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Genetic variation in in vitro response of elite aromatic and non aromatic rice varieties

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ABSTRACT

The genetic modification approach in rice can successfully address both biotic and abiotic stresses through minimization of yield losses; can also enhance the nutritional status of the rice grain thus ensuring both food and nutritional security. As indica rice genotypes are known to vary in their response to culture, production of embryogenic callus having high regeneration potential is a pre requisite for achieving high transformation rates in indica genotypes. The present study is an assessment of the response of new and popular rice genotypes that include both aromatic and non aromatic rice. Results suggest that though significant genotypic differences exist for both callus induction and regeneration, new genotypes like Tapaswini and Satya Krishna were identified that can be used for transformation studies in indica rice as they match the Pusa Basmati 1, the most frequently used genotype for rice transformation, in their in vitro response.

Key words: *indica rice, callus induction, regeneration, transformation*

Rice is grown under a wide range of climatic conditions. Through the semi dwarf HYVs of the green revolution era, high yields were realized ushering in food security and self sufficiency. Though rice production in India is increasing slowly over the years, the rate of increase is lagging behind the rate at which Indian population is increasing, new developments are needed to augment the conventional methods to improve both production and productivity of rice. With shrinking land resources and ever increasing demands of food production, the production levels of the rice varieties has to be increased substantially to raise the overall rice production through stabilization of yield levels. The recent advances in biotechnology provide the rice breeders a great opportunity to achieve their objectives in a short time with high precision. Of these, the genetic engineering technology can be employed to broaden the genetic base of rice through transfer of agronomically important traits across living organisms.

Production of callus and its subsequent regeneration are the prime steps in crop plant to be manipulated by biotechnological means and successful genetic transformation in rice is possible only when efficient and reproducible plant regeneration protocols are available for the target genotypes (Rachmawati and Anzai, 2006). In *indica* rice, several applicable tissue culture methods for subculture and regeneration have been reported (Aldemita and Hodges 1996; Rashid *et al.,* 1996; Khanna and Raina, 1998). Even though a great deal of progress has been made in *in vitro* studies of rice, the results have been still unsatisfactory in producing transformants as it is difficult to induce embryogenic calli in *indica* rices as its calli tend to get brown easily (Qian *et al.,* 2004). Furthermore, their response to culture is poor due to the recalcitrant nature of genotypes (Aldemita and Hodges, 1996; Cho *et al.*, 2004). The most desirable strategy is to improve the regeneration rates using appropriate media that can induce embryogenic calli and also the green plant regeneration (Wanichananan *et al.,* 2010). The potential for callus formation and regeneration have been reported to be a varietal characteristic and efficient regeneration in *indica* rice still poses a major problem for genetic manipulation through innovative approaches (Toki, 1997). So the present work was

carried out to study the efficiencies of callus induction and regeneration of twenty popular rice cultivars and some of which are likely to be used in the transformation experiments and the present study is an attempt to identify the best genotype of *indica* rice for transformation studies.

MATERIALS AND METHODS

The experimental material included twenty rice cultivars *viz.* IR-64, Anjali, Gayatri, Naveen, Pooja, Pusa 44, Sahabhagidhan, Samba Mahsuri, Satabdi, Swarna, Tapaswini, Vandana (popular varieties); Basmati 370,CR Sugandh Dhan 907, Dubraj, Geetanjali, Poorna Bhog, Pusa Basmati1 (aromatic varieties); Phalguni and Satyakrishna (doubled haploid varieties). Mature dehusked seeds of these cultivars are surface sterilized (Vijayachandra *et al.,* 1995) and were inoculated twelve per plate in three replicas in culture petri dishes containing semisolid callus induction (CI) medium. MS medium(Murashige *et al.,* 1962) supplemented with three different concentrations of 1.5 mg l^{-1}, 2.0 mg l^{-1} and 2.5 mg l^{-1} of 2, 4-dichlorophenoxy acetic acid (2, 4-D), 30 g l^{-1} maltose and 2.6g l^{-1} of Gel-rite (Sigma Aldrich, Bangalore India). The culture petri dishes inoculated with seeds were incubated in dark at 25 ± 1ºC for a period of 3 weeks. Scutellum-derived calli were excised and sub cultured on the same CI medium for another 4 days and clusters of highly embryogenic compact calli (3–5 mm in diameter) were transferred to regeneration medium supplemented with MS salts and vitamins, 30 g l^{-1} maltose, 0.5 mg l^{-1} naphthalene acetic acid, 0.5 mg l-1 kinetin, 1.5 mg l^{-1} benzylaminopurine, and 9g l^{-1} of agar agar (Sigma Aldrich, Bangalore India). pH 5.8 was maintained and grown in dark for 15 days and transferred to light for regeneration. Each replication constitutes ten culture tubes and the callus induction (CI) and regeneration (RF) frequencies were recorded and statistical analyses were performed using SAS software.

