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Short Communication

Development of superior somaclones of aromatic local cultivar of rice (*Oryza sativa* L.)

Bidhan Roy

Department of Seed Science and Technology, Faculty of Agriculture, Uttar Banga Krishi Viswavidyalaya, Pundibari, Cooch Behar, West Bengal 736 165

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Abstract

The traditional local aromatic rice cultivars are poor combiners. Here we report development of superior somaclones plants through tissue culture. The somaclones, TC 4/8 had the maximum yield followed by TC 5-1, TC 4/4, TC 4/5, and TC 4/7. Yield increase of TC 4/8, TC-5-1 was 54.75% and 50.33% more over the yield of parental cultivar, respectively. The plant tissue culture thus may be recommended to create genetic variability in rice as a trustworthy biotechnological tool.

Key words: Local cultivars, aromatic rice, somaclones, biotechnological tool

Rice is the most important staple food grain and stands next to wheat in the global food grain production. A number of scented and non-scented rice land races are available in India in general and Northeastern India in particular (Talukdar et al. 2012). The traditional local aromatic land races of rice possess very low combining ability with the modern high yielding varieties.

Somaclonal variations (Larkin and Scowcroft 1981) that occur spontaneously during tissue culture cause changes in a variety of plant traits (Veena et al. 2018). The somaclonal variation may be an additional tool for crop improvement parallel with conventional breeding approaches (Neelakandan and Wang 2012). In the background of the above circumstances, the present study was undertaken to compare the performance of four local aromatic rice cultivars in respect of their callusing and regeneration ability in MS medium with various supplements and subsequently identification of somaclone(s) at field level for desirable characteristics.

The seeds of four aromatic local cultivars of rice, namely Kalo Nunia, Tulsimanjury, Kalojeera and Kalturey were used in this experiment. Two hundred fully matured and healthy seeds of each genotype were used for callus induction and subsequently plantlet regeneration. The seeds were dehusked manually, thoroughly washed in sterile water and surface sterilized with freshly prepared 0.1% $HgCl_2$ for 15 minutes followed by three washes in sterile distilled water and inoculated on MS (Murashige and Skoog 1962) medium in culture tubes (25 x 150 mm) and plugged with non-absorbent cotton wrapped in a layer of cheese cloth. The cultures were kept in dark at 25±2^{o}C. The experiment was repeated two times. On the 15th day, callus induction (%) was recorded. The calli were separated from explants and placed onto the same medium. After 28 days of inocubation on callus induction medium, callus from individual seed was sub-cultured on freshly prepared callus maintenance medium (MS + 1 mg/L of 2,4-D). They were kept for 28 days for callus proliferation.

Regeneration potential of the varieties was assessed by culturing the selected calli on regeneration medium (MS + 1 mg L^{-1} kinetin + 1 mg L^{-1} BAP + 0.5 NAA). The cultures were kept at 25±2 °C under 16/8h light/dark cycle. Plantlet regeneration was recorded up to 60th day after inoculation on regeneration medium and percentage was calculated as per Roy et al. (2011).

*Corresponding author's e-mail: bcroy10@yahoo.com

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Regenerated plantlets (S_0) following after hardening transplanted in earthen pots standard cultural practices. The seeds were harvested on individual plant basis and were grown (S1) following plant-to-plot method in the paddy field.

Observations were recorded crop-wise (S_3 and S_4) for each individual clone for two consecutive years (*Kharif* 2014 and 2015) and observations were taken on quantitative and qualitative characters. Aroma was evaluated through cooked rice aroma test as described by Allidawati dan Kustianto (1989). The score of each sample was recorded keeping Kalo Nunia as standard by a panel of experts. Completely randomized design (CRD) experiment was laid out for the laboratory experiment. The field experiment was conducted using Randomized Block Design with two replications. The data were subjected to standard statistical methods of analysis of variance (ANOVA) using AgRes Statistical Software, (c) 1994 Pascal Intl Software Solutions, Version 3.01 and significant differences were compared by LSD.

The analysis of variance showed significant differences among the varieties and treatment for germination of seed on synthetic medium. All the varieties for all the treatments showed high germination on callus induction medium and it ranged from 83.75% to 98.25%. Light, yellow globular calli were found to initiate at mesocotyle region of germinating seeds.

