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Effect of Plant Growth Regulators on Callus Induction and Plant Regeneration in Anther Culture of Rice

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Abstract: An experiment was conducted to find the effects of different concentrations and combinations of growth regulators viz. 2, 4 D, IAA, α-NAA and Kinetin on callus induction from the anthers of a commercial hybrid rice line IR-69690, developed by Bangladesh Rice Research Institute and subsequent plant regeneration. N_6 medium was used as basal medium. Callus induction frequencies in different media combinations ranged from 1.2 to 35.5%. The medium supplemented with 2, 4 D 1 mg L^{-1}, α-NAA 2 mg L^{-1} and Kinetin 1 mg L^{-1} was found most effective for callus induction (35.5%). Regeneration of plants from the callus on agarified MS medium supplemented with α-NAA 0.5 mg L^{-1} and Kinetin 3 mg L^{-1} was also variable and ranged from 16.7 to 69.3%. Calli derived from the media supplemented with 2, 4 D 1 mg L^{-1}, α-NAA 2 mg L^{-1} and Kinetin 1 mg L^{-1} also showed better performance for plant regeneration (69.3%) and among these plants 56.14% were green. However the callus induction medium containing α-NAA 1 mg L^{-1} and Kinetin 1 mgL^{-1} directly produced green regenerated plants in higher frequency (70%), without transferring the calli on to the regeneration medium; but rate of callus induction in this medium was very low (6%).

Key words: Hybrid rice, anther, callus, plant regeneration, haploid

INTRODUCTION

Rice (*Oryza sativa* L., 2n=2x=24) is one of the most important food crop and ranks second to wheat in area harvested; but it ranks first as a food crop, providing more calories ha^{-1}. Its importance as staple food emphasizes its improvement undoubtedly. A considerable improvement has been done by conventional rice breeding methods. Many countries are now employing different biotechnological method including anther culture for varietal development of crop plants[1,2]. Anther culture as a tool in plant breeding has several advantages. It speeds up the breeding cycle by fixing homozygosity in one generation. It allows an increase in selection efficiency due to better discrimination between genotypes within any generation of desirable genes in later generations

This technique can be considered complimentary to mutation induction because both dominant and recessive genes will be phenotypically expressed allowing easier isolation of desirable recessive mutations[3].

Since the use of anther culture technique in rice, there has been a steady increase not only in efficiency but also in varieties and hybrids where androgenesis is possible, whereas earlier only Japonicas were reported to be capable of regenerating sufficient number of doubled haploids, on which selection can be practiced[4]. It is now also possible to induce high regeneration efficiency in indicas[5].

While 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 explants[6] the growing conditions of donor plants[7] the developmental stage of the microspores[8,9] the culture methods[8,11] the culture conditions[10,18] the media and its components specially growth regulators[8,12]. In these circumstances the present study was undertaken with the following objectives:

To study the ability of a commercial hybrid rice line under study in callus induction from anthers and subsequent plant regeneration.

To determine the most suitable combination and concentration of growth regulators that enhances callus induction and plant regeneration.

MATERIALS AND METHODS

The present experiment was conducted in 2001 at the laboratory of Genetics and Plant Breeding of Agrotechnology Discipline, Khulna University. A commercial hybrid rice variety (IR-69690) developed by

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BRRI was used as source plant for anthers. The panicles were collected from the demonstration plot located at Dumuria Upazilla, Khulna. 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°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. The panicles were then surface sterilized by immersing them in 70% ethanol for 20 seconds followed by immersing in 0.2% $HgCl_2$ solution for 10 min. The treated materials 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 appropriate nutrient medium. The cultures were incubated in the dark at 26±1°C for one month and then under 16 hours photoperiods at about 3000 Lux.

The induced calli were transplanted into test tubes containing MS medium[13] supplemented with α-NAA 0.5 mg L^{-1}, Kinetin 3 mg L^{-1}, 50 g L^{-1} sucrose, 8 g L^{-1} agar. The plated calli were incubated in a growth chamber at 26±1°C with 16 h of light, at a light intensity of about 2000 Lux.

pH of the media both for callus induction and plant regeneration was adjusted to 5.6.

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 different combinations and concentrations of the selected growth regulators on callus induction.

RESULTS

Callus induction: In the present experiment anthers from a commercial hybrid rice line were used to study their callusability on different combinations and of 2, 4 D, α-NAA, IAA and Kinetin. Six different combinations of these four growth regulators were studied to determine the most suitable combination and concentration for callus induction and subsequent plant regeneration from plated calli. Data on callus induction was recorded twice-after 30 days and 45 days of inoculation of anthers.

The callusing ability of anthers on various combinations of growth regulators was significantly different ($p<0.001$). The results on callus induction are presented in Table 1. Callus induction frequencies were variable and ranged from 1.2 to 35.5%, with an average of 10.5%. Media Combinations 1, 2, 3, 4 and 6 showed very poor performance in inducing callus, which were 1.2, 4.5, 6.0, 2.7 and 13.2%, respectively. However highest frequency (35.5%) of callus induction was obtained on the medium 5, supplemented with 2, 4 D-1 mg L^{-1}, α-NAA-2 mg L^{-1} and Kinetin-1 mg L^{-1} and it was significantly higher than other combinations (Table 1).

