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Callus Induction, Regeneration and Hygromycin Selection of Rice (Super Basmati)

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Abstract: A protocol has been developed for callus induction, regeneration and hygromycin selection of rice (*Oryza sativa* L. cv. Super Basmati). Mature seeds of rice were used as starting material for callus induction and cultured on Murashige & Skoog medium (MS) and N6 medium having different levels and combinations of 2,4-Dicholro Phenoxy Acetic Acid (2,4-D). 2,4-D @ 2 mg/l gave good response to callus induction. For regeneration, calli were cultured on MS medium having different concentrations of Naphthalene Acetic Acid (NAA) and Benzyl Amino Purine (BAP). Medium containing NAA @ 1mg/l with BAP 5 mg/l showed best results with regeneration frequency of 45.3%. Hygromycin at the concentration of 50 mg/l proved to be lethal for secutellum-darived calli of Super Basmati.

Key words; Rice, callus, regeneration, hygromycin, Super Basmati

Introduction

Rice is the most important crop at the global level, as it is used as a staple food in most countries of the world. *Indica* type rice feeds more than two billion people, predominantly in developing countries. In the coming 30 years the world will require 70% more rice than that it requires today. According to conservative estimates, 800 million tons of rice will have to be grown with considerable reduction in the input of agrochemical under sustainable conditions (IRRI, 1992).

Super Basmati, the best quality scented rice produced in Pakistan commands the international market have four times greater price than in the domestic market. Pakistan exports 7 percent of the total world market (Rashid *et al*., 1996). The production of Super Basmati is severely affected by various stresses, including diseases. Bacterial blight, is one of the most destructive diseases of rice caused by *Xanthomonas oryzae*. Super Basmati is also susceptible to this pathogen. Production of rice has been reduced to 50% by this disease. For producing resistant cultivars through gene transfer, a resistant gene is required. Xa21 gene cloned from wild type rice, which introduced into japonica-rice by genetic transformation, has shown resistance against this pathogen (Wang *et al*., 1996).

Basmati varieties were once recalcitrant to *in vitro* regeneration, but due to the consistent efforts of scientists a high frequency transformation system by using *Agrobacterium*-mediated transformation has been developed (Rashid *et al*., 1996).

Enthused by the success in *Agrobacterium* mediated transformation a study was initiated at Agricultural Biotechnology Institute, National Agricultural Research Centre, Park Road, Islamabad, Pakistan in 1999, to develop high frequency callus induction and regeneration system to select the lethal dose of hygromycin, for genetic manipulation of Super Basmati.

Materials and Methods

Indica rice (*Oryza sativa* L.cv. Super Basmati) seeds were used as explant source. The seeds were exposed to different kinds of media for callus induction, 2,4-D singly (0,1.0, 2.0, 3.0 and 4.0) and in combination with BAP (0.5 mg/l) was used for callus induction. Compact calli were excised and were transferred to maintenance medium for a period of 4 weeks. Followed by their transfer to regeneration media. For regeneration Naphthalene Acetic acid @ 0.0, 0.1 and 1.0 mg/l in combination with BAP @ 0.0 2.5 and 5.0 mg/l was used. Another experiment was performed to optimize the lethal dose of hygromycin from 3 weeks old *scutellum-derived* calli. For selection were cultured on media having different concentrations of hygromycin i.e., 0.0, 10.0, 25.0 and 50.0 mg/l. The composition of different media used is give (Table 1).

Table 1: Composition of different media used

Medium	Composition
CI-1	N6 & vitamins (Ch *et al*., 1975). 30 g/l sucrose, different concentrations of 2,4-D & BAP, 5 g/l agar, pH - 5.8
CI-2	MS salts & vitamins (Murashinge & Skoog, 1962), 30g/l sucrose, different concentrations of 2,4-D & BAP, 5 g/l agar pH-5.8
Re	MS salts & vitamins (Murashige & Skoog, 1962), 30 g/l sucrose, 30g/l sorbitol, 2 g/l casamino acids, 4 g/l gelrite, different concentrations of NAA & & BAP, pH-5.8
Hy	N6 salts & vitamins (Chu *et al*., 1975), 1 mg/l 2,4-D different concentrations of hygromycin, pH-5.8.

