Plant Tissue Cult. & Biotech. **22**(2): 163-169, 2012 (December) **PTC&B**

Plant Regeneration Efficiency of Two Scented Indica Rice Varieties Pusa Basmati 1 and Kalanamak

Aniruddha Kumar Tiwari, Md Shamim, Ravi Prakash Saxena[1], and Kapil Deo Narayan Singh*

Department of Plant Molecular Biology and Genetic Engineering, Narendra Deva University of Agriculture & Technology, Kumarganj, Faizabad-224 229 (U. P.), India

Key words: Scented rice, Regeneration efficiency, Growth hormone

Abstract

This study was undertaken to establish a regeneration protocol for two scented indica rice varieties, namely Pusa Basmati1 and Kalanamak. Callus culture in Pusa Basmati1 was initiated in MS containing 2.0 mg/l of 2,4-D. Optimum requirement of growth hormones for callus induction in Kalanamak was 1.5 mg/l of 2,4-D supplemented with 0.1 mg/l each of NAA and BAP in MS. Shoot regeneration in Pusa Basmati1 initiated in MS containing 2.0 mg/l BAP, 0.5 mg/l each of NAA and Kn. There was no shoot initiation in MS with same composition in Kalanamak. The shoot regeneration was successfully initiated and achieved in Dl medium supplemented with 2.0 mg/l BAP, 0.2 mg/l each of IAA, NAA and Kn, 500 mg/l each of proline and glutamine and 800 mg/l casein hydrolysate. The calli derived from mature seed embryo produced fertile green plants. The plants were successfully transferred to field with normal flowering.

Introduction

Rice (*Oryza sativa* L.) is the important staple food crop of the world and feed to more than one third of world's population mainly in the tropics. Traditional breeding system consumes longer time, needs greater diversity in base population for wider scope of selection and has its own limitation. Although a variety of germplasms needed for breeding programs are available, the demand for generation of variability for breeding programs is still the current demand for breeding programs. Generation of variability through *in vitro* culture can be utilized for crop improvement. Variability in callus induction and plant regeneration in rice have been reported in different cultivars/varieties (Nishi et al.

*Author for correspondence: <kapildeos@hotmail.com>. [1]Department of Vegetable Science, Narendra Deva University of Agriculture and Technology, Kumarganj, Faizabad, 224 229 (U. P.) India.

1968, Maeda 1967, Nakano et al. 1975, Larkins and Scowcroft 1981, Gonzales 2000, Tariq et al. 2008). *Indica* rice is less amenable to tissue culture than *Japonica* type of rice (Reddy 1982, 1987 , Nam and Heszeky 1984, Hoque et al. 2007) and this is one of the reasons that progress towards the transfer of useful genes into *Indica* rice has been slow than *Japonica* types.

Among scented rice varieties, Pusa Basmati1 and Kalanamak are known for their aroma and are being exported to other countries. The cultivation of these two varieties faces both biotic and abiotic stresses that reduce yield. Biotechnological approaches can be useful in introducing useful genes in crop improvement program. The purpose of this study was to develop an efficient protocol for callus induction from mature seeds, plant regeneration and how plant regeneration differs at optimal requirement of hormones in different nutritionally defined media in two different rice cultivars/varieties.

Materials and Methods

Breeder grade seeds of rice varieties Pusa Basmati1 and Kalanamak were collected from Crop Research Station, Masodha, N.D. University of Agriculture & Technology Faizabad, India.

MS and Dl media (Lin and Zhang 2005) were used supplemented with 1.0 mg/l of thiamine hydrochloride, 3 per cent sucrose and 0.8 per cent agar agar. Regeneration medium Dl_{R4} contained 500 mg each of proline and glutamine and 800 mg/l of casein hydrolysate (Lin and Zhang 2005). pH of the culture medium was adjusted to 5.8 before autoclaving. Mature dehusked seeds of both the varieties were given a quick dip of 1 min in 70% alcohol (v/v). The seeds were then surface sterilized with 0.1% mercuric chloride (w/v) solution for 10 min under aseptic conditions. The seeds were then washed thrice with pre-sterilized distilled water and aseptically transferred to culture medium in test tubes and Petri dishes containing 20 ml of culture media. Each Petri dish contained 10 seeds and each test tube contained one seed for callus induction. The calli induced this way were pooled down for further studies. Weight of callus and number of plants regenerated were recorded.

