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## An Improved Method for Shoot Regeneration from Calli of Indica Rice (Basmati)

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**Abstract:** Shoot regeneration from calli of indica rice remains a limiting factor for undertaking transformation experiments. In this study, callus cultures were initiated from seeds of Basmati 370, Basmati 385 and Basmati 6129. A 2,4-D concentration at 2 mg/l was found to be suitable for callus induction in Basmati 370 and Basmati 385 and was 3 mg/l for Basmati 6129 in N6 medium. Callus induction frequency ranged from 23.1%-100% for Basmati 370, 73-100% for Basmati 385 and 40-100% for Basmati 6129. Inclusion of Benzyl antipyrine (BAP) in callus induction medium has a negative effect for the frequency of callus induction and callus growth. Shoot regeneration was obtained from calli in Basmati varieties between 40%-55.7% in MS medium with 3% sucrose, 3% sorbitol, 2g/l casamino acids, 5 mg/l BAP, 1 mg/l Naphthalene acetic acid (NAA) and 4 g/l gelrite. A maximum shoot regeneration was found in Basmati 370, Regeneration frequency was increased 2-7 folds by the addition of sorbitol and gelrite.

**Key words:** Basmati, callus, regeneration, sorbitol

### Introduction

Successful plant regeneration from rice callus has been reported by several researchers (Raghava Ram and Nabors, 1985; Abe and Futsuhara, 1989; Hartke and Lorz, 1989; Ozawa and Komamine, 1989; Tsukahara and Hirose, 1992). However, there are several limitations that need to be overcome. Instability of regeneration frequency is one of the most serious limitations, particularly in indica rice. Efficient plant regeneration depends upon several factors including the composition of the culture media and especially the genotype of material plants. Some other factors that also affect plant regeneration frequency from rice callus are concentration of gelling agents, osmoticum and the specific combinations of plant growth regulators (Tsukahara and Hirose, 1992). This study was initiated on Basmati cultivars which comprise an important group of indica rice and was hampered by lack of regeneration system. Now we have developed a reproducible regeneration method by exploiting specific combinations of growth regulators, addition of osmoticum and high concentration of gelling agent.

### Materials and Methods

Mature rice seeds (*Oryza sativa* L. ssp. *indica* cvs. Basmati 370, Basmati 385 and Basmati 6129) were kindly supplied by Dr. Muhammad Akram, Rice Breeding Programme, National Agricultural Research Centre (NARC), Islamabad, Pakistan. They were dehulled, washed by tap water, first surface sterilized with 70% ethanol for 1 minute and then with 2.5% sodium hypochlorite for 20 minutes. The seeds were further rinsed three times with sterile distilled water. Callus cultures were initiated from seeds by placing them into N6 (Chu *et al.*, 1975) medium (pH 5.8), supplemented with various concentrations (1.0-4.0 mg/l) of 2,4-D singly or in combination with 0.5 mg/l BAP, 30 g/l sucrose and 4 g/l gelrite. The cultures were incubated in the dark at 25°C for two weeks and then shifted to 16 hours photo period light for next two weeks for proliferation.

The callus contained both embryogenic (white to light yellow in colour, compact and friable) as well as non-embryogenic mucilaginous and smooth parts. The embryogenic sections were carefully excised for further transfer, whereas

non-embryogenic parts for percentage of callus induction and callus growth (more than 5 mm in size). These calli were transferred independently to fresh medium.

For plant regeneration, embryogenic sections of the callus were cultured on MS medium (Murashige and Skoog, 1962) supplemented with various additives. These were placed at 23°C ± 2°C with 16/8 h photo period with light intensity of 60 μmol.m<sup>-2</sup>.s<sup>-1</sup>. The percentage of regenerated calli was based on 50-70 replicates per treatment. All the experiments were conducted at least twice.

