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TISSUE CULTURE STUDIES IN *ORYZA SATIVA L. CVS.* BASMATI 385 AND SUPER BASMATI

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Abstract

The main objective of the present study was to investigate and optimize callus induction frequency and regeneration in two rice cultivars viz., Basmati 385 and Super basmati and to monitor the effect of mannitol and sorbitol on regeneration. Callus induction was achieved by using different concentrations of 2, 4-D (1, 2, 3, 4 mgL^{-1}) on MS and N6 media with 2 mgL^{-1} producing the best results. Regeneration was studied only on MS medium containing BAP, NAA and 3% sucrose with or without either 3% mannitol or sorbitol. Super Basmati showed better results both for callusing and regeneration. Regeneration frequency decreased by the addition of either mannitol or sorbitol.

Introduction

Basmati rice is an important export commodity of Pakistan. Its production is limited by various stresses. Thus there is a need to improve the existing germplasm of Basmati cultivars. Traditional breeding system consumes a longer time and has its own limitation. Developments in the plant tissue culture techniques offer possibilities of introducing variability into plants that could be utilized for crop improvement.

The genetic manipulation of rice is being performed both by direct and indirect DNA transfer methods by scientists all over the world in attempts to improve the yield and quality of this important crop. Applications of *in vitro* transfer techniques to plant improvement depend on regeneration capacity (Higuchi & Maeda, 1990).

Different methods of sterilization have been reported by many researchers; with the aim to limit the development of microorganisms during callogenesis. Piernas & Guirand (1997) reported that sodium hypochlorite and ethanol were very effective for seed sterilization.

Successful callus induction and improvements have been reported in rice by several researchers (Gonzales, 2000; Marasi *et al*., 1996; Valdez *et al*., 1997; Xie *et al*., 1995 and Rashid *et al*., 1996) from time to time. However high frequency regeneration in *Indica* in general and in fine rice in particular, is highly desirable. Present study was, therefore, carried out to develop a high frequency callus induction and regeneration system in two commercial cultivars of rice i.e. Basmati 385 and Super basmati in order to investigate the effect of basal media and growth regulators on them.

Materials and Method

Seeds of *Oryza sativa* L., cvs. Basmati 385 and Super basmati were obtained from Agricultural Biotechnology Program, NARC, Islamabad, Pakistan and were used as

explant source. Mature and healthy seeds were selected by physical appearance and then dehusked manually.

Disinfection: Seeds were washed with detergent, rinsed thrice with tap water and finally with distilled water. Then under aseptic conditions in laminar flow cabinet seeds were treated with 70% Clorox (5.25 sodium hypochlorite) for surface sterilization.

Culture media: MS (Murshige & Skoog, 1962) and N6 (Chu, 1978) media were used for callus induction. Different concentrations of 2,4-D were used for callus induction (1,2,3,4 mgL^{-1}).

Inoculation: Sterilized dried seeds were inoculated into test tubes under aseptic conditions in laminar flow cabinet. Cultures were kept at 25 ± 3°C with 2000 lux intensity for 16 hour photoperiod.

Screening: Screening was done after every week and finally seeds were observed for callus induction after three weeks. Healthy callus (Granular, light yellow in color) were subcultured on fresh callus proliferating media for another three weeks. Calli obtained were maintained on the callus induction medium for a period of four weeks.

Regeneration: Under aseptic conditions in a laminar flow, the maintained calli were transferred from proliferating medium onto the regeneration medium. Regeneration was performed with three different combinations of MS medium i.e., MR (MS medium containing 3% sucrose, 1.0 mg L^{-1} NAA, 5.0 mg L^{-1} BAP, and 2 gm L^{-1} casamino acid), MRS (MS medium containing 3% sucrose, 1.0 mg L^{-1} NAA, 5.0 mg L^{-1} BAP, 2 gm L^{-1}casamino acid and 3% sorbitol) and MRM (MS medium containing 3% sucrose, 1.0 mg L^{-1} NAA, 5.0 mg L^{-1} BAP, 2 gm L^{-1} casamino acid and 3% mannitol).

MRS and MRM were used to assay the effect of these sugar alcohols on regeneration. After one month, some part of the callus that was healthy developed into green primordia and after another two weeks shoots were produced from these green spots. Later these shoots also developed roots and appeared as complete regenerated plants.

Statistical analysis: ANOVA was applied to see which variety was best and which media or combination at which concentration of growth regulators, provided the higher callus induction frequency and regeneration.

