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Induction of Haploid Rice Plants Through *in vitro* Anther Culture

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Abstract: Anthers of three rice cultivars having late uninucleate microspores were studied for induction of haploid rice plants. Anthers were cultured on Z_2 medium supplemented with various combinations and concentrations of auxins and kinetin (cytokinin). The best callusing from cultured anthers obtained in Z_2 medium containing 2,4-D 2 mg l^{-1}, NAA 2.5 mg l^{-1} and kin 0.5 mg l^{-1}. For regeneration of haploid plantlets anther derived calli were transferred to modified MS medium enriched with kin 1 mg l^{-1}, NAA 1 mg l^{-1}, BAP 1.0 ml l^{-1} and incubated at 25±1 °C in light. BRRI Dhan-29 produced both green and albino plants while BR-3 produced green plants only. Green plantlets obtained from BRRI Dhan-29 were examined and found to be 69.2% haploid plants.

Key words : Androgenesis, haploids, microspore, chromosome

Introduction

The production of haploid plants from immature pollen grains technique offers a rapid development of homozygous lines for early release of new crop varieties. Development of cultivar through anther culture has been advantages over the conventional breeding techniques (Han, 1985 and Snape, 1981). The induction of haploid rice plants has been receiving attention by the rice breeders all over the world during the last few years (Chen and Lin, 1976; Sunderland, 1974; Chu, 1982; Collins, 1977 and Oono, 1975). The androgeneic haploid plant was achieved for the first time by (Guha and Maheshwari, 1964). In the subsequent year Bourgin and Nitsch (1967) raised androgenic haploidy of *Nicotiana tabaccum*. (Niizeki and Oono, 1968) reported the first production of haploid rice plants from anther culture. Haploid plants have the genotypic number of chromosomes, that a single set of chromosomes in the sporophyte. Haploids may be induced by different techniques, the most promising and successful one is microspore androgenesis. In culture, microspore undergo various modes of androgenesis which lead to the formation of haploids either directly or indirectly via callus formation. The haploid plants are very important for the production of homozygous plants and induction of mutations. Their significance for plant improvement and as a tool in various disciplines of plant sciences has been stressed (Kasha, 1974).

Androgenic haploids have been produced more than 50 genera but the greatest effort has been given to economically important crops such as cereals and vegetables (Cao *et al*., 1995). The novel innovative approach can be attained by spontaneous doubling of the chromosome complements of haploid material, which is much quicker in method of inducing homozygosity and stability in the plant genome, compared to the conventional breeding approach. Haploids, with their unique genomic constitution as a results of meiotic recombination, have potential for accelerating or shortening the time required for the development of homozygous new improved rice varieties (Zapata *et al.,* 1983, Chen, 1977; Chen *et al.,* 1991). In China 100 varieties and lines of rice were reported to have been developed through anther culture (Yin *et al.,* 1976). Through anther culture techniques have been utilized in rice breeding programmes in China but not much works have been reported in induction of haploid rice plant from anther culture in Bangladesh. The purpose of the study was to produce haploids rice plants through *in vitro* anther culture for possible utilization in rice breeding programme.

Materials and Methods

Three indica rice cultivars namely, BR-3, BR-10 and BRRI Dhan-29 were grown in the experimental Garden of Regional Rice Research Institute, Rajshahi, Bangladesh during February-May, 2002.

Closed flower buds (Boots) of rice cultivars having the late uninucleate microspores suitable for induction of androgenesis were collected for conducting the investigation. Identification of late uninucleate stage was made as described by Challef and Stolarz (1981). Panicles thus collected were wrapped in a moist muslin cloth, sealed within polyethylene bags and then subjected to cold shock at 6°C for 7 days in the dark.

Cold treated panicles were cleaned. Individual spikelets from the middle of the panicles were taken out and put in clean and sterilized petridishes. The spikelets were

sterilized by dipping into 70% ethanol for 3-5 seconds. Anthers were picked up from the central spikelets of these panicles by sterilized forceps and then placed horizontally on the culture medium Z_2, modified H_5 (Huang *et al.*, 1978) aseptically for induction of callus. Precaution was taken during inoculation to avoid injury to the anthers. Adequate measure was also taken to avoid contamination and operation should be done in a 'Laminar air flow' chamber. The culture was incubated at 26±1 °C in the dark. After 3-4 weeks of incubation the pollens of the responsive anthers of the cultivars started to produce callus. For regeneration of haploid rice plantlets anthers derived calli were transferred to modified MS (Murashige and Skoog, 1962) medium supplemented with kin 1.0 mg l^{-1}, NAA 1.0 mg l^{-1} and BAP 1 mg l^{-1}. Rice root tips of regenerated plants were prepared for determination of haploidy chromosome as described by Rush (1981). Chromosome numbers were determined by chromosome counting of root tips.

