

Full Length Research Paper

# Anther culture response in *indica* rice and variations in major agronomic characters among the androclones of a scented cultivar, Karnal local

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Effect of organic adjuvants, synthetic plant hormones and diverse carbon sources in influencing anther culture response in five *indica* rice genotypes (IR 72, Mansarovar, Taraori Basmati, Pusa Basmati and Karnal local 95) were assessed. Androgenic callus induction as well as green plantlet regeneration was more when callus was induced on N6 fortified with 100 mg L<sup>-1</sup> YE. However, coconut water (5 and 10% concentrations from Pink Dwarf variety) encouraged green plantlet regeneration only in Karnal local 95. High callus induction was observed with synthetic hormones in comparison to the control set. However, green plantlet regeneration was observed when callus induction medium (CIM) was supplemented with 2, 4-D and IBA in Taraori Basmati and Karnal local 95. Among the varieties Pusa Basmati and Karnal local 95 showed better callus inductions on N6 supplemented with 6% maltose. Maximum callus induction was observed in Pusa Basmati and Karnal local 95 when 6% sucrose or maltose were used as carbon source. Total plantlet regeneration was cent percent in Taraori Basmati when CIM was supplemented with maltose (6%). Maximum green plants were obtained when CIM was added with maltose (6%) for Karnal local 95. Plantlet regeneration in the present study was found to be very low. Androgenic plantlets derived from the scented *indica* rice, Karnal local 95, were evaluated under field condition to assess variability among segregating A<sub>2</sub> generation. Overall mean values in respect of some major agronomic characters viz. plant height, panicle length, number of filled grains per panicle, spikelet sterility (%) and grain yield per plant were reduced except number of panicles per plant. High CV was observed for filled grains per panicle, grains yield per plant and number of panicles per plant suggesting the existence of high variation among the androclones for those characters. Positive selection seems to be improvising those characters. The frequency distribution for number of panicles per plant and panicle length varied largely among the androclones in both direction of the parental mean. Whereas, all the androclones were found to be shorter than the parent and low yielder too. This study elucidates that the genetic modulation through exploitation of androclonal variation is a feasible proposition in scented *indica* rice.

**Key words:** Scented rice, anther culture, androclonal variation, genetic improvement.

## INTRODUCTION

In the recent past anther culture in rice has been improved substantially. However, detailed study on various factors governing culture response of anthers under *in vitro* condition especially in *indica* rice is extremely limited. Generally green plant regeneration from androgenic calli is very low irrespective of race. Low anther culture response, high percent of albino plantlet

regeneration and abundance of haploids are the principal constraints in establishing successful anther culture in rice.

Plantlets developed directly from microspores provide ample scope in developing homozygous doubled haploids (DH) without interference of heterozygosity. Anther culture provides an easy to handle *in vitro* selection for genetic improvisation of characters (Jahne et al., 1991) for superior performance. Androclonal variation seems to have immense prospect albeit it was quantified in a very few cases in rice (Mandal et al.,

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2000). DH lines produced from microspore culture and/or anther culture were proved to be highly potential in genetic improvement by broadening the genetic diversity through production of homozygous lines within a short time (Morrison and Evans, 1988; Foroughi-wehr and Wenzel, 1989), in contrast to numerous cycles of inbreeding or back crossing as conventionally practiced *en route* plant breeding. Owing to the importance of anther culture in speeding up the breeding process, plants with special agronomic characters such as development of earliness, increased grain weight, superior grain quality, disease resistance (Zhan et al., 1984; Zhang, 1989), dwarf plant type and abiotic stress tolerances have been developed (Alejar et al., 1995). Unlike somaclonal variation, utilization of androclonal variation (AV) in rice genetic improvement is reported to be exceedingly less. Upon culturing anthers from an inbred line or from  $F_1$  recombinants, one might generate androclones with altered characters different from the parent due to culture induced AV. Thus, anther culture prospects amply in rice genetic improvement. Considering the above aspects of anther culture, the present study was aimed at increasing the green plantlet regeneration by optimizing phytohormones, organic adjuvants and carbon sources and to assess the range and magnitude of variation in pollen derived plants of a scented traditional cultivar, Karnal local 95 which is widely grown in the Haryana State and is popular for its high domestic and international market owing to high consumers preference.