Results and Discussion

Seeds of Basmati 385 and Super basmati were treated with different concentrations of Clorox for 30 min for sterilization. Undiluted Clorox when applied for 30 minutes showed average 97% sterilization but produced poor callus. It was observed that decreasing Clorox concentration resulted in a decrease in sterilization with a concomitant improvement in callus health/quality (Table 1). It was therefore concluded that 70% Clorox was more suitable for sterilization as it did not cause any browning and inhibition of germination as seen in other concentrations. Both the varieties tested were similar in their response towards sterilization treatment.

Table 1. Effect of clorox on sterilization of mature seeds for callus induction in rice.

Clorox %	Time in min.	Super Basmati		Basmati 385	
		Sterilization %	Callus growth	Sterilization %	Callus growth
100	30	96	+	98	+
90	30	82	++	88	++
80	30	70	+++	75	+++
70	30	66	++++	69	++++

+ = Poor, ++ = Moderate/Average, +++ = Good ++++ = Excellent

Table 2. Genotypic responses to callus induction conditions.

Varieties	No. of Inoculated seeds	Callus formation	Percent callus induction	Appearance
Basmati 385	68	56	82	Yellowish brown, small in size and loose
Super Basmati	80	75	94	Creamy white, granular and compact

Both varieties were treated for callus induction frequency on MS and N_6 media with different concentration of 2, 4-D (1, 2, 3, 4 mg L^{-1}). In N_6 media 2, 4-D at 2 mg L^{-1} was found to be suitable for callus induction in both varieties especially for Super basmati (Fig. 1 and 2). Highly significant difference was found between MS and N_6 media, while N_6 was found better in both varieties. These results are somewhat similar to earlier reports suggesting that callus induction on N_6 media is relatively more efficient in rice [Naqvi *et al*., 1989; Gul *et al*., 2000].

Embryogenic calli were selected on the basis of their physical properties. Both varieties proved good for callus induction. However, Super basmati has more potential towards callus formation than Bamati-385 (Table 2). The observed difference was not only in callus induction frequency but also in its quality. Callus produced by Super Basmati was more embryogenic. Significant difference between two varieties for callogenesis under the same nutritional condition indicates that callus induction quality is genotype dependent. These findings are in accordance with the reports of Navraj *et al*., (1999) and Gul *et al*., (2000). It signifies that tissue culture conditions need to be studied for individual genotypes.

The highest regeneration percentage for both cultivars was observed in MR as 81% (Table 3, 4). It was therefore concluded that the use of sorbitol and mannitol caused osmotic stress which affected callus growth, shoot regeneration, somatic embryogenesis and metabolism of specific compounds. However, these different media combinations MR, MRM and MRS were used for regeneration. Significant differences were observed both among the media and genotypes.

Increase in regeneration frequencies of some genotypes by use of these sugar alcohols has been reported earlier (Alkhayri *et al*., 1996; Pande & Bhojwani, 1999). Recently, study of two non-aromatic cultivars of *Indica* origin (KS-282 and Pakhal) under the influence of sorbitol and mannitol has revealed increase in the green spots and number of shoots emerging per callus; albeit frequency of the regenerating calli was decreased (Sultana, 2003). However in the present study neither regeneration frequency nor any signs of regenerative ability displayed any improvement. This finding is suggestive of the fact that tissue culture responses are genotype dependent and that fine/aromatic rice material of *Indica* origin is more recalcitrant to tissue culture manipulation.