Results and Discussion

The anthers of three cultivars of rice viz. BR-3, BR-10 and BRRI Dhan-29 were collected at late uninucleate stage and cultured on Z_2 medium containing 2,4-D 2 m l^{-1}, NAA 2.5 mg l^{-1} and kin 0.5 m l^{-1} for induction of callus. All the three cultivars responded to callus formation. The results are presented in Table 1.

The frequency of induction of callus accounted to 3.2% in BR-3, 1.85% in BR-10 and 8.23% in BRRI Dhan-29 (Table 1).

From results in Table 1 it was observed that BRRI Dhan-29 responded the highest callus induction frequency (8.23%). This showed that BRRI Dhan-29 was more responsive to callus induction than the other two tested cultivars. The frequency of anther forming calli varied from 1.85% to 8.23% depending upon the genotype. The anther derived calli was compact, and white in colour. The development of callus is shown in Fig. 1.

The results of the induction of callus observed in the study are in agreement with the results obtained by Chen and Lin (1976) and Hakim *et al.* (1991) who studied indica rice for callus induction. They found poor callusability in indica rice variety.

For regeneration of haploid rice plantlets anther derived calli were transferred to a modified MS medium enriched with kin 1.0 mg l^{-1}, NAA 1.0 mg l^{-1} and BAP 1.0 mg l^{-1}. For each regeneration experiment two replicate dishes were used and the results are averaged. Out of three cultivars BR-3 produced green plant, BR-10 produced albino only while BRRI Dhan-29 produced both green and albino plantlets. The data on the regeneration of haploid plants are recorded in Table 2.

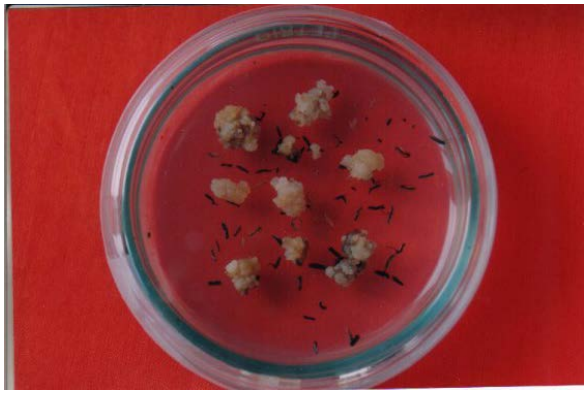

Fig. 1 : Induction of callus from cultured rice anthers

Fig. 2 : Regeneration of haploid rice plantlets from callus

Table 1: Induction of callus from the anther of three cultivars of rice

Treatment mg l^{-1}	Cultivars	No. of anthers inoculated	No. of anthers formed callus	Frequency of callus (%)	Days to initiation	Texture	Colour
Z_2+2,4-D 2mg l^{-1}+	BR-3	425	14	3.20	20-22	Friable	White
NAA 2.5 mg l^{-1}+	BR-10	325	06	1.85	22-24	Compact	White
kin 0.5 mg l^{-1}	BRRI Dhan-29	425	35	8.23	18-21	Compact	White

Table 2: Regeneration of haploid rice plantlets from the anther derived calli of three cultivars

Treatment mg l^{-1}	Cultivars	No. of callused anther plated	No. of plants regenerated			% of haploid rice plants obtained
			Green	Albino	Total	
MS + Kinetin 1.0mg l^{-1}+	BR-3	14	6	1	7	66.6%
NAA 1.0 mg l^{-1}+	BR-10	6	0	2	2	-
BAP 1.0 mg l^{-1}	BRRI Dhan-29	25	13	3	16	69.2%

The highest callus inducing cultivars BRRI Dhan-29 also had a higher regeneration frequency (69.2%) of haploid plantlets. 16 plants were regenerated from anther derived calli of BRRI Dhan-29 where 13 were green and 3 were albinos (Table 2).

In regeneration medium some calli lost their ability to produce plant and died while the others differentiated into green haploid plantlet and albino. Significant variation was observed among the tested cultivars in regeneration of green and albino plants. Occurrence of albino plantlets is a common phenomenon in anther culture of cereals (Fig. 2).

During induction of haploid plants by using anther culture technique microspores contained within immature anthers were induced to form callus from which haploid rice plants were subsequently regenerated. The green plantlets regenerated from the anther calli were examined for their ploidy level. Out of 13 green plants regenerated from BRRI Dhan-29, it was possible to identify 9 as haploid plants (chromosome number, n=12) and 4 in BR-3 (Table 2). The frequency of regeneration of haploid in BRRI Dhan-29 was found to be 69.2% followed by 66.6% in BR-3. The findings in the present investigation are consistent with the results obtained by Raina (1997), Raina and Zapata (1997) and Zapata *et al*. (1983).

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