Plant Regeneration: Induced calli (along with directly regenerated plants) were transferred to MS medium for their differentiation into whole plant. Results of plant regeneration from plated calli are presented in Table 2. Calli induced on media 1 and 4 were not cultured on regeneration medium due to their inconvenient size and numbers. Results of plant regeneration showed that calli

Table 1: Callus induction from rice anthers cultured on different media concentrations based on N_6 Basal medium

	Concentrations of growth regulators (mg L^{-1})					
Media	2, 4 D	α-NAA	IAA	Kinetin	No. of anthers inoculated	Frequency of callus induction (%)
1	2.0	0.0	0	0.05	250	1.2a±1.20
2	2.5	0.0	0	0.5	250	4.5ac±7.72
3	0.0	1.0	0	1.0	250	6.0ab±3.37
4	0.0	0.0	1	1.0	250	2.7a±1.76
5	1.0	2.0	0	1.0	250	35.5d±1.76
6	2.0	0.75	0	1.5	250	13.1bc±4.96
Mean						10.5±2.67

Note: Frequency followed by the same letter is not significantly different

Table 2: Regeneration of plants from plated calli on MS medium

Calli derive from the media	No. of calli plated	Frequency of plant regeneration		
		Green	Albino	Total
2	12.0	0.00	16.70	16.70
3	10.0	70.00*	0.00	70.00
5	114.0	56.14	13.16	69.30
6	22.0	4.55	50.00	54.55
Mean	39.5	32.67	19.97	52.64

* Plants regenerated directly on callus inducing medium

induced on all the media combinations studied, produced both green and albino plants except media 2 which produced only albino plants. Plant regeneration frequencies ranged from 16.7 to 70% with a mean value of 52.64%. Among the regenerated plants 32.67 and 19.97% were green and albino respectively. Calli induced on the medium combination 3 showed maximum plant regeneration capacity (70%) and all these plants were green and directly regenerated on the callus induction medium. Calli produced on medium 5 were also found highly efficient in plant regeneration. 69.3%of calli from this combination regenerated into whole plants, among these 56.14% were green and only 13.16% were albino plants (Table 2).

DISCUSSION

In the present investigation efficiency of different growth regulators at various concentration s were examined for callus induction from anthers and subsequent plant regeneration. The first two media compositions in the table 2, composed of 2,4D and kinetin did not show any significant differences for callus induction and overall callus production in these media was very low. However a number of authors reported that 2, 4 D in combination with kinetin responded efficiently both in callus formation and plant regeneration[17,8]. This dissimilarity may be due to use of different concentration level of these growth regulators.

Media compositions 3 and 4 were devoid of 2,4 d and this growth regulator was replaced by α-NAA 1 mgL^{-1} (medium 3) and by IAA 1 mg L^{-1} (medium 4). Concentration of Kinetin for both the media was 1 mg L^{-1}. In this case no significant increase in callus induction was found in comparison with previous media compositions. However callus induction rate was somewhat increased with the addition of α-NAA (Table 2) and a higher percentage of direct regenerated plants was obtained from this medium (Table 3).Inoue and Maeda[14] reported that 2, 4 D and α-NAA are equally effective in inducing callus, but 2, 4 D may be inhibitory to morphogenesis in the callus, but α-NAA has not such kind of effect. It was also noticed in the present study that addition of 2, 4 D or α-NAA individually in combination with kinetin have more or less similar effect in callus induction, but addition of α-NAA singly has much better effect on the formation of plant from the calli.

Media supplemented with 2, 4 D, α-NAA and kinetin resulted in significant increase in callus induction and their subsequent differentiation into plant. In this study the medium containing 2, 4 D 1 mg L^{-1}, α-NAA 2 mg L^{-1} and kinetin 1 mg^{-1} most efficiently influenced on callus formation, as well as on plant regeneration. Islam *et al.*[15] also observed that application of 2, 4 D and α-NAA in combination with kinetin could lead to an increase of callus induction and their subsequent plant regeneration in rice and wheat anther culture.

Albinism is a serious problem in graminae especially in rice anther culture[16]. In our investigation calli derived from the best combination containing 2, 4 D 1 mg L^{-1}, α-NAA 2 mg L^{-1} and kinetin 1 mg^{-1}, Showed very promising response in green plant regeneration and here the frequency of green plant production was 56.14% and only 13.16% of plants were albino.

Anthers collected from a commercial hybrid rice line (IR-69690) were inoculated on N6 basal medium supplemented with various combinations and concentrations of 2, 4 D, IAA, α-NAA and kinetin to study the callusability of the anthers and to determine the best combination and suitable concentrations of the growth regulators for maximum callusing and subsequent plant regeneration. The frequency of callus induction on different media, ranged from 1.2 to 35.5%. Plant regeneration frequency from the plated anthers was also variable and ranged from 16.7 to 70%. Calli derived from the medium supplemented with α-NAA 1 mg L^{-1} and kinetin 1 mg L^{-1} produced 70% green plants and all these plants were directly regenerated on callus induction medium without transplanting them into regeneration medium, but callus induction rate from this combination was only 6%. Combination with 2, 4 D 1 mg L^{-1}, α-NAA 2 mg L^{-1} and kinetin 1 mg L^{-1} was found most pronounced both in callus formation (35.5%) as well as in plant regeneration (69.3%) and among these, green and albino plants were 56.14 and 13.16%, respectively.

From the present investigation it can be concluded that the commercial hybrid rice line (IR-69690) is competent for anther culture study and by the addition of 2, 4 D 1 mg L^{-1}, with α-NAA 2 mg L^{-1} and kinetin 1 mg L^{-1} to callus induction medium a higher callusing and plant regeneration response can be obtained.

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