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Original Article

EFFECT OF COLD PRE-TREATMENT ON RICE ANTHERS FOR CALLUS REGENERATION FREQUENCY OF INDICA RICE (*Oryza sativa* L.) VARIETIES IR 28 AND IR 66

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Abstract: Present study was carried out with the objective to explore the healthy or detrimental effects of cold pre-treatment on callus regeneration frequency of indica rice varieties IR 28 and IR 66. It was found that cold pre-treatment had healthy and promotive effect on the rice anthers in both the varieties, yet varying temperature levels and durations of cold pre-treatment had significant impacts. Both the varieties IR 28 and IR 66 performed well with higher callus induction percentage of 7.5 and 6.0 which were produced at duration of 64.75 and 68.25 days, respectively, when the panicles of both the varieties were subjected to store at 8°C for 8 days.

Key words: Anther culture, Callus initiation, Indica rice

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important cereal crop in the world. Rice plays an important role in the growth of annual GDP in India by 15% and provides essential nutrition with 43% calorie to more than 70% of the total population [1]. To elevate the yield potential in rice, which has become static since the green revolution, hybrid rice was considered as an alternative to increase productivity per unit area. However, despite its yield advantage over inbreds, the hybrid rice technology has not found favours with the farmers of India.

The development of high yielding rice varieties with improved grain quality begins with the combined knowledge of biotechnology along with traditional breeding practices which can produce faster and more accurate results [1].

Anther culture is a revolutionary technique that can be utilized for the rapid development of homozygous plants. Pollen within the cultured anthers may be induced to form callus and subsequent organogenesis which will later regenerate doubled-haploid plants [2]. Research efforts on the enhancement of response to anther culture have been confined mostly on manipulation of callus induction and plant regeneration protocols [3]. Giving cold treatment to the rice panicles has collective advantage on callus and subsequent regeneration frequency [4].

The objective of the experiment on hand was to explore the healthy or otherwise effects of cold pre-treatment on callus regeneration frequency of both indica rice varieties IR 28 and IR 66. This study was carried out at Plant Tissue Culture Laboratory, Anand Agricultural University, Anand.

MATERIALS AND METHODS

Collection of explants: Anthers from the unopened flower buds (panicles) were collected to use as explants. Both the varieties IR 28 and IR 66 were

periodically grown and maintained in the nursery bed to safeguard the continuous supply of anther explant. The flower buds of rice plants were collected from vigorously growing diploid plants when the distance between the base of the flag leaf and the auricle of the last leaf was 3-6 cm [5]. Anthers collected at the time when the panicle length having 3-6 cm distance between the base of the flag leaf and the auricle of the last leaf were at uni-nucleate stage [2].

Cold pre-treatment: The panicles harvested between 8.30 am to 9.00 am, were placed in the flask containing distilled water and brought to the laboratory facility. They were then kept in polyethylene bag for cold pre-treatment in cold cabinet facility at 4° and 8°C for three varying durations viz., 4, 8 and 12 days [6].

Glassware maintenance: All the glasswares were autoclaved for 30 minutes at 121°C (under 15 PSI) and then rinsed with tap water followed by initial cleaning by soaking in potassium dichromate solution for 12 hours. They were washed thoroughly with tap water jets with the objective to completely remove all the traces of dichromate solution. They were then soaked in detergent solution (Laboline 0.1%) overnight, thoroughly washed with tap water and rinsed twice with single distilled water. The glasswares were then dried in a hot air oven at 100°C for 24 hours [2].

Media composition and preparation: Prescribed standard procedures were followed to prepare N_6 media [4]. The stock solutions for both macro and micro nutrients, vitamins, iron EDTA and growth regulators were prepared with sterile distilled water. A known quantity of glass distilled water was added into the beaker and required quantities of sucrose, inositol and KH_2PO_4/ $NaH_2PO_4.H_2O$ weighed, added as solids and dissolved fully and then volume was made up to one liter with distilled water.

The pH of the solution was adjusted to 5.75 using 0.1N NaOH or 0.1N HCl before autoclaving. Media was gelled with agar (0.8%) and then distributed to test tubes (150 mm x 25 mm) at the rate of 14 ml each. The medium was allowed to cool down to room temperature and stored at a cool-dry place until use. The growth regulator was dissolved in 2 to 5 ml NaOH with slight heating and gradually diluted to 50 ml with double distilled water. Two auxins *viz.*, 2,4-D and NAA along with cytokinins *viz.*, kinetin at varying concentrations were used. All the media compositions were supplemented with 6 per cent sucrose and 0.8 per cent Agar.

Aseptic manipulation and surface sterilization: All the aseptic manipulations such as surface sterilization of explants, preparation and inoculation of explants and subsequent sub-culturing were carried out in the laminar air flow (LAF) chamber which was exposed to UV-light for 30 minutes and then it was left for 10 minutes without UV-light and unattended prior to use. To ensure maximum sterilization, the LAF bench was flame sterilized by 70 per cent alcohol/protozan.