### Results and Discussion

Basmati varieties were tested for callus induction frequency (CIF) on N6 medium containing various concentrations (0, 1, 2, 3 and 4 mg/l) of 2,4-D singly or in combination with 0.5 mg/l BAP. Callus induction frequency ranged from 23.1%-100% for Basmati 370, 73-100% for Basmati 385 and 40-100% for Basmati 6129 (Fig. 1, 2 and 3). A 2,4-D at 2 mg/l was found to be suitable for callus induction in Basmati 370 and Basmati 385 and was 3 mg/l for Basmati 6129. Furthermore, inclusion of BAP with 2,4-D in callus induction medium decreased CIF in both Basmati 370 and 6129 but CIF of Basmati 385 was not significantly affected. Moreover, callus growth was very efficient for Basmati 385. Our results are contrary as reported earlier that addition of cytokinin in induction medium greatly contributed to CIF (Hu and Liang, 1979; Chen *et al.*, 1982). It also revealed that the genotype of the explant may be also an important factor in callus induction. Compact and friable parts of calli were further selected and subcultured, whereas non-embryogenic parts were discarded.

Various regeneration media were tested. A combination of specific dose of auxin with cytokinin with sorbitol as osmoticum has played a significant role for enhancing regeneration from Basmati calli. Shoot formation was obtained from all of them. Regeneration frequency varied between 40, 55.7%. A maximum shoot regeneration was found in Basmati 370. NAA at 1 mg/l and BAP 5 mg/l with basic regeneration medium (MS salts and vitamins with 3% sucrose, 3% sorbitol, 2 g/l casamino acids and 4 g/l gelrite) was suitable for all varieties. However a less frequency (23.0%-38.6%) of regeneration was also obtained on 1 mg/l

Table 1: Plant regeneration from calli of different varieties of rice on the basic reegeneration medium consisting of MS salts and vitamins, 3% sucrose, 3% sorbitol, 2 g L<sup>-1</sup> casamino acid and 4 g L<sup>-1</sup> gelrite

Hormones Used (mg L <sup>-1</sup> )		Basmati 370			Basmati 385			Basmati 6129		
NAA	BAP	Total Celli Planted	Shoot forming calli	Regeneration (%)	Total Celli Planted	Shoot forming calli	Regeneration (%)	Total Celli Planted	Shoot forming calli	Regeneration (%)
0.0	0.0	52	0	0.00	60	0	0.00	60	0	0.00
1.0	2.0	70	27	38.57	60	15	25.00	95	22	23.15
1.0	5.0	70	39	55.71	60	24	40.00	60	32	53.33

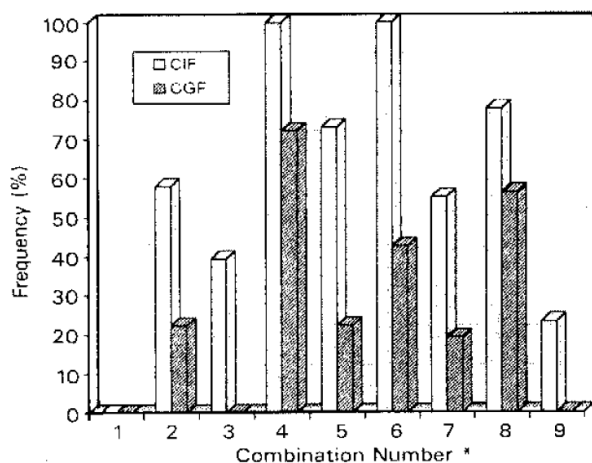


Fig. 1: Comparison of percentages of callus induction (CIF) and callus growth (CGF) in Indica rice cv. Basmati 370 on N6 medium supplemented with 2,4-D in combination with BAP, 30 g/l sucrose and 4 g/l gelrite. Results of replicated test with 100-120 seeds

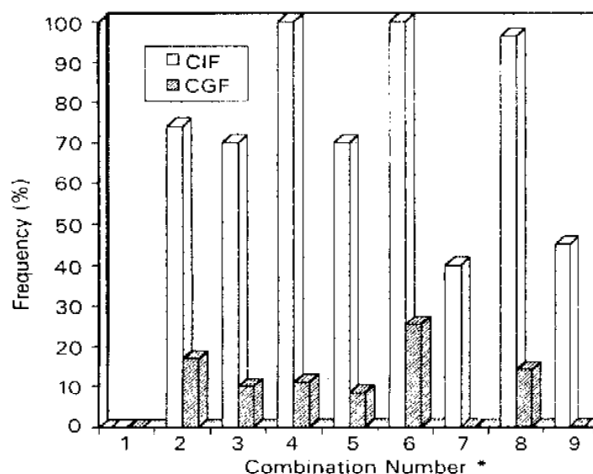


Fig. 3: Comparison of percentages of callus induction (CIF) and callus growth (CGF) in Indica rice cv. Basmati 6129 on N6 medium supplemented with 2,4-D in combination with BAP, 30 g/l sucrose and 4 g/l gelrite. Results of replicated test with 100-120 seeds