Cultures were incubated at 25 ± 2^0C at 55 - 65% relative humidity and 2000 Lux of light intensity using Philips 40w cool white florescent tubes at 16 hr of photoperiod. For callus induction one replication contained five Petri dishes containing ten seeds/Petri dish or in ten test tubes containing 1 seed/test tube in five replications. Fifty test tubes were inoculated with 25 - 50 mg of callus for regeneration studies in each treatment and in five replications. All regenerated shoots were rooted in half strength of MS containing iron in full strength. The regenerated plantlets were hardened in 1/10^{th} strength of MS with full strength of

chelated iron for two weeks before transferring to polycups containing pre-autoclaved soil : sand : FYM mixture (2 : 1 : 1) and irrigated with Hoagland's solution. The per cent callus induction and plant regeneration was calculated (Islam et al. 2005). The data were statistically analyzed.

Results and Discussion

High concentration of 2,4-D in the MS caused inhibition in germination of rice seeds, root emergence and shoot growth over controls. Differences in the frequency and weight of callus have been observed in both the varieties i.e. Pusa Basmati1 and Kalanamak at the same concentrations of hormones (Table 1). The callus induction frequency differed at the same concentration of 2,4-D and was genotype specific. Maximum callusing was observed at 2.0 mg/l of 2,4-D. Ninety two per cent callus induction was observed in rice seed explants at 2.0 mg/l of 2,4-D in Pusa Basmati1 whereas it was 45% in Kalanamak. Addition of 0.1 mg/l each of NAA and BAP to the culture medium increased the callusing frequency up to 1.5 mg/l of 2,4-D, and reduced later at 2/0 mg/l of 2,4-D (Table 1). However, maximum callusing frequency in Kalanamak was recorded in MS containing 1.5 mg/l of 2,4-D supplemented with 0.1 mg/l each of NAA and BAP. Callus was yellow first in Kalanamak and later it turned whitish whereas in Pusa Basmati1 it remained yellow during the culture.

Table 1. Per cent callus induction Pusa Basmati1 and Kalanamak.

Medium code	MS + growth regulator (mg/l)	Callus induction (%)	
		Pusa Basmati1	Kalanamak
MS_1	1.0 2,4-D	65	50
MS_2	2.0 2,4-D	92	45
MS_3	3.0 2,4-D	70	57
MS_4	1.0 2,4-D + 0.1 NAA + 0.1 BAP	52	82
MS_5	1.5 2,4-D + 0.1 NAA + 0.1 BAP	85	90
MS_6	2.0 2,4-D + 0.1 NAA + 0.1 BAP	48	50
SEM (±)		0.57	0.75
CD (0.05)		1.24	1.68
CV (%)		3.97	4.33

Callus formation in both the genotypes differed. Maximum weight of callus (186.96 mg) was produced in Pusa Basmati1 as compared to 100.66 mg in Kalanamak at 2.0 mg/l of 2,4-D in MS, which decreased with the increase in concentration of 2,4-D to 3.00 mg/l. Weight of callus increased by addition of 0.1 mg/l each of NAA and BAP and 2,4-D from 1.0 mg/l to 1.5 mg/l in Kalanamak and recorded highest callus weight (136.82 mg) at 1.5 mg/l of 2,4-D which decreased by increasing the concentration of 2,4-D to 2.0 mg /l in both varieties.

2.0 mg/l of 2,4-D has been found to be the optimal requirement for callus induction in both *Indica* and *Japonica* rice cultivars as observed in present findings of Pusa Basmati1 (Paul and Ghosh 1984). Two distinct types of callus observed were also reported earlier (Singh et al. 1991). Variability in callusing in different genotypes of cereals from scutellum of mature seeds of different rice varieties has observed (Larkins and Scowcroft 1981, Singh et al. 1991). Moreover, these variations in callus induction, formation, growth rate and morphology in different genotypes also have been reported to be genetically controlled (Shirai et al. 1984). The differences in induction/production of callus in two varieties at same concentration may be attributed to the differences in endogenous levels of hormones in the explants specially cytokinin(s)/auxin(s) levels that is expressed by the requirement of auxin/cytokinin for callus induction through culture media in two different genotypes (Sundaru et al. 1983).

Regeneration studies in both the varieties were successful and required different medium composition (Table 2). In Pusa Basmati1 maximum shoot formation of 42% was observed in MS_{R2} (2.0 mg/l BAP and 0.5 mg/l Kn) with one albino shoot. Withdrawal of BAP (2.0 mg/l) from culture medium checked regeneration in both the varieties, suggests that BAP is essentially required for plant regeneration. Low frequency of 26% of shoot formation in Pusa Basmati1

Table 2. Plant regeneration efficiency in Pusa Basmati 1 and Kalanamak.

