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MANIFESTATION OF SOMACLONAL VARIATIONS IN REGENERATED *ORYZA SATIVA* CV. *BASMATI 370* THROUGH PRETREATMENT OF MATURE SEEDS WITH 2,4-DICHLOROPHENOXYACETIC ACID

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With the aim of studying the effect of pre-treating the explants before culturing, a set of pretreatment experiments were performed on mature seeds of *indica* rice variety *Basmati 370*. Liquid pretreatment medium with different concentrations of 2,4-D was used. Each pretreatment was given for durations of 24, 48 and 72 hours. It was observed that upon transfer of callus derived from pretreated seeds to regeneration medium, a lot of variations in terms of ratio of regenerated albino to green shoots occurred. Highest number of green shoots were obtained from seeds which were pretreated for 48 hours on pretreatment medium supplemented with 2.5 mg/l 2,4-D. However albino shoots were also produced. Maximum number of albinos were produced from seeds pretreated for 48 hours on pretreatment medium containing 1 mg/l 2,4-D. When the pretreatment period was increased to 72 hours, the ratio of albino shoots to green shoots increased. Since pretreatment results in increased stress conditions, somaclonal variations in the form of production of albino plants resulted in the cultures.

Keywords: Albino rice; Basmati 370; Pretreatment; Somaclonal variations.

Introduction

Tissue culture of rice is a routine exercise and is being used as a tool for not only mass propagation but also to aid genetic manipulation studies. But it has been felt that, though tissue culture systems have a lot of advantages, they come with inherent problems that at times drastically reduce the efficiency of the system. One such problem is the production of albino plant, a conspicuous manifestation of somaclonal variations. Somaclonal variations could be used in breeding. Moreover, somaclonal variations offer advantages that the variation can be induced in improved and agronomically acceptable cultivars without disrupting the genetic background and linkages of commercial advantages[1,2]. Thus, somaclonal variations can be used as a genetic manipulation tool. However, the biggest drawback is that it has yet to be worked out as to which particular set of conditions will effect which particular genotype and in what particular way. We studied the effect of 2,4-D pretreatment of seeds on regeneration of green/albino shoots.

Materials and Methods

Surface sterilization: Mature seeds of *indica* rice cv. *Basmati 370* were dehusked and surface sterilized using 0.1 percent (w/v) $HgCl_2$ solution for 4 to 6 minutes and subsequently rinsed thoroughly four times with sterile distilled water.

Pretreatment: 2,4-D (1, 2.5 and 5.0 mg/l) in the four types of pretreatments, each for a duration of 24, 48 and 72 hours were given to see the effect on callus induction, growth and subsequent regeneration response. Pretreatment was given using 3 liquid media as described in Table 1.

After pretreatment the seeds were cultured on MS medium[3] supplemented with 2,4-D (2.5 mg/l), sucrose (30 g/l) solidified with agar (8 g/l) at pH 5.8. The cultures were incubated at 26± 2°C under 1400 lumen per sq. m. light intensity at 16h photo period. The calli were sub-cultured on regeneration medium (RM1) supplemented with BAP (92 mg/l), NAA (0.5 mg/l), sucrose (30 g/l), solidified with agar (8 g/l) under the incubation conditions as mentioned earlier.

Results and Discussion

Callus induction occurred in all experiments. Percent explant undergoing callusing ranged between 91.1 to 99.8%. The control gave a callusing response of 95.2%. Maximum callus fresh weight (0.2936 g) was recorded from seeds pretreated for 24 hours with PTM-1. In general, higher fresh weight between 2.0 to 2.9 g was recorded from seeds that were pretreated for 24 hours on PTM-1, PTM-3 and PTM-4, for 48 hours on PTM-3, PTM-4 and for 72 hours on PTM-3. While minimum callus fresh weight (0.1138 g) was recorded from callus derived from seeds pretreated for 72 hours with PTM-1 (Table 2).

Table 1. Composition of pretreatment media.

Pretreatment medium	Composition
PTM-1 (Control)	MS salts + vitamins + sucrose (30 g/l)
PTM-2	MS salts + vitamins + sucrose (30 g/l) + 2,4-D (1.0 mg/l)
PTM-3	MS salts + vitamins + sucrose (30 g/l) + 2,4-D (2.5 mg/l)
PTM-4	MS salts + vitamins + sucrose (30 g/l) + 2,4-D (5.0 mg/l)

Table 2. Effect of pretreatment of mature seeds of *O. sativa* cv *Basmati 370* on callus induction and growth response.

Pretreatment time	Pretreatment media	Percent explant undergoing callusing (%)	Callus fresh weight per explant in gm. (Mean ± S.E).
0 Hour	No pretreatment	95.2	0.223±0.013
24 Hour	PTM-1	93.9	0.294±0.012
	PTM-2	91.1	0.142±0.021
	PTM-3	98.2	0.254±0.016
	PTM-4	99.8	0.269±0.034
48 Hour	PTM-1	95.0	0.131±0.009
	PTM-2	97.4	0.123±0.018
	PTM-3	98.8	0.243±0.022
	PTM-4	92.0	0.218±0.014
72 Hour	PTM-1	96.6	0.114±0.024
	PTM-2	98.1	0.191±0.008
	PTM-3	97.0	0.209±0.016
	PTM-4	94.0	0.141±0.040

Table 3. Shoot regeneration response from pre-treated mature seeds of *O. sativa* cv *Basmati 370.*