RESULTS AND DISCUSSION

The callus induction frequencies were in the range of (16.91) to (97.22) (Table 1). The genotypic response varied with the concentration of 2, 4-D with majority of them (11cultivars) responding well at 2.0mg l^{-1} concentration while five showed good callus induction at 2.5 mg l^{-1} and the other four responded well at 1.5mg l^{-1} (Fig. 1). However, differences were not significant between the three levels of 2, 4-D in the medium. The compactness of the calli increased and the size of the calli decreased with the increase in concentration of 2, 4-D in almost all the varieties. From the physical features of the callus like colour, compactness, growth pattern, it was apparent that concentration of 2, 4-D plays a crucial role in callus induction in different cultivars.

Among the 12 popular rice cultivars, Tapaswini showed high callus induction and rated as first category, varieties like Samba Mahsuri, Vandana, Swarna, Naveen, and IR-64 comparatively showed slightly lower level of induction (second category), Pooja, Pusa 44, Gayatri and Sahabahgidhan constitute the third category, while Anjali and Satabdi showed very low callus induction rates (fourth category). Among the aromatic rice, very good callus was induced from Basmati 370 and Pusa Basmati1 (A), Dubraj, CR Sugandh Dhan 907 and Geetanjali were under B category, while Poornabhog was categorized as C. Both the doubled haploid varieties (Satya Krishna and Phalguni) showed good callus induction rates (Fig. 1). The analysis of variance confirms the presence of significant differences among the genotypes while differences were not significant between the replications (Table 2).

Large scale differences were observed for both callus induction and regeneration frequencies in the genotypes tested. The analysis of variance confirm the presence of significant differences among the genotypes while between the replications, differences were not significant (Table 2). When the varieties are ranked according to their response (%) using Duncan's multiple range test (DMRT), Tapaswini, Basmati 370, Pusa Basmati 1, Satyakrishna and Samba mahsuri showed highest callus induction while Satabdi, Poornabhog and Anjali showed poor callus induction (Table 1 and 3).

Significant differences were observed between the genotypes in green plant regeneration also. Of the genotypes tested, Satyakrishna, Pusa Basmati 1, Sahabhagidhan, Gayatri and Basmati 370 showed highest regeneration rates while Anjali, Geetanjali and Dubraj showed low regeneration rates (Table 1 and 3).