## MATERIALS AND METHODS

### Plant materials and callus induction

IR 72, Mansarovar, Pusa Basmati, Taraori Basmati, and Karnal local 95 were employed for this study. The panicles with boot leaf sheath were washed thoroughly in tap water and spread with 70% ethanol. They were covered with moist tissue paper, kept in polyethylene bag and cold shocked at 8°C for eight days in a BOD incubator prior to anther plating. On the day of culture, selected spikelets were surface sterilized in tissue culture bottles with 0.1% freshly prepared  $HgCl_2$  solution for 10 min. The  $HgCl_2$  was drained off and the panicles were washed four times in sterile distilled water. Fifty to sixty spikelets were cut at a time on sterile petri dishes under laminar airflow (LAF) bench. Individual spikelets were cut at the base to free the anthers from the filaments. They were plated aseptically onto radiation-sterile petri dishes (60×15 mm diameter, Tarsons make) containing callus induction medium (CIM) - N6 (Chu et al., 1975) supplemented with different concentrations of phytohormones (2,4-D at 0.5, 1.0, 2.0 and 3.0 mg L<sup>-1</sup>; NAA at 0.5, 1.0, 2.0 and 3.0 mg L<sup>-1</sup>; IBA at 0.5, 1.0, 2.0 and 3.0 mg L<sup>-1</sup>), organic adjuvants (Yeast extract, YE at 200, 400 and 1000 mg L<sup>-1</sup>; casein hydrolysate, CH at 50, 250 and 500 mg L<sup>-1</sup>; coconut water, CW at 5, 10 and 15%) and carbon sources (sucrose, maltose, dextrose, glucose, galactose and cane sugar at 3 and 6% singly) and 0.8% agar. The cultures were sealed with parafilm and kept in dark at 25±2°C. The plates were examined periodically at weekly interval to observe the progress in respect of callus formation. Embryogenic calli of at least ~2 mm diameter were transferred to 25×150 mm culture tubes (Borosil) containing 10 ml regeneration

medium consisting of MS supplemented with 1 mg L<sup>-1</sup> BAP, 1 mg L<sup>-1</sup> Kinetin, 0.5 mg L<sup>-1</sup> NAA, 3% (w/v) sucrose and 0.8% (w/v) agar. The pH of the medium was adjusted to 5.8 with 1N HCl or 1N NaOH before adding agar and autoclaving. The culture tubes were plugged with non-absorbent cotton wrapped in one layer of cheesecloth. Inoculated cultures were kept for four weeks under 16/8 h light (~130  $\mu E m^{-2} s^{-1}$ ) /dark at 25±2°C.

### Field evaluation of the androclones

The regenerated plantlets were hardened and transplanted in cement pots under glass house condition. At maturity seeds were harvested ( $A_1$ ) individual plant wise and stored for field trails in the succeeding generation ( $A_2$ ). Seeds harvested from Karnal local 95 were used to assess the variability among the progenies. Seeds were grown in raised nursery bed. Seedlings of 25-day old were transplanted at a spacing of 20 × 15 cm. Standard cultural practices compatible to the humid tropics of Bay Islands were adopted to ensure a good crop growth (Majumder et al., 1992). Major agronomic characters viz. plant height, number of panicles per plant, panicle length, number of filled grains per panicle, spikelet sterility and grain yield per plant were recorded at maturity of the crop. The data were statistically analyzed to assess the extent of variability among the androclones.

## RESULTS AND DISCUSSION

### Effect of phytohormones

Callus induction was more in Taraori Basmati and Karnal local 95 in comparison to others. Maximum callus induction (3.38%) was observed in Taraori Basmati, when N6 was fortified with 0.5 mg L<sup>-1</sup> of 2,4-D followed by Taraori Basmati (2.58%) at 2.0 mg L<sup>-1</sup> of 2,4-D and Karnal local 95 (2.06%) at 1.0 mg L<sup>-1</sup> of 2,4-D. Green plantlets were generated more in Taraori Basmati and Karnal local 95 from the androgenic callus induced on CIM supplemented with 1.0 mg L<sup>-1</sup> of 2,4-D.

