albino
BR-28	30	0	0	0	0	0	0
BR-30	36	5	0	5	13.9	0	13.9
Chini atab	184	104	25	79	56.5	13.6	42.9
Ratna	24	8	3	5	33.3	12.5	20.8
Total	274	117	28	89	42.7	10.2	32.5

Frequencies of regenerated calli of the varieties BR-30, Chini atab and Ratna were 13.9%, 56.5% and 33.3% respectively. Out of these three varieties Chini atab and Ratna produced both green and albino plants, but BR-30 produced only albino plants. The frequency of green regenerated plants was very low, which counted to 13.6% for Chini atab and 12.5% for Ratna.

Discussion

In the present experiment six rice varieties were tested on liquid and semisolid N_6 medium to study their ability to callus formation and subsequent plant regeneration. The mean frequencies of callus induction on the two states of media were found insignificant. However, the variety BR-30 produced significantly higher rate of callus on liquid medium than that of on semisolid medium and the variety Ratna showed significantly higher performance in callus induction on semisolid medium than on liquid medium. Although, many authors reported that liquid medium was found to be superior to agarified medium for embryo/callus induction in cultured anthers of wheat (Lazen *et al.,* 1985), rice (Xie *et al.* 1995) and several other cereal crops.
The average mean frequencies of callus induction on the two media, studied in the present study varied from 1.2% to 64% and was significantly depended on genotypes (Table 1.). Several workers have already studied the factors affecting callus induction. Besides, genetic variation, stage of pollen, constituent of culture medium, pretreatment of anthers with cold shock (Nitzsche and Norrel, 1973) and environment (light, temperature etc.) of incubation period influence callus induction. However, the genotype of the pollen plant has the greatest influence on the formation of callus (Chu, 1982).

Plant regeneration frequency in this experiment varied from 0 to 56.5% and a total of 42.7% calli regenerated into plant, out of this only 10.2% plant were green and 32.5% were albinos. The variation in plant regeneration potential among the varieties, studied indicated that the dissimilar response was perhaps due to different genotypes. Earlier researchers (Abe and Futsufara, 1984; Guo and Cao, 1982; Rahim *et al.,* 1991; Samad *et al.,* 1996) also reported that genotypes played an important role in callus induction and plant regeneration. Occurrence of albino seems to be a common phenomenon in pollen plants of Gramineae. The high frequency of albino plants formation from microspore-derived calli has been a formidable obstacle to use the anther culture technology in rice breeding (Hakim *et al.* 1991). Several investigators reported that the formation of albino plants lacking chlorophyll could have a genetic (Loo and Xu, 1986) or a physiological origin (Torrizo and Zapata, 1986). Culture temperature also influences in the frequency of albino formation. A rise in temperature increases the frequency of the albinos (Wang *et al.,* 1977).

Conclusion

The results of this study revealed that callus induction and plant regeneration are depended on genotypes of the varieties. The anther culture technology offers enormous opportunities like mutant plant selection, development of homozygous lines within one year, which reduces total breeding cycle from three to four years. The results from this study suggest a need for increasing callus and plant regeneration in the materials

studied and point toward the need to optimize the other necessary requirements like media composition, incubation environment etc. to maximize the frequency of callus induction and regeneration of green plants.

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