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Short Communication

Doubled haploid production through culture of anthers in rice

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Abstract

Rice anthers were cultured in liquid N6 medium containing various concentration of growth harmones. Haploid plants were treated with different doses of colchicine to develop doubled haploid (DH) plants. The DH plants were transferred to soil and seeds were harvested from mature plants. The DH plants would be suitable for genetics and molecular breeding activities.

Key words: Rice, anther, hybrid, microspore culture, salinity tolerance

Haploid technology is easy and efficient selection method of promising hybrids [1]. Kazakhstan is one of the northern most countries of the world where rice is grown successfully. For development of high yielding rice in Kazakhstan, anther culture technology has high promise [1]. The main objective of this study was therefore to regenerate doubled haploid rice plants through anther culture of local rice *in vitro.*

Hybrids of different generations of sorts of rice *viz*., SGP-180F_1, HS-205F_1, HS-206F_1, HS-207F_1, HS-208F_1, SPE-21F_5, HS-176F_5, HS-180F_5, HS-186F_5, BR-1-F_1, BR-3-F_1, BR-4-F_1, BR-5-F_1, BR-6-F_1, BR-7-F_1, BR-8-F_1, BR-9-F_1 and BR-10-F_1 grown in Kazakhstan and Russia were used in the study. The parents namely, Marjan, Madina, Backnassk, Khazar, Regal, Rapan Violetta, Analoguez, Akdla and Lider were involved in the hybrids.The phenological observations were recorded out on the hybrid (1st and 5th generations) as per standard procedures [2]. The panicles were collected at booting stage and sterilized with 70% ethnol. The anthers were pre-treated at 8^{o}C for 3 days. The N6 medium with 2 mg/L of 2,4-dichlorophenoxyacetic acid (2,4-D) was used for callusogenesis of rice anthers. Rice regerants were grown on the modified MS medium. Colchicine treatment was given to double the number of chromosomes in the regenerated plants [3].

It was found that cold pretreatment facilitated synchronisation of cell division and maintained viability of embryogenic microspores. Liquid MS medium supplemented with 10 mg PAA/L, 12% of Ficoll400 and 90 g maltose/L was used to induce anther culture in suspension. The first androgenic structures, i.e. embryos, were observed on the 20th day of culture. The embryos were cultured for another 18 days to increase their weight and on the 38th day they were transferred to agar MS medium supplemented with 0.5 mg NAA/L, 1mg BAP/L and 500 mg proline/L. This medium however appeared to be inefficient in regenerating embryoids.

Subsequently, the calluses grown on the N6 medium were transferred to agar MS medium containing various concentrations of plant hormones (BAP, kinetin, NAA, IAA). The medium containing 0.5 mg/L of NAA +1 mg/L of BAP +500 mg/L of proline however, produced albino-plants in some genotypes (HS-176F5, HS-207F1). Formation of albino plants is

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Fig. 1. Green regenerants hybrid rice genotypes BR F1-4 and HS-207F1

linked to mutations in the genes controlling the development of chlorophyll, chloroplast DNA [4], nuclear genome [5] and cytoplasmic genes [6]. Green regenerants of HS-176 F5, HS-207F1 and HS -208F1 genotypes showed no roots formation till 47 days. It indicated that these genotypes might require higher concentration of auxin.

Increase of concentrations of BAP up to 2 mg/L and addition of casein hydrolyzate and amino acids (proline and glutamine) stimulated regeneration of full green plants with well developed root and aerial parts for BR-4 F1 and HS-207F1 genotypes (Fig. 1).

The obtained results are consistent with findings of other researchers concluding that rice calluses are very responsive to the concentration of phytohormones and their regeneration are genotypic dependent.

Colchicine in combination with various hormones and cryoprotectants (gibberellin, DMSO and Tween 80) was used in our experiments to promote cell division. The first set of treatment included 0.05% of colchicine + 2% of DMSO + 10mg/L of gibberellin; and the second set included 0.25% of colchicines + 2% of DMSO + 20 drops Tween 80/L. After colchicine treatments, 20 plants out of 26 green regenerants (77%) survived. Such plants were transplanted under controlled sunlight and sprayed with hormone containing water (gibberellic acid 2 mg/L + nicotinamide 3 mg/L + kinetin 0.5 mg/L) for 5-6 days. New leaves appeared 6-8 days later indicating survival of the genotypes. Increased frequency of doubled haploid plant generation has been reported in rice earlier [7]. Seeds from the dihaploid plants were obtained from genotypes HS-207F1 and BR-4 F1 at maturity of the crop. The doubled haploids produced in this study would be suitable for genetics and molecular breeding activities in rice.

The next study was focused on the intensity of early growth (IEG) of dihaploid regerants (HS-207F1 and BR-4 F1). The regerants were collected in the saline solution of 0.75% NaCl. Typically, samples with high IEG showed high level of sprouts in rice field. Table 1 presents IEG data in comparison to the

Table 1. Height of 14-days old plants (IEG) grown in saline medium

Type/line	Height (cm)	Percent to standard
Marjan-standard	16.8	100
Dihaploid BR-4-F_1	16.1	96
Dihaploid BR -4 F_1	17.7	105
Dihaploid ÃÑ-207 F_1	19.3	115
Analogue 2	13.9	83
Akdala	11.8	71
Violetta (Krasnodar region)	6.6	39
HCP_{05}	0.94	-

standard sort Marjan and other regional sorts of rice.

Previously, a standard heterosis on average of 15-17% (115-117% of standard Marjan) was observed for the F_1 hybrids; however, it appeared in not more than 5-7% of the combinations. It is possible that the heterosis effect was fixed in combinations of BR-4-F_1 and GS-F_1 on the homozygous level as a result of androgen manipulation. Anther culture of rice *in vitro* produced green dihaploid plants with complete formation of seeds. Optimization of the nutrient medium to stimulate morphogenesis in isolated anther culture of rice for a wide range of genotypes is carried out. It is necessary to take into account the characteristics of different genotypes of a given crop culture, the pollen development stage, cold pretreatment duration, selection and optimisation of the nutrient medium, and cultivation conditions are the factors affecting the yield of green rice regenerants.

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