Panicles of both the varieties were initially treated with Twin-20 detergent solution followed by 70% ethanol for 10 seconds and 0.1% $HgCl_2$ for 3 minutes. Autoclaved doubled distilled water was used to remove adherent chemicals at every stage of surface sterilization.

Inoculation of anther explants: Anthers kept for cold pre-treatment at 4° and 8°C temperature for 4, 8 and 12 days from both the varieties IR 28 and IR 66 were subjected to media inoculation at precise uninucleate stage using pointed forceps. Initially the inoculated cultures were incubated in the dark at 25 ± 1°C in an air-conditioned culture room to facilitate the callus initiation. Later on after callus initiation, they were incubated at the same temperature with a 16 hours' photoperiod (3000 lux) supplied by white fluorescent tubes [2].

RESULTS AND DISCUSSION

Cold pre-treatment is the method to arrest the stage of anthers which is vital for the callus induction. Both the varieties IR 28 and IR 66 under androgenic investigation were kept under cold pre-treatment at varying temperatures for varying periods of time. The panicles of rice were subjected to two different cold treatments (at 4° and 8 °C temperatures) for three different durations (4, 8 and 12 days) in order to determine the optimal cold pre-treatment which would be effective for callus induction. The results thus obtained are presented in Table 1. Both the varieties IR 28 and IR 66 performed well with higher rates of callus induction per cent 7.5 and 6.0 and days to callusing 64.75 and 68.25, respectively when the panicles of both the varieties were treated with C_5 (8°C for 8 days) in the cold cabinet facility.

It is clear from the Table 1 that, at 8°C temperature, both the varieties performed almost similarly with non-significant difference, however, IR 66 took longer duration to produce callus with lower callus induction per cent which is indicative of genotype specific response under all the similar set of treatments [7]. Cold pre-treatment above 8 days duration produced critically low callus for both the varieties (2.5% callus induction). Out of two, 8°C temperature showed statistically high callus induction per cent (4.17) after 10 weeks of inoculation (Table 2) [8]. In the present study, temperature as well as days of pre-treatment was found to influence early callus induction (Table 3). Cold pre-treatment of 8°C for 8 days showed early callus initiation (66.50 days) as compared to other treatments irrespective of varieties under consideration. Thus best cold pre-treatment was 8°C for 4-8 days to achieve more callus initiation within short duration in both the varieties under consideration [9,10].

The response to cold pre-treatment is genotype dependent. However, a temperature shock has been reported to improve the androgenetic response in rice [1,2]. Mode of division of microspores can be changed by giving cold temperature shock treatment which inhibits the spindle formation causing abnormal first meiosis resulting into arrested cellular stage and also by activating the explants for androgenesis and increasing the number of responding anthers [2]. The induction of microspores to sporophytic instead of gametophytic pathway was strongly influenced by cold pre-treatment of the anthers before culture [2,6].

CONCLUSIONS

From the above discussed results, it can be summarized that temperature had an insightful influence with both the intensity and duration of exposure, greatly controlling callus induction response. Low temperature pre-treatment was found to be advantageous in improving the androgenic response towards callus induction in both the varieties *viz.*, IR 28 and IR 66. IR 28 provided significantly early callusing under the similar set of cold pre-treatment than IR 66.

Treatment code	Temperature	Duration of exposure	Days to first callus induction		Callus induction (%)*	
			IR-28	IR-66	IR-28	IR-66
C_1	4°C	4 days	70.75	73.25	2.5 (1.716)	1.5 (1.363)
C_2	4°C	8 days	68.75	71.75	3.5 (1.958)	3.5 (1.986)
C_3	4°C	12 days	73.25	75.25	3.0 (1.851)	1.0 (1.144)
C_4	8°C	4 days	69.75	68.75	4.0 (2.093)	2.5 (1.716)
C_5	8°C	8 days	64.75	68.25	7.5 (2.824)	6.0 (2.534)
C_6	8°C	12 days	71.5	73.25	2.5 (1.716)	2.5 (1.716)
C_7	Control		0	0	0 (0.7071)	0 (0.7071)
S. Em.±			0.418		0.623 (0.172)	
C.D. at 5%			1.196		NS (0.890)	

Table 1: Effect of cold pre-treatment on callus induction in rice. * Square root (x +0.5) transformed values in parenthesis

Temperature (°C)	4°C	8°C
Callus induction per cent	2.50	4.17
S. Em.+	0.254	
C.D. at 5%	0.73	

Table 2: Callus induction per cent after 12 weeks of inoculation at different temperatures of cold pre-treatment

Duration of cold treatment (C)	Temperature (B)	
	4°C	8°C
4 days	72.00	69.25
8 days	70.25	66.50
12 days	74.25	72.37
S. Em.±	0.296	
C.D. at 5%	0.598	

Table 3: Temperature (B) and duration of cold pre-treatment (C) interactions for days to callus induction

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