Initially, the cultivars were cultured on MS medium supplemented with different doses of 2,4-D, Picloram and Dicamba potentiality. It was found that 2,4-D @ 2.0 mg/L resulted better callus induction in all the local cultivars (Table 1). Across the medium compositions, MS medium fortified with 2,4-D @ 2.0 mg/L and NAA @ 05. Mg/L was found to be the best combination for callus induction. Cultivarwise, highest callus induction was recorded for Kalo Nunia followed by Tulsimanjury when MS medium was supplemented with 2,4-D @ 2.0 mg/L + NAA @ 0.5 mg/L (Table 1). Kalo Nunia also showed high callus induction ability when MS medium was fortified with 2,4-D @ 2.0 mg/L.

The cultivars, response to tissue culture was also considerably different. Callus induction (Fig. 1A), plantlet regeneration (Fig. 1B,C), number of plantlets (Fig. 1C) and number of plantlets survived after hardening were high in Kalo Nunia followed by Tulsimanjury, Kalojeera and Kalturey (Table 1). Highest mean values of four genotypes on eight treatments were observed for callus induction, plantlet regeneration, number of plantlets and number of plantlets survived after hardening when callus were induced on MS medium fortified with 2,4-D @ 2.0 mg/L and NAA @ 0.5 mg/L following regeneration on MS medium supplemented with 1 mg/L kinetin + 1 mg/L BAP + 0.5 NAA (Table 1).

No morphological variation was observed for the somaclones derived from Tulsimanjury and Kalturey in S_0 and S_1. Morphological variations were observed in 13 somaclones of Kalo Nunia and five somaclones of Kalojeera in S_0 generation (Fig. 1E). Considering the field performance of S_2 generation in terms of yield attributing characters and presence of aroma, eight individual somaclones with superior characters of Kalo Nunia were given special attention during the next year field trials. But, we could not identify any desirable somaclone from Tulsimanjury in S_1 and S_2 generations. Those eight somaclonal lines of Kalo Nunia were passed to the evaluation trials for another two successive seasons (S_3 and S_4 generations) along with Kalo Nunia.

A large amount of variability was observed among the eight somaclones and the mother cultivar-Kalo Nunia for all the characters except flag leaf width and decorticated grain width. Somaclones performed better in the field trials than the parental cultivar in respect of most of the yield attributing characters and also retained the aroma (Table 2b). Reduced plant height as compared to Kalo Nunia was observed in four somaclones, namely, TC 4/3, TC 4/4, TC 5-1 and TC 4/6 (Table 2a). Days to 50% flowering also reduced in all the somaclones except TC 4/8, however, it showed insignificant difference with parental cultivar. The panicle emergence of the somaclones TC 4/3, TC 4/4, and TC 5-1 was about 10 days earlier to parental cultivar. All the somaclones produced more tillers as compared to parental cultivar. Highest number of panicle per plant was observed for TC 4/6 followed by TC 4/7, TC 4/3, TC 4/4 and TC 5-1. Highest number of filled grains per panicle was recorded for TC 4/5 (157.80) followed by TC 4/8, TC 5-1 and TC 4/3.

Grain length, grain breadth and grain L:B ratio were used to determine the grain type. According to the classification of ICAR, the somaclones-TC 4/2, TC 4/5, TC 4/7 and TC 4/8 were found to be long slender, TC 4/3, TC 4/6, and TC 5-1 were found to be medium slender, whereas, TC 4/4 was short bold (Table 2b).