Callus induced more when N6 was fortified with different concentrations (0.5, 1.0, 2.0 and 3.0 mg L<sup>-1</sup>) of NAA. Maximum callus induction was observed in Taraori Basmati (6.09 and 2.15% at 0.5 and 1.0 mg L<sup>-1</sup> of NAA, respectively), followed by Karnal local 95 (1.51% at 1.0 mg L<sup>-1</sup>). Very low callus induction (%) was observed in Mansarovar and IR 72. The embryogenic calli were transferred to RM for plantlet regeneration. Pusa Basmati, Taraori Basmati and Karnal local 95 developed plantlets profusely. No green plantlet regeneration was observed, when N6 with NAA was used as CIM. Different concentrations (0.5, 1.0, 2.0 and 3.0 mg L<sup>-1</sup>) of IBA was used to facilitate callus induction and plantlet regeneration. Callus induction was observed over all concentrations of this synthetic hormone for Taraori Basmati and Karnal local 95. Maximum (5.08%) callus induction was observed at 2.0 mg L<sup>-1</sup> of NAA in Karnal local 95. Pusa Basmati, Mansarovar and IR 72 showed poor callus induction. Compact embryogenic calli of ~2 mm diameter were transferred to RM for further



**Figure 1.** Anther culture and plantlet regeneration in rice variety (Karnal local-95. a) Plated anthers showing androgenic callus induction on N6 medium supplemented with 15% coconut water, b) Stereomicroscopic view of the emerging androgenic calli by rupturing the anther wall, c) A close view of androgenic calli harbouring embryo like structure on N6 medium supplemented with 15% coconut water, d) Regeneration of albino plantlets on RM consisting of MS with  $1 \text{ mg L}^{-1}$  Kinetin,  $1 \text{ mg L}^{-1}$  and  $0.5 \text{ mg L}^{-1}$  NAA, e) Regenerating androgenic green plantlets on RM containing MS with  $1 \text{ mg L}^{-1}$  Kinetin,  $1 \text{ mg L}^{-1}$  BAP and  $1 \text{ mg L}^{-1}$  NAA, f) Fertile plants developed through anther culture on cement pot.

poor callus induction. Compact embryogenic calli of ~2 mm diameter were transferred to RM for further regeneration. Albino plantlets were obtained in all the varieties.

### Effect of organic adjuvants

Except Taraori Basmati and Karnal local 95, all other varieties produced androgenic callus in N6 supplemented with three different concentrations (200, 400 mg L<sup>-1</sup> and 100 mg L<sup>-1</sup>) of YE. High callus induction (%) was observed at low concentration (200 mg L<sup>-1</sup>) of YE in all anther culture responsive varieties. In Taraori Basmati, callus induction from cultured anthers was observed only at 200 mg L<sup>-1</sup> of YE. This showed that lower concentration of YE facilitated androgenic callus initiation. The result also indicates substantial variation in callus induction among the varieties as well as among different concentrations of YE. Maximum albino plantlets (6) were observed in Mansarovar followed by Pusa Basmati (3). IR 72 developed more plantlets from the callus induced on N6 containing 200 mg L<sup>-1</sup> of YE, whereas Pusa Basmati developed more plantlets from the callus induced on N6 fortified with 400 and 1000 mg L<sup>-1</sup> of YE, respectively.

Callus initiation was observed in all varieties, which were cultured on MS fortified with different concentrations of CH except in Karnal local 95 at 250 mg L<sup>-1</sup>. However, this organic adjuvant showed null relationship between the callus induction and its concentration. Maximum callus induction was observed in Karnal local 95 (1.19 and 1.39% at 50 and 500 mg L<sup>-1</sup> of CH, respectively), followed by Mansarovar (0.61, 1.14 and 0.89% at 50, 250 and 500 mg L<sup>-1</sup> of CH, respectively). The plantlet regeneration varied between 30.77-100%. The beneficial effect of CH might be due to the influence of undefined organic nitrogenous compounds in CH, which generally favour embryogenic callus induction. However, the dose of CH added to the medium may vary according to the rice genotypes.