Medium code	Plant growth regulator (mg /l)				Pusa Basmati 1		Kalanamak	
	BAP	NAA	Kn	IAA	Total plantlets regenerated	Green plants regenera-tion	Total plantlets regene-rated	Green plants regene-ration
MS_{R1}	2.0	0.5	0.0	0.0	2	4.0	-	0.0
MS_{R2}	2.0	0.5	0.5	0.0	21	42.0	1	2.0
MS_{R3}	0.0	0.5	2.5	0.0	-	-	-	-
Dl_{R4}	2.0	0.2	0.2	0.2	13	26.0	31	62.0
SEM (±)						0.48		1.03
CD (0.05)						1.06		2.26
CV (%)						25.33		41.5

was observed in Dl_{R4} medium. In Kalanamak the change in culture medium constitution from MS to Dl medium increased frequency of shoot formation to 62% without formation of any albino shoot. All the microshoots of about 3 - 4 cm long rooted after transfer to half strength of MS containing full strength of chelated iron. More than 80% of hardened plants were recovered successfully in

pots. These plants upon transfer to pots in green house remained healthy and erect. The growth of plants was normal, produced fertile plants and viable seeds. Fig. 1 has been presented to show callus induction and *in vitro* regeneration of plants in cases of both Kalanamak and Pusa Basmati1.

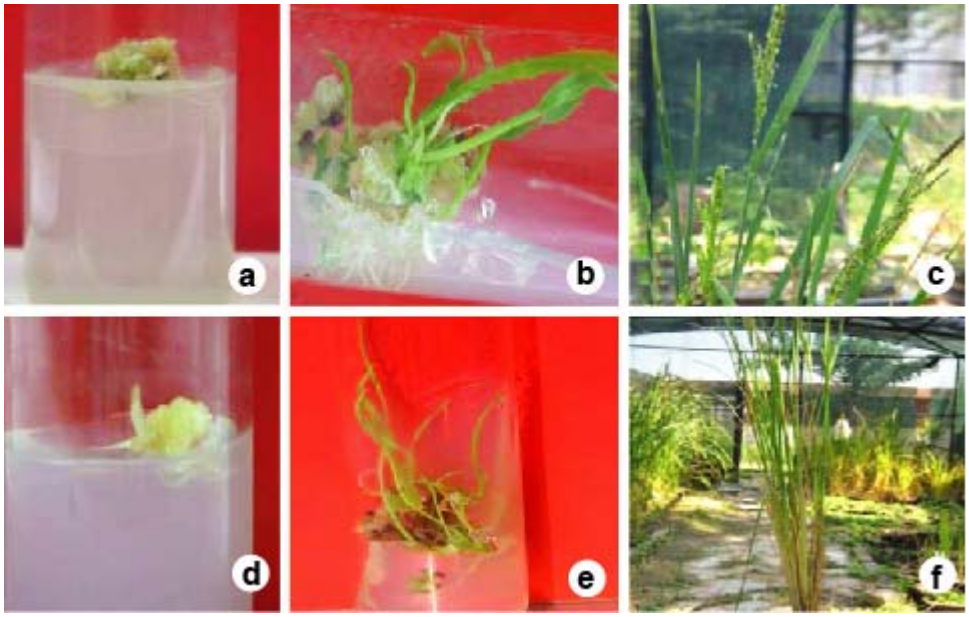

Fig. 1. (a) Callus induction in MS containing 1.5 mg/l of 2,4-D supplemented with 0.1 mg/l each of NAA and BAP in Kalanamak after 30 days of incubation, (b) shoot formation in Kalanamak in Dl medium containing 2.0 mg/l BAP, 0.2 mg/l each of IAA, NAA and Kn, (c) *in vitro* raised plants of Kalanamak showing flowering in pots, (d) callus induction in Pusa Basmati1 on MS containing 2.0 mg/l of 2,4-D, (e) shoot formation Pusa Basmati1 in MS containing 2.0 mg/l BAP, 0.5 mg/l each of NAA and Kn and (f) an *in vitro* raised flowering plant of Pusa Basmati1 in pot.

Success has been achieved in efficient plant regeneration in both the varieties of rice in the present study. The study clearly points out that salts and hormone requirement in both varieties were different for callus induction and plant regeneration. Use of BAP and NAA in Dl medium in Kalanamak has been found to be optimal for efficient shoot regeneration over Pusa Basmati1 in the MS. The stimulatory effect of BAP in combination with NAA has been reported to facilitate regeneration of rice callus cultures and presence of high concentration of BAP has been instrumental for regenerating more shoots/callus (Kartikeyan et al. 2009). Moreover, the callus formation and plant regeneration have been reported to be genetically determined (Khalequzzaman et al. 2005, Hoque and Mansfield 2004). Success in transfers of tissue culture raised plants has also been reported (Kartikeyan et al. 2009).