Table 1. Mean values on regeneration and number of plantlet obtained

Treatment	*Callus ability		No. of callus responded to regeneration		Regeneration (%)	No. of plantlets regenerated		No. plant survive after hardening		Survival (%)	Variability
V1T1	27.00	op	6.50	lmno	24.07	12.00	lmnop	9.50	no	79.17	Variable
V1T2	107.50	c	68.00	b	63.26	138.00	b	103.50	b	75.00	
V1T3	22.00	q	4.50	mopq	20.45	6.50	p	4.50	rst	69.23	
V1T4	29.50	mno	6.00	nop	20.34	13.00	klmno	8.50	op	65.38	
V1T5	33.00	l	11.50	hi	34.85	19.00	hij	17.50	j	92.11	
V1T6	39.50	k	12.00	h	30.38	22.00	hi	17.00	jk	77.27	
V1T7	21.50	qr	11.00	hi	51.16	16.50	ijklm	13.50	lmq	81.82	
V1T8	133.50	a	107.5	a	80.52	225.50	a	209.50	a	92.90	
V2T1	21.50	qr	9.50	ijk	44.19	18.00	hijk	12.50	mq	69.44	True-to-type
V2T2	97.00	d	44.50	d	45.88	96.00	c	63.50	c	66.15	
V2T3	16.50	s	4.50	opq	27.27	7.00	p	2.50	t	35.71	
V2T4	23.50	q	4.00	pqr	17.02	8.00	op	3.50	st	43.75	
V2T5	24.00	pq	6.50	lmno	27.08	18.00	hijk	11.50	mn	63.89	
V2T6	30.00	lmno	6.50	lmno	21.67	18.00	hijk	15.00	kl	83.33	
V2T7	27.00	op	8.50	jkl	31.48	14.50	jkln	7.50	op	51.72	
V2T8	125.50	b	51.00	c	40.64	86.50	d	62.50	c	72.25	
V3T1	18.50	s	6.50	lmno	35.14	10.00	mnop	6.50	pr	65.00	Variable
V3T2	84.00	e	31.50	e	37.50	87.00	d	55.00	d	63.22	
V3T3	28.00	no	8.00	klm	28.57	14.50	jklmn	7.00	pqr	48.28	
V3T4	31.00	lm	5.50	nop	17.74	14.50	jklmn	4.50	st	31.03	
V3T5	49.00	j	10.50	hij	21.43	23.50	h	18.00	j	76.60	
V3T6	52.50	i	4.50	opq	8.57	9.50	nop	4.00	t	42.11	
V3T7	65.50	g	18.50	g	28.24	31.50	g	23.50	i	74.60	
V3T8	76.50	f	31.50	e	41.18	67.00	e	42.00	e	62.69	
V4T1	31.00	lm	7.50	klmn	24.19	15.50	jklm	6.50	pqrs	41.94	True-to-type
V4T2	63.50	g	21.00	f	33.07	52.50	f	32.50	g	61.90	
V4T3	31.00	lm	3.00	q	9.68	8.50	op	7.00	pq	82.35	
V4T4	32.50	lm	2.00	r	6.15	7.50	op	5.50	qrs	73.33	
V4T5	28.50	o	2.50	qr	8.77	8.00	op	3.50	t	43.75	
V4T6	21.50	qr	5.50	nopr	25.58	8.00	op	3.00	t	37.50	
V4T7	57.50	h	20.50	fg	35.65	36.50	g	26.00	h	71.23	
V4T8	74.50	f	42.50	d	57.05	66.00	e	35.00	f	53.03	

*No. of germinated seed produced callus; Values bearing same letter in the column are not significantly different at P=0.01 of LSD; Group 'a' is the best performing treatment(s) and the performance of treatment(s) in Group 'b', 'c', …. are in decreasing order, respectively

V1: Kalo Nunia; V2: Tulsimanjury; V3: Kalojeera; V4: Kalturey

T1: MS + 2,4-D @ 1.0 mg/L T2: MS + 2,4-D @ 2.0 mg/L

T3: MS + Picloram @ 0.50 mg/L T4: MS + Picloram @ 1.0 mg/L

T5: MS + Dicamba @ 0.50 mg/lL T6: MS + Dicamba @ 1.0 mg/L

T7: MS + 2,4-D @ 2.0 mg/L + NAA @ 0.25 mg/L T8: MS + 2,4-D @ 2.0 mg/L + NAA @ 0.5 mg/L

Stem thickness has direct positive correlation with lodging resistance. Three smoaclones had significantly different thicker culm as compared to Kalo Nunia. Highest 100-grain weight was recorded for TC 4/8 followed by TC 4/2 and TC 4/5. Grain yield of all selected somaclones increased over the parent (Table 2b). TC 4/8 had the maximum yield followed by TC 5-1, TC 4/4, TC 4/5, and TC 4/7. Yield increase of TC 4/

Fig. 1. ***In vitro*** **development of plantlets from an** ***indica*** **local rice, Kalo Nunia involving mature seed embryos and the performance of somaclones at field (A) Stereomicroscopic view of callus proliferation on callus maintenance medium (MS + 1 mg L^{-1} 2,4-D). (B) Embryogenesis, producing roots and shoots simultaneously in regeneration medium (MS + 1 mg L^{-1} kinetin + 1 mg L^{-1} BAP + 0.5 mg L^{-1} NAA). (C) Regenerated plantlets in culture tubes. (D) S_0 plant after hardening on earthen pot in net-house. (E) S_2 plants in the field. (F) S_4 somaclonal of population of TC4/8 derived from Kalo Nunia. (G) S_4 somaclonal of population of TC-5-1 derived from Kalo Nunia**

Table 2a. Mean vales of quantitative and qualitative characters of eight somclones