Callus induction varied from 0.07 to 1.57% in Taraori Basmati and Karnal local 95 (Figures 1a, b and c). IR 72 showed high androgenic callus induction at all the concentrations of CW (5, 10 and 15%). Pusa Basmati showed very poor *in vitro* culture response (0.07%) at 15% CW on N6. Green plantlet regeneration was 57.14 and 66.66% in Karnal local 95, when compact embryogenic calli were transferred to RM from CIM consisting of N6 with 5 and 15% of CW, respectively.

### Effect of carbon sources

Diverse carbon sources viz. sucrose, glucose, maltose, dextrose, galactose and cane sugar were used to pinpoint the appropriate compound for prolific callus induction and facile green plantlet regeneration in anther

culture system. Maximum callus induction (4.47%) was observed in Pusa Basmati on N6 with 3% maltose. Callus induction was exceedingly low in Mansarovar. However, callus induction was observed in all the treatments for all varieties. Maximum callus induction was recorded when N6 was supplemented with 3 and 6% of maltose. The embryogenic calli were transferred to RM for regeneration of plantlets. High percent of albino plantlets were observed from all the *in vitro* culture responsive cultivars. In some varieties \*cent percent\* plantlet regeneration was observed, however, majority were found to be albino (Karnal local 95 on RM supplemented with 3% glucose and Taraori Basmati with 6% maltose). Green plantlet regeneration was 13.04 and 20% in Pusa Basmati and Karnal local 95, respectively on RM, when N6 was fortified with 6% maltose.

Plantlet regeneration was observed in all the varieties in this study. Number of responding calli was high in Taraori Basmati, Karnal local 95 (Figure 1e) and Mansarovar. Extremely low culture response was observed in IR 72 followed by Pusa Basmati. Among the treatments, more culture response was recorded when medium was supplemented with organic adjuvants like CH, YE and CW, phytohormones (2, 4-D, NAA and IBA) and maltose as carbon source. The regenerated plantlets were found to be rootless indicating their organogenetic development. When such shootlets were transferred onto MS with 1 mg L<sup>-1</sup> IAA/IBA, they put forth prolific roots in a short time.

Manipulation of chemical and physical culture environments is essential to provoke microspore to switch on to an embryogenic rather than an gametophytic pattern of development (Srivastavae and Johri, 1988). The effect of different treatments and varieties on green plantlet regeneration showed green plantlet regeneration in only three varieties viz. Taraori Basmati, Karnal local 95 and IR 72. It was also found that CW (5 and 15%), 2,4-D (2 mg L<sup>-1</sup>) and 6% maltose in CIM promoted green plantlet regeneration. N6 supplemented with organic adjuvants like YE, CH and CW showed enhanced androgenic callus induction in *indica* rice to a considerable extent. Null relationship was discernable between callus induction/plantlet regeneration and different concentrations of medium/organic adjuvants, phytohormones, carbon sources and genotypes. It indicates that the callus induction and subsequent green plantlet regeneration were stringently genotype specific. However, application of 6% maltose as carbon source in the medium inflated plantlet regeneration substantially. Green plantlet regeneration was found to be very low. It is mentionable that the success of plantlet regeneration under *in vitro* culture system depends upon the type of medium used in each phase of culture starting from callus induction, proliferation to plantlet regeneration and the type and dose of different growth regulators especially auxins, and cytokinins used in cereal anther culture. Among the auxins, 2,4-D was found to be useful