The present investigation reveals an efficient *in vitro* plant regeneration from mature seed derived callus culture of popular two scented *Indica* rice varieties Pusa Basmati1 and Kalanamak that could be conveniently used in undertaking studies for genetically modified plants. The study has clearly pointed out that media composition and their interaction with growth regulators largely affect of callus induction and plant regeneration that require different salt compositions.

References

Gonzales MC (2000) Effect of different growth regulators on in vitro culture of rice cultivar. Tropicales **21**: 23-37.

Hoque EH and **Mansfield JW** (2004) Effect of genotype and explants age on callus induction and subsequent plant regeneration from root derived callus of *Indica* rice genotypes. Plant Cell Tiss. Org. Cult. **78**: 217-223

Hoque ME, Ali MS and **Karim NH** (2007) Embryogenic callus induction and regeneration of elite Bangladeshi rice cultivars. Plant Tiss. Biotech. **17**(1): 65-70.

Islam MM, Ahmed M and **Mahaldar D** (2005) *In vitro* callus induction andplant regeneration in seed explants of rice (*Oryza sativa* L). Res. Jour. Agric. Biol. Sci. **1**(1): 72-75.

Kartikeyan A, Thevar S, Pandian K and **Ramesh M** (2009) High frequency plant regeneration from embryonic callus of a popular *Indica* rice (*Oryza sativa* L.). Physiol. Mol. Biol. Plants **15(4):** 371-375.

Khalequzzaman M, Haq, Hoque ME and **Aditya TL** (2005) Regeneration efficiency and genotypic effect of 15 *Indica* type Bangladeshi rice (*Oryza sativa* L.) Landraces. Plant Tiss. Cult. **15:** 33-42.

Larkins RJ and **Scowcroft WR** (1981) Somaclonal variation. A novel source of variability from cell culture for plant improvement. Theo. Appl. Genet. **60**(4): 197-214.

Lin YJ and **Zhang Q** (2005) Optimizing the tissue culture conditions for high efficiency transformation of *Indica* rice. Plant Cell Rep. **23:** 540-547.

Maeda E (1967) Varietal differences in callus formation of rice seed under sterile condition. Proc. Crop Sci. Soc. Jpn. **34**: 139-147.

Nakano H,Tashiro T and Maeda E (1975) Plantlet differentiation in callus tissue from immature endosperm of rice (*Oryza sativa* L.) Z pflanzenphysiol. **70** : 444-449.

Nam Li SU and **Heszeky LE** (1984) High frequency of callus induction and regeneration in cultured explants of rice varieties. *In:* Proceedings Int. Sym. Plant cell tissue and Cell culture application to crop improvement held at Olomoque Czeckslovakia. Sept 24-29 1984, Novak FJ Kavel L and Dolozei HJ (Eds) Czecklovakia Acad. Sci. Prague.

Nishi T, Yamada J and Takashashi E (1968) Organ redifferentiation and plant regeneration in rice callus. Nature **21**(9): 508.

Paul NK and **Ghosh PD** (1984) Callus induction and plant induction from embryo tissue of rice. Int. Rice Res. Note **9**(2): 13.

Reddy GM (1982) Callus intitiation and plant regeneration from haploid internodes in rice. *In:* Plant cell culture in crop improvement. Giles KL and Sen SK (eds). Plenum Publication Co. 113-118.

Reddy GM (1987) Cell and tissue culture studies in rice improvement. *In:* Proc. Symp. Plant cell and tissue culture of economically important plants. Reddy GM Hyderabad. 247-257.

Shirai S, Mori K and **Kinoshi** T (1984) Variation in callus growth among isogenic lines of rice. Rice Genet. Lett. **3:** 111-112.

Singh S, Saxena RP, Maurya DM, Singh, HP and **Singh, AK** (1991). Variability in different genotypes of rice to callusing. *In:* Proc. Int. Conf. Pl. Physiol. Banaras Hindu University, India. pp. 261-269.

Sundaru M, Baba I, Tami F and **Motoda Y** (1983) Varietal difference of Indonesian rice plants in their susceptibility to 2,4-D injury and interrelationships with ethylene. Japanese Crop Sci. **52:** 323-330.

Tariq M, Ali G, Hadi F, Ahmed S, Ali N and Shah AA (2008) Callus induction and *in vitro* plant regeneration of rice (*Oryza sativa* L.) under various conditions. Pak. J. Bio. Sci. **11**(2): 255-259.