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Plant Regeneration from Seed Derived Callus of three varieties of Basmati Rice

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Abstract

Mature seeds of three rice varieties : Basmati 370, Basmati 385 and KS 282 were cultured on MS medium supplemented with 2.0 mg/l 2,4-D. The vareity KS 282 exhibited high callus induction efficiency (31.3%) followed by Basmati 385 (17.6%) and Basmati 370 (6.5%). Calli were maintained for 90 days by subculturing at 15 days interval on the same modified MS. Calli were transferred onto MS with different combinations of auxin and cytokinin. The highest freqeucny of plant regeneration was 71.42% for Basmati 370 and (57.14%) for Basmati 385 on MS supplemented with 0.5 mg/l NAA, 1.0 mg/l BAP. KS 282 showed the highest regeneration efficiency (75%) on the same medium but at lower concentration i.e., 0.4 mg/l NAA and 0.8 mg/l BAP.

Introduction

Rice (*Oryza sativa* L.) is an important cereal crop of Pakistan for both domestic consumption and export. Current Basmati varieties possess excellent cooking and eating qualities (Awan et al. 1998). The grain yield of these varieties is low. Tissue culture techniques provide a powerful tool for the improvement of important agronomic traits in rice such as productivity. The potential for crop improvement through cell culture of cereals depends upon easier and more efficient techniques for selecting cultured cells with desired characteristics as possible with conventional plant breeding (Yamada 1982). This is well documented that practical advantges of somaclonal variation techniques for plant breeding are associated with mutations of somatic cells (Sondah et al. 1984). Somaclonal variation, induced by in vitro culture, has been reported in many plant species (Bouharmont et al. 1991). Plant regeneration by cell and tissue culture techniques can complement conventional breeding procedure, provided plants can be regenerated in large numbers and the success of this

breeding technology depends upon the selection efficiency of the cultured cells for desired characteristics. Selection efficiency in rice can be enhanced by increasing genetic variability for agronomic and quality traits. Tissue culture itself generates genetic variability during passage through the culture medium (Larkin and Scowcraft 1981). The objectives of the present study was to induction of plantlets from callus culture of rice and examine the influence of genotype and the media on callus induction and plant regeneration.

Materials and Methods

Mature seeds of three cultivars *viz :* Basmati 370, Basmati 385 and KS 282 were dehusked manually and soaked in 70% ethanol for one min, immersed in 45% chlorox for 20 min and rinsed several times with sterilized distilled water. These were inoculated aseptically on MS supplemented with 2.0 mg/l, 2.4-D. Cultures were incubated in dark at 25_C. Callus induction efficiencies were recorded and calli were subcultured at 15 days intervals to obtain embryogenic callus. After three months, calli were transferred to MS for regeneration and kept under continuous light for four to five weeks. Regenrated plants were hardened in Youshida's culture solution (Youshida 1976) and grown to maturity in greenhouse along with parents and analyzed for inter plant variability affecting seed set, number of grains/panicle and fertility.

Results and Discussion

Callus growth was observed from the mature seeds 21 - 33 days on the callus induction medium. Callus formation varied widely among the rice varieties examined. Seeds that developed calli in percentage ranged from 6.5 to 31.25 (Table 1). Basmati 370 callused relatively poor in the induction medium and the calli were less regenerative efficiency of 75%. Callusing efficiency was found to be genotype dependent. The results are in agreement with Chung (1982) who reported that rice varieties differed in the degree of callusing. Similar observations were also reported by Nguyen (1984). However, basmati varieties exhibited maximum callus induction frequency on N6 medium (Rashid et al. 2001).

Callus pieces of KS 282 developed green sectors. When green sectors were transferred to regnerative medium, shoots rapidly developed from such segments. Regeneration efficiencies of callus as observed in all the three varieties and the effects of different combinations of auxin and cytokinin their regeneration are presented in Table 2. The data showed a positive effect on genotypic x media on regeneration. All combinations of cytokinin and auxin

inhibited callus development. It is evident from Table 2 that on MS supplemented with 0.5 mg/l NAA and 1.0 mg/l BAP, the frquency of plant regeneration was high in Basmati 370 (57.14%). KS 282 gave the highest regeneration frequency (75%) on the same medium supplemented with 0.4 mg/l NAA and 0.8 mg/l BAP. The genotypes also showed variability in their ability for differentiation into roots and shoots. These findings are in line with Abe and Futsuara (1991) who reported that callus growth and organ differentiation are under genetic control. Almost similar level of regeneration was achieved on MS medium containing NAA, BAP, sorbitol and casamino acids (Rashid et al. 2000).

Table 1. Response of rice cultivars to callus induction.

Variety	No. of exaplants	Callus induction	Callus induction frequency (%)
Basmati 370	656	43	6.50
Basmati 385	476	84	17.60
KS 282	800	250	31.25

Table 2. Effect of NAA and BAP on regeneration of rice.

Variety	Regeneration frequency (%)						
	M1	M2	M3	M4	M5	M6	M7
Basmati 370	0.00	57.10	36.36	43.75	40.00	5.10	0.00
Basmati 385	0.00	71.42	38.00	45.00	38.09	20.80	0.00
KS 282	47.61	68.18	75.00	24.85	60.86	40.90	26.00

M1 = 0.93, 2.25; M2 = 0.5, 1.0; M3 = 0.40, 0.80; M4 = 0.3, 0.60; M5 = .20, 0.40; M6 = 0.1, 0.2; M7 = 0.00, 0.00; NAA and BAP, respectively.

A number of regenerated plants died before maturity. Often clumps of shoots originated from a single callus piece. When potted as a group they produced a mosaic of normal and aberrant panicles. The plants were examined for inter plant variation among the callus derived regenerants in a number of parameters including seed set and fertility. The panicles of these plants differed in seed set and fertility (Table 3). Plants originating from seeds were normal in growth, development, fertility; they produced 1320 seeds/plant for Basmati 370, 1875 for Basmati 385 and 1800 seeds/plant for KS 282. However, regenerants from callus showed reduced fertility, smaller number of seeds per plant and per panicle accompanied by shrivelled seeds. In case of KS 282, pale green shoots originated from single callus.

Table 3. Fertility of rice plant regenerated from callus cultures.

Cultivar	Days culture	Culture trans-ferred	Plants regenerated	No. of plants matured	No. of panicles examined	Seeds/ panicle	Fertility (%)	No. of seeds/ plant
Bas. 370	a. 90	200	43	20	140	70	79.5	560
	b. 30	20	70	20	140	110	85.0	1320
Bas. 385	a. 90	200	84	50	140	110	83.35	1080
	b. 30	20	20	20	140	75	90.0	1875
KS 282	a. 90	200	150	90	140	85	81.03	595
	b. 30	20	20	20	140	100	95.0	1800

a. = Plant regenerated from seed derived callus. b. = Plant grown from seed. Bas. = Basmati.

A close examination of the data obtained in the present study demon-strates that in rice the frequency of somatic embryo formation and of plant regeneration is under genotypic control. Similar genotype-dependent differences in the ability for embryogenic callus formation and plant regeneration have been described for various cereals. The data also revealed that besides genotypic effects, the composition of culture media also influences callus growth.

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