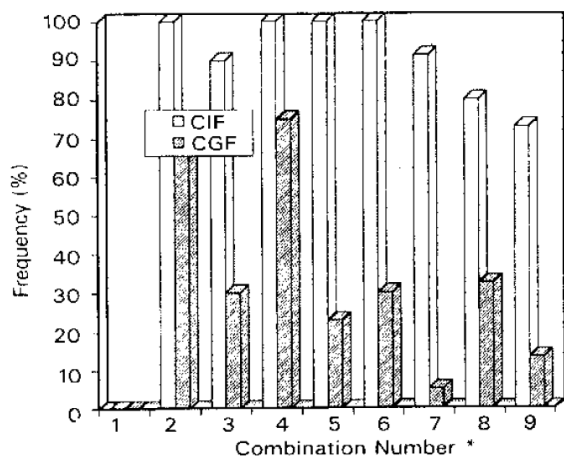


Fig. 2: Comparison of percentages of callus induction (CIF) and callus growth (CGF) in Indica rice cv. Basmati 385 on N6 medium supplemented with 2,4-D in combination with BAP, 30 g/l sucrose and 4 g/l gelrite. Results of replicated test with 100-120 seeds

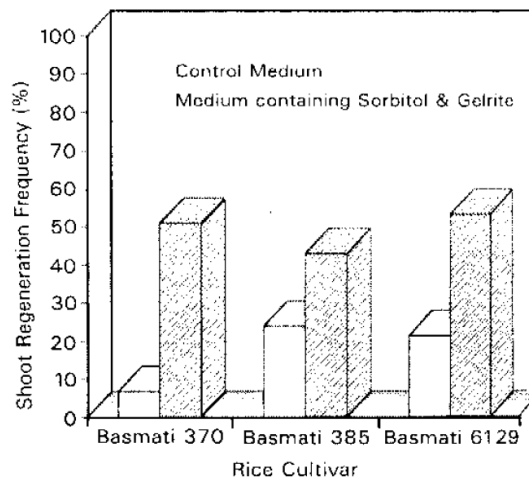


Fig. 4: Effect of osmolarity on shoot regeneration frequency of rice callus. Shoot regeneration medium was supplemented with 3% sorbitol and 4 g/l gelrite. Control was without sorbitol and gelled with 8 g/l agar. Percentage of shoot forming calli was scored after 4-5 weeks of transfer

NAA and 2 mg/l BAP (Table 1). Shoots developed roots on regeneration medium without any hormone after 2-3 weeks. Lee *et al.* (1989) showed that addition of BAP in regeneration medium has a positive effect on regeneration frequencies and number of plants produced. Our study also confirmed this

observation. Higuchi and Maeda (1991) also reported the stimulation of regeneration frequency by high osmolarity of the growth medium. Albino plantlet formation was seen in all cultivars with a high frequency of 31.1% in Basmati 6129 and

17-19% in other two varieties.

In this study, effect of 3% sorbitol with 4 g/l gelrite in the regeneration medium was also tested. Control was gelled with 8 g/l agar. A high regeneration percentage was recorded on medium containing sorbitol. Inclusion of sorbitol significantly increased regeneration about 7 folds in Basmati 370. A similar 2-3 folds was also documented in other genotypes (Fig. 4). Lai and Liu (1988) earlier reported that inclusion of mannitol and a high concentration of agar was one of the elemental factors for efficient regeneration of rice callus.

Our study shows that it is now possible to obtain high frequencies of regeneration in Basmati cultivars which belongs to indica group. The method described is simple and starting material is easy to obtain at any time during the year. The regeneration frequencies achieved should allow transformation procedures to improve these cultivars.

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