Table 1. Callus induction and regeneration frequencies in different indica rices

Genotype	CI at different Concentrations of 2,4-D			Regeneration
	1.5mg l^{-1}	2mg l^{-1}	2.5mg l^{-1}	
High yielding varieties				
IR-64	61.66 ± 7.26	72.22 ± 2.77	63.88 ± 19.44	42.06 ± 4.82
Anjali	27.77 ± 2.77	19.44 ± 7.34	41.66 ± 4.81	10.80 ± 1.28
Gayatri	27.22 ± 9.24	48.61 ± 6.05	33.33 ± 4.81	79.16 ± 11.02
Naveen	58.33 ± 4.81	72.22 ± 5.55	73.88 ± 3.88	22.35 ± 9.98
Pooja	54.05 ± 2.40	72.22 ± 2.77	47.22 ± 5.55	25.63 ± 10.04
Pusa 44	44.19 ± 1.26	41.66 ± 4.81	31.56 ± 3.39	50.87 ± 0.87
Sahabhagidhan	36.86 ± 4.79	27.77 ± 2.77	27.77 ± 2.77	79.41 ± 15.09
Samba Mahsuri	75.00 ± 4.81	77.77 ± 2.77	72.22 ± 2.77	46.10 ± 2.08
Satabdi	19.44 ± 5.55	16.91 ± 4.59	19.44 ± 2.77	66.66 ± 16.66
Swarna	61.11 ± 5.55	77.77 ± 2.77	63.88 ± 2.77	64.79 ± 3.99
Tapaswini	86.11 ± 2.77	97.22 ± 2.77	97.22 ± 2.77	55.49 ± 1.12
Vandana	78.18 ± 7.92	70.51 ± 6.57	68.18 ± 8.34	51.90 ± 17.14
Aromatic Varieties				
Basmati 370	91.67± 4.81	94.64 ±2.77	88.89 ± 2.77	75.00 ± 2.88
CR Sugandh Dhan 907	46.96 ± 1.51	61.11 ±13.88	58.33 ± 12.72	48.53 ± 0.73
Dubraj	52.77 ± 7.34	52.77 ± 2.77	76.76 ± 6.56	15.45 ± 3.42
Geetanjali	45.45 ± 4.54	50.00 ± 9.62	36.11 ± 5.55	14.71 ± 1.91
Poorna Bhog	24.34 ± 6.08	42.65 ± 7.34	19.44 ± 2.77	20.65 ± 2.00
Pusa Basmati 1	77.77 ± 2.77	88.88 ± 2.77	86.11 ± 2.77	84.73 ± 0.99
Doubled haploid varieties				
Phalguni	66.66 ± 8.33	69.50 ± 3.09	47.22 ± 2.77	61.54 ± 2.31
Satyakrishna	81.41 ± 5.44	73.07 ± 1.92	72.22 ± 16.89	91.50 ± 1.37

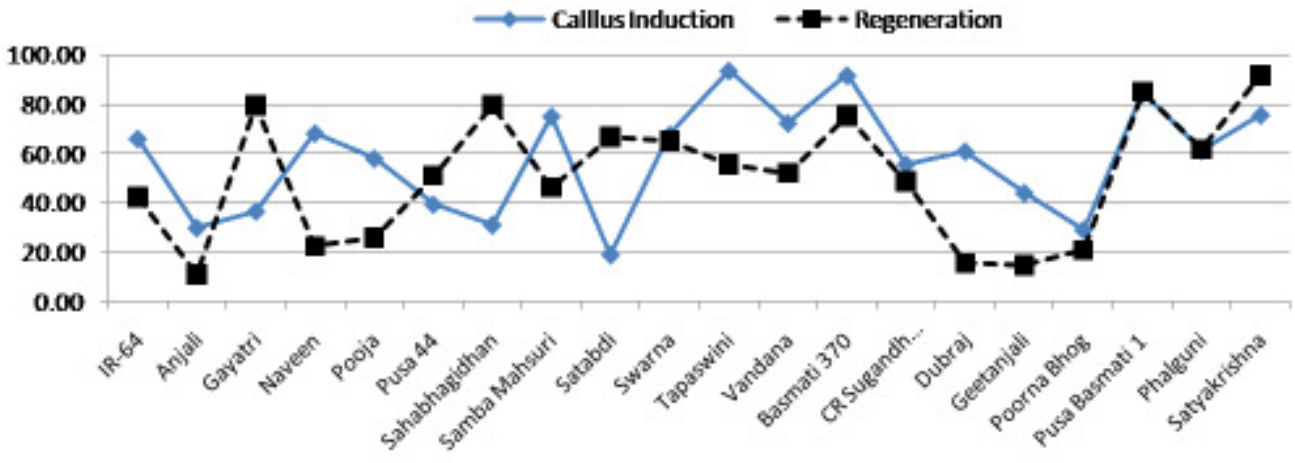

Fig. 1. The callus induction and regeneration frequencies of rice genotypes

The critical evaluation of *in vitro* response of eighteen recently developed rice varieties for both callus induction and regeneration suggest that large scale genotypic differences exists for both steps. The DMRT and analysis of variance clearly points out such significant differences and the results are in agreement with the earlier reports on genotypic differences in *indica* rices to culture (Jain 1997). As japonica rice genotypes are known to be superior in their response to culture than *indica* rices and for this reason, major advances in rice anther/tissue culture are well documented in *japonicas* (Abe and Futsuhara, 1986 and Jain 1997).