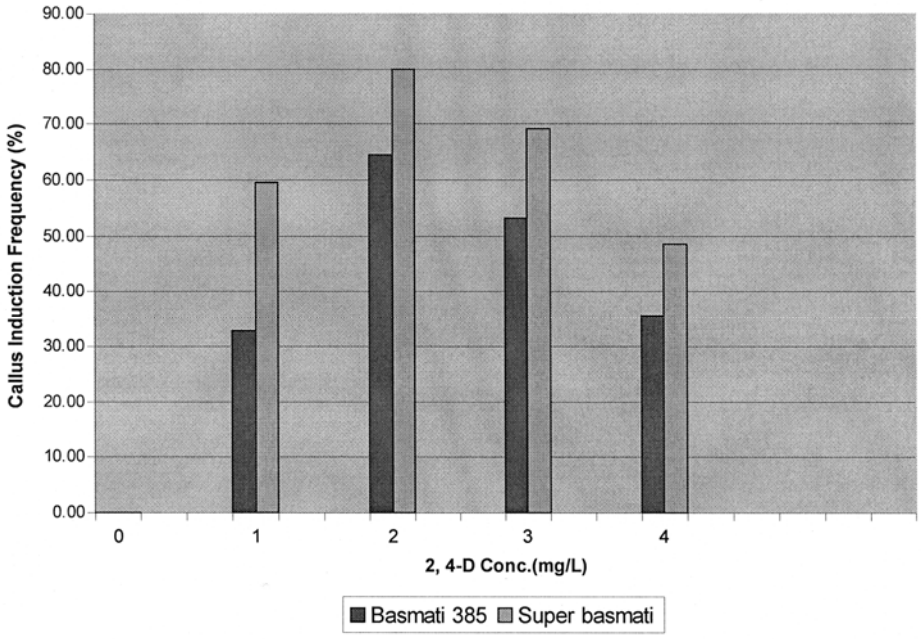

Fig. 1. Effect of 2, 4-D on callus induction frequency in Basmati 385 and Super Basmati using N6 media.

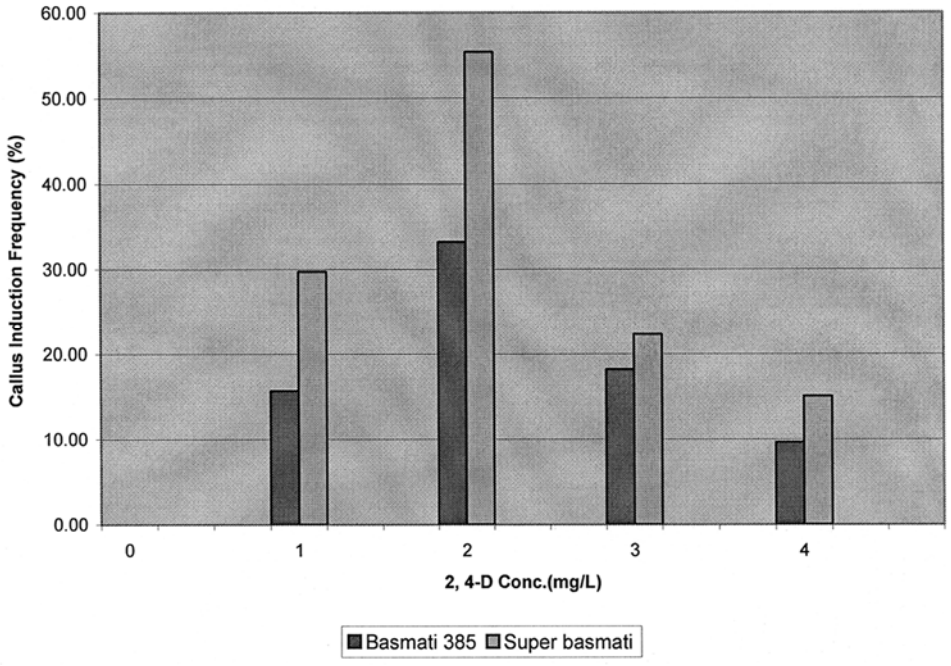

Fig. 2. Effect of 2, 4-D on callus induction frequency in Basmati 385 and Super Basmati using MS media.

Table 3. Effect of different media combinations (MR, MRM and MRS) on regeneration of Super basmati.

Combinations	Total calli	No regeneration	Green spots*	Plantlets regenerated	Percent regeneration
MR	48	4	44	40	83
MRM	48	9	39	32	66
MRS	48	14	34	30	62

*Appearance of green spots on calli indicating appearance of photosynthetic tissues.

Table 4. Effect of different media combinations (MR, MRM and MRS) on Basmati 385.

Combinations	Total calli	No regeneration	Green spots*	Plantlets regenerated	Percent regeneration
MR	48	6	42	38	79
MRM	48	13	35	29	60
MRS	48	18	30	25	52

*Appearance of green spots on calli indicating appearance of photosynthetic tissues.

It would suggest that the tissue culture response of Super Basmati is better than Basmati-385. Optimum concentration of 2,4-D for callus induction is 2 mg/L both in MS and N6 media. For callus induction N6 was found better than MS. Mannitol and Sorbitol adversely affected both the development of vital signs for regeneration and regeneration frequency.

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