CI = Callus Induction. Re = Regeneration.
Hy = Hyromycin.

Results and Discussion

Callus induction frequency in Super Basmati ranged from 54.6 to 87.7% on N6 medium and 23.9 to 68.2% on MS medium. Relatively more callus growth was observed on N6 medium as compared to MS medium. The highest callus induction frequency with maximum callus growth was noted by using 2,4-D @ 2 mg/l irrespective of the basic medium. Further inclusion of BAP with 2,4-D in callus induction medium decreased callus induction frequency and callus growth significantly. The results coincide with the results reported earlier that a high callus induction frequency was achieved in Basmati cultivars on N6 medium with 2,4-D 2 mg/l (Rashid *et al*., 2000). These results are however contrary to earlier discussion that addition of cytokinins in induction medium increased callus induction frequency in rice (Hu and Liang, 1979; Chen *et al*., 1982; Zhu *et al*., 1996). On the basis of above discussion it can be concluded that better response of N6 medium may be due to the presence of more nitrogenous contents. 2,4-D @ 2 mg/l was optimal for callus induction and growth because callus is formed due to the abnormal growth of the cell, which is the result of high transcription rate of cell caused by the 2,4-D. Higher levels of 2,4-D showed inhibitory affects on callus induction and growth, which can be attributed to the high concentration of 2,4-D which probably have damaged the cells. BAP has negative effect because it is

Table 2: Percent callus induction & callus growth

Hormones (mg/l)		N6		MS	
2, 4-D	BAP	% Callus induction	Callus growth	% Callus induction	Callus growth
0.0	0.0	0.0	--	1.0	--
0.0	0.0	59.0	+ +	66.9	+ + +
0.5	0.5	66.4	+ + + + +	35.5	+ +
0.0	0.0	87.7	+ + + + + +	68.2	+ + + +
2.0	0.5	47.7	+	23.9	+
3.0	0.0	77.5	+ + +	50.4	+ + +
3.0	0.5	73.9	+ +	27.9	+
4.0	0.0	74.1	+ + +	32.8	+ +
4.0	0.5	54.6	+ +	62.6	+ + +

Callus not induced = - - Very poor = + Poor = + + Satisfactory = + + +
Good = + + + + Excellent = + + + + + +

Table 3: Regenerative response of calli to different levels of NAA and BAP

Hormones (mg/l)		Total No. of calli plated	No. of Calli green spotted	No. of shoots formed	Percent plant formation
NAA	BAP				
0.0	0.0	75	10	1	1.3
0.1	2.5	75	10	8	10.6
0.1	5.0	75	23	18	24.0
1.0	2.5	75	15	13	17.3
1.0	5.0	75	35	34	45.3

NAP = Naphthalene Acetic Acid BAP = Benzyl Amino Purine

Table 4: Response of calli at different concentrations of hygromycin

Concentration (mg/l)	Response
0.0	Well proliferating
10.0	Average proliferating
25.0	Poor proliferating
50.0	Calli died

a growth enhancer and its interaction with 2,4-D might have slowed down the activity of 2,4-D (Table 2).

Regenerative calli were transferred to media having different levels of NAA and BAP. The media containing NAA @ 1mg/l with BAP 5 mg/l was found to be the optimal for regeneration (Table 3). Rashid *et al.* (2000) reported that shoot regeneration from calli in Basmati varieties was in the range 40-55%. Super Basmati also had a high frequency of shoot regeneration in this range, which confirmed that all calli of basmati cultivars showed similar response to regeneration, which was best at 1mg l^{-1} NAA and 5 mg l^{-1} BAP. It appeared that the presence of a high concentration of BAP enhanced the growth of the plant and low concentration of NAA, proved to be a weak auxin.

Three weeks old, *scutellum-derived* calli were exposed to various levels of hygromycin for of selection of a lethal dose for transformed. It was noted that 50 mg l^{-1} proved to be lethal, because at this concentration the calli died (Table 4). Similar results were already reported for Basmati 370, Basmati 385 and Basmati 6129 (Rashid *et al.*, 1996).

In summary, a protocol has been developed for callus induction regeneration and hygromycin selection which will serve as a base for further *Agrobacterium* mediated transformation studies in Super Basmati.

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