Pretreatment time	Pretreatment medium	Shoots per callus Mean ± S.E.	
		Green	Albino
0 Hour	PTM-0	6.7	5.3
24 Hour	PTM-1 (Control)	0.0	0.0
	PTM-2	0.0	4.0
	PTM-3	0.0	0.0
	PTM-4	0.0	0.0
48 Hour	PTM-1 (Control)	0.0	13.0
	PTM-2	0.2	13.2
	PTM-3	9.8	4.0
	PTM-4	8.2	3.2
72 Hour	PTM-1 (Control)	0.0	1.8
	PTM-2	0.0	0.0
	PTM-3	5.2	7.8
	PTM-4	3.4	5.0

The resulting calli were transferred to the regeneration medium (RM1). It was observed that no green shoots regenerated from callus derived from 24 hour pretreated seeds. However, an average of 4 albino shoots per callus were produced from callus derived from seeds pretreated for 24 hours on PTM-2. Callus derived from seeds pretreated for 48 hours on PTM-3 gave the best regeneration response producing an average of 13.8 shoots per callus, out of which an average of 9.8 were green and 4 albinos. This was followed by 48 hour pretreatment on PTM-4, where an average of 8.2 green and 3.2 albino shoots were produced. When the pretreatment period was increased to 72 hours, the number of albino shoots per callus produced was more than the green ones if at all. Copntrol (PTM-1) produced an average of 6.7 green and 5.3 albino shoots/callus (Table 3; Fig. 1).

It is clear from the results that upon transfer of callus derived from pretreated seeds to regeneration medium there was a lot of variation in regeneration response as well as scale of somaclonal variation manifested as production of albino plants. Though maximum amount of callus was obtained from 24 hour pretreated seeds on control (hormone free medium) upon transfer to regeneration medium, there was no regeneration at all or all regenerating plants were albino. Highest number of green shoots were obtained from seeds which were pretreated for 48 hours with 2.5 mg/l 2,4-D. However albino shoots were also produced. Maximum number of albino plants were produced from seeds pretreated for 48 hours on pretreatment medium containing 1 mg/l 2,4-D. When the pretreatment period was increased to 72 hours, the ratio of albino shoots to green shoots increased as evident from Fig. 1.

The factors that have been reported to influence the emergence of albinos are genotype and physiological status of the anther donor plants; development stage of microspores; culture temperature for callus induction; callus selection; growth regulator combination; addition of amino acids and sucrose concentration in combination of growth regulators[4-7].

Since pretreatement results in increased stress conditions, somaclonal variations in the form of production of albino plants resulted in our cultures. According to Tsukhara *et al.*[8], based upon their microscopic observations of regeneration process, it seems that both green and albino plants were regenerated via somatic embryogenesis. They further reported that a reduction in frequency of albino plants resulted on liquid regeneration medium used. It appears that not only the composition of the medium used at various stages of regeneration protocol, but the physical factors also have a role to play, which remain an enigma yet.

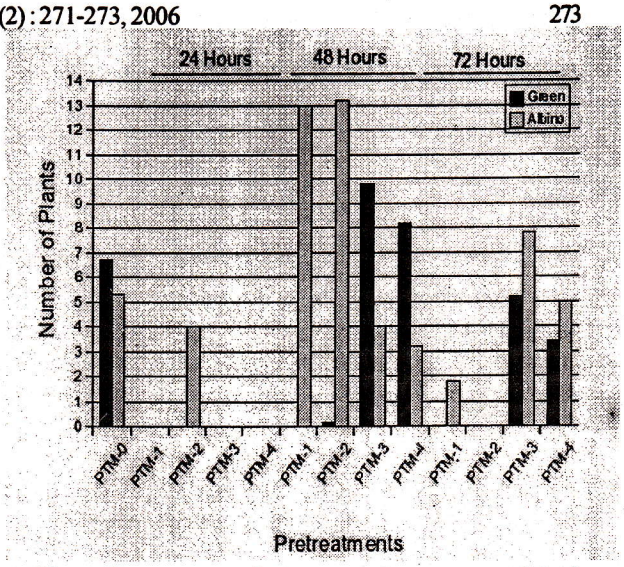

Fig. 1. Shoot regeneration response (green versus albino) from pre-treated mature seeds of *O. sativa* cv Basmati 370.

References

1. Chopra V L and Balanarasimhulu S 1986, Somaclonal variations: A new resource for crop improvement. *Science Reporter* **1** 8-10
2. Karp A 1991, On the current understanding of somaclonal variation. In: *Oxford survey of plant molecular and cell biology,* Vol.7 (Ed.) Miflin BJ, Oxford University Press, pp 1-58.
3. Murashige T and Skoog F 1962, A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant.* **15** 473-497
4. Genovesi A D and Magill C W 1979, Improved rate of callus and green plant production from rice anther culture following cold shok. *Crop Sci.* **19** 662-664
5. Bjornstad A, Opsahl-Ferstad HG and Aasmo M 1989, Effects of donor plant, environment and light during incubation on anther cultures of some spring wheat (*Triticum aestivum* L.) cultivars. *Plant Cell, Tiss. and Organ Cult.* **17** 27-37.
6. Ziegler G, Dressler K and Hess D 1990, Investigations on anther culturability of four german spring wheat cultivars and the influence of light on regeneration of green vs. albino plants. *Plant Breeding* **105** 40-46.
7. Rout J R and Sharma N P 1991, Anther callus induction and green plant regeneration at high frequency from an interspecific rice hybrid *Oryza sativa* L. X *O. rufipogon* Griff. *Euphytica* **54** 155-159.
8. Tsukahara M, Osanai E, Hirosawa T and Muragama H 1996, Comparison of somaclonal variation between two regeneration methods in rice (*Oryza sativa* L.). *Plant Tiss. Cult. Lett.* **12** 61-64.