The growth regulators, mainly the auxins and cytokinins are known to control the dedifferentiation and differentiation processes in the *in vitro* cultures and through manipulation of plant growth regulators, the success rates can be enhanced (Mandal and Gupta, 1995). In the study, significant differences were not observed between the three levels of 2,4-D used in the callus induction experiments suggesting that low levels of 2,4-D can be used to ensure higher levels of regeneration as the regeneration rates are lower in the callus generated on higher levels of 2,4-D.

One of the major aspects of *in vit*ro studies is the relation between callus induction and regeneration as the genotypes that had high callus induction rates may not have high regeneration and vice versa. High callus induction frequencies observed in both Basmati 370 and Pusa Basmati 1 did confirm their high *in vitro* response (Raina 1987) while the mutant varieties Geetanjali (from Basmati 370) and Poorna Bhog (from Pusa Basmati 1) derived from these genotypes, did not possess the high *in vitro* response of their respective parents. Though both mutants are superior to parents in yield and possess good grain quality but their *in vitro* response is relatively poor thus confirming the

Table 3. Ranking of rice genotypes for callus induction and regeneration as per DMRT test

Cultivar	DMRT Rank
Callus induction	
Tapaswini	A
Basmati 370	A
Pusa Basmati 1	AB
Satyakrishna	BC
Samba Mahsuri	BC
Vandana	BCD
Naveen	CDE
Swarna	CDE
IR-64	CDE
Phalguni	DE
Dubraj	DE
Pooja	E
CR Sugandh Dhan 907	EF
Geetanjali	FG
Pusa 44	GH
Gayatri	GH
Sahabhagidhan	GHI
Anjali	HI
Poorna Bhog	HI
Satabdi	I
Regeneration	
Satyakrishna	A
Pusa Basmati 1	AB
Sahabhagidhan	ABC
Gayatri	ABC
Basmati 370	ABC
Satabdi	BCD
Swarna	BCD
Phalguni	BCD
Tapaswini	CD
Vandana	D
Pusa 44	D
CR Sugandh Dhan 907	DE
Samba Mahsuri	DEF
IR-64	DEFG
Pooja	EFGH
Naveen	FGH
Poorna Bhog	GH
Dubraj	HI
Geetanjali	HI
Anjali	I

Table 2. Analysis of variance for different traits

Source		Callus Induction						Regeneration					
	DF	SS	MS	F	P	CV	CD	SS	MS	F	P	CV	CD
Between all varieties	19	82276.62	4330.35	34.81	<.0001	19.29	9.92	34840.78	1833.73	10.08	<.0001	27.68	22.29
Between popular all varieties	11	52119.58	4738.14	42.00	<.0001	19.46	8.271	15652.53	1422.96	4.9	0.0008	34.36	28.86
Between aromatic varieties	6	25801.84	4300.31	25.41	<.0001	21.14	12.80	11036.94	1839.49	95.85	<.0001	11.43	7.79
Between doubled haploid varieties	2	973.32	486.66	1.38	0.2884	27.79	18.22	3720.786	1860.39	127.98	0.0002	5.86	8.64

Table 4. Clustering of genotypes based on callus induction and regeneration

Cluster	Genotypes in the cluster	Features of the cluster
1	Gayatri, Sahabhagidhan , Satabdi	Low callus induction and moderate regeneration
2	IR 64, Swarna, Tapaswini, Vandana, Basmati 370, CR Sugandh dhan 907, Pusa Basmati 1, Phalguni, SatyaKrishna, Pusa 44, Samba Mahsuri	Moderate to good callus induction and moderate to good regeneration
3	Dubraj, Geetanjali, Poornabhog, Anjali, Naveen, Pooja	Low to moderate callus induction and low regeneration

genetic nature of *in vitro* response. In general, aromatic rices possess high *in vitro* response while in this study, Geetanjali, Poorna Bhog (improved genotypes) and Dubraj (a native landrace) showed very low levels of regeneration suggesting that the genotypic differences also exist in aromatic rices.

Since Pusa Basmati 1 is the most frequently used genotype for transformation by many laboratories, Tapaswini and Satya Krishna (a doubled haploid) are the two genotypes among the newly developed varieties that can be employed for transformation purposes. Since PHB 71, a hybrid that shows high levels of callus induction in anther culture, Satya Krishna, a derivative from PHB 71 also had higher levels of *in vitro* response in callus cultures.

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