Genotypes	Plant height (cm)	Days to 50% flowering	No. of panicles/ plant	Flag leaf length (cm)	Flag leaf width (cm)	Panicle length	No. of filled grains/ panicle	No. of chaffy grains/ panicle	Sterility (%)
TC-4/2	158.80 c	136.67 c	19.33 d	28.98 bc	1.38	27.17 bc	94.73 e	65.80 e	40.95 e
TC-4/3	142.07 a	128.67 a	25.53 b	27.40 cd	1.34	22.96 f	124.53 c	42.53 c	25.42 bc
TC-4/4	143.60 a	128.33 a	23.07 c	26.40 d	1.34	24.97 e	114.20 d	31.07 a	21.39 a
TC-4/5	165.67 d	134.00 b	18.73 d	30.58 ab	1.27	28.29 ab	157.80 a	51.87 d	24.75 b
TC-4/6	155.87 c	131.67 b	34.87 a	24.26 e	1.41	26.66 cd	114.40 d	34.60 ab	23.14 ab
TC-4/7	159.00 c	126.33 a	26.27 b	30.61 a	1.32	28.79 a	81.53 f	40.40 bc	33.10 d
TC-4/8	164.47 d	138.67 c	15.40 e	30.18 ab	1.24	28.06 abc	147.20 b	38.87 bc	20.87 a
TC-5-1	147.80 b	128.00 a	22.33 c	29.10 ab	5.45	24.35 e	128.80 c	35.73 abc	21.70 a
Kalo Nunia	157.36 c	136.67 c	13.80 e	29.83 ab	1.40	25.19 def	98.57 e	37.95 abc	27.79 c

8 (Fig. 1F), TC-5-1 (Fig. 1G) was 54.75% and 50.33% more over the yield of parental cultivar, respectively (Table 2a,b). All the somaclones also retained strong aroma as that of the parental cultivar- Kalo Nunia.

Table 2b. Mean vales of quantitative and qualitative characters of eight somclones

Genotypes	Grain length (mm)	Grain width (mm)	Grain L:B ratio	Grain type	Dehusked grain length (mm)	Dehusked grain width (mm)	Stem thickness	100-grain weight	Grain yield	Yield advantage over parent (%)
TC-4/2	8.02 a	2.28 bc	3.53 ab	LS	5.90 a	1.97	5.06 cd	1.61 b	2.34 cd	26.07
TC-4/3	5.67 d	2.12 ab	2.69 de	MS	4.05 d	1.89	4.02 h	1.20 fg	2.12 e	18.52
TC-4/4	5.91 cd	2.47 c	2.41 e	SB	4.79 bcd	2.19	5.65 a	1.23 ef	2.45 c	29.39
TC-4/5	7.08 b	1.96 a	3.63 a	LS	5.48 abc	1.69	4.49 f	1.51 c	2.42 c	28.51
TC-4/6	6.35 c	2.24 abc	2.83 cd	MS	4.02 d	1.95	5.20 b	1.16 g	2.22 de	21.95
TC-4/7	6.48 c	2.09 ab	3.12 abc	LS	4.90 bcd	1.82	4.72 e	1.21 efg	2.38 c	27.21
TC-4/8	7.95 a	2.44 c	3.27 b	LS	5.70 ab	2.05	5.15 bc	1.77 a	3.82 a	54.75
TC-5-1	6.25 cd	2.29 bc	2.74 cde	MS	4.57 cd	1.94	4.35 g	1.27 e	3.48 b	50.33
Kalo Nunia	7.62 ab	2.27 bc	3.35 ab	LS	6.01 a	2.27	5.00 d	1.41 d	1.73 f	-

*Values bearing same letter in the column are not significantly different at P=0.01 of LSD. Group 'a' is the best performing somaclone(s) and the performance of somaclone(s) in Group 'b', 'c', …. are in decreasing order, respectively.

For ranking the somaclones the values of plant height, days to 50% flowering, number of chaffy grains per panicle and sterility (%) were arranged in ascending order and the values of other characters were arrange in descending order.

LS = Long slender; MS = Medium slender and SB = Short bold

The selections of somaclone(s) having desirable qualitative and quantitative characteristics with high yielding potentiality are being received great attention.

Morphological variations of the somaclones were observed only in Kalo Nunia and Kalojeera (Table 2a,b). Bunnag and Suwanagul (2017) developed drought tolerant somaclones in rice. Saleh et al. (2019) reported somaclonal variation in rice. They have determined the variations through cytological analysis of *in vivo* and *in vitro* grown rice plantlets.

Significant yield increase of somaclones over parent might be due to increase in number of panicles per plant, panicle length, number of filled grains per panicle and 100-grain weight along with reduction in spikelet sterility of the somaclones. Mandal et al. (2000) also found somaclones with more yield over parent coupled with salt tolerance ability. The elite performance of TC 4/8 and TC 5-1 was probably due to inherent improvisation of yield attributing traits or higher accumulation of culture induced variation facilitating higher yield.

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