**Table 1.** Androclonal variation for major agronomic characters in Karnal local-95.

Character	Karnal local 95 (parent)	Androclonal variation			
		Range	Mean	SD	CV
Plant height (cm)	131.60	96.0 - 125.0	111.36	8.47	7.84
Panicle/plant	6.80	3.0 - 21.0	8.63	4.33	50.21
Panicle length (cm)	24.43	17.0 - 25.0	22.02	1.87	8.51
Filled grain/panicle	116.13	3.2 - 45.0	14.36	8.62	60.09
Sterility (%)	27.88	18.42 - 89.28	62.51	15.13	24.21
Yield/plant (g)	12.03	0.48 - 8.65	2.66	2.31	87.12

for callus induction and subsequently in green plantlet regeneration. Albino plantlet regeneration was very high (Figure 1d) in all the varieties. Interestingly, number of responding calli was high in Taraori Basmati, Karnal local 95 and Mansarovar. Very low culture response was observed in IR 72 followed by Pusa Basmati. Among the treatments, more culture response was observed when medium was added with organic adjuvants like CH, YE and CW, phytohormones (2, 4-D, NAA and IBA) and maltose as carbon source. Only three varieties (Taraori Basmati, Karnal local 95 and IR 72) showed green plantlet regeneration. It was also found that androgenic callus induced on CIM containing CW (5 and 15%), 2 mg L<sup>-1</sup> 2,4-D and 6% maltose promoted green plantlet regeneration upon their transfer to RM to a considerable extent. The regenerated plantlets were rootless initially. However, they were rooted on MS containing 1 mg L<sup>-1</sup> IAA/IBA as root promoting hormone. After two weeks on RM, profuse tillers were observed. Those rooted plantlets were shifted to experimental glass house for hardening. After one week of hardening, plants were finally transferred to cement pots (Figure 1f). The androgenic plants obtained from Taraori Basmati showed flowering in time, however, produced \*cent percent\* spikelet sterility owing to their haploid nature. The plants developed in Karnal local 95 also flowered and produced filled grains with high sterility perhaps due to their genesis and development on relatively harsh *in vitro* environment. The androclones obtained from IR 72 were maintained and multiplied under *in vitro* culture condition following large-scale microtillering, which bears tremendous importance in generating tillers of true to parent nature.

### Androclonal variation

To quantify AV precisely, androclones derived from Karnal local 95 were used. Wide variation among the androclones was observed for major agronomic characters (Table 1). AV was earlier described by Oono (1984), Raina (1989) and Mandal et al., (2000). It was suggested that the variation in regenerants from haploid somatic tissue would be greater than any other chromic mutagenic treatments. Overall mean value of andro-

clones was found to be reduced except panicle per plant in comparison to the parent. It was also observed that the panicle length displayed almost no variation among the androclones and the parental line. This result corresponds with the findings of Mandal et al. (2000) for this character. Plant height of the androclones reduced as compared to the parent. The reduced plant height is desirable since the parental line was tall and lodgingsusceptible. High spikelet sterility (%) was observed in the androclones, which varies from 18.42 to 89.29% probably because of the growth and development of androclones under artificial *in vitro* culture condition. Owing to high spikelet sterility, yield of androclones reduced drastically and ranged in between 0.48-8.65 g per plant. Owing to high spikelet sterility, yield of androclones reduced drastically and ranged in between 0.48-8.65 g per plant.

SD of mean, a reliable parameter to measure uniformity of a character was found to be more uniform for panicle length (1.87) across the androclone families in comparison to other characters as evident from their low SD and narrow range of variation (17.00-25.0). High CV was observed for grain yield per plant (87.12) followed by number of filled grains per panicle (60.09) and panicles per plant (50.21). It elucidates that the genetic modulation of those characters through exploitation of AV is a feasible proposition in rice.

The frequency distribution for number of panicles per plant and panicle length varied greatly among the androclones (Table 2). Of the 180-androclone families, all were found to be shorter than the parent. About 45 and 36% of the androclones showed more panicles per plant and increased panicle length, respectively than the parent. However, no androclone yielded more than the parent. This may be due to high spikelet sterility (%) among the anther derived clonal populations. In all, large variations were observed among the androclones. Those variations may be due to physiological factors, chemical constituents of the culture medium, biological condition of the explants, departure duration of the unorganized calli and reorganized plantlets. Gene amplification, changes in gene distribution, gene conversion, somatic recombination, and movement of transposable elements, position effect mutation and other chromosomal rearrangements

**Table 2.** Distribution of androclones based on major agronomic characters in comparison to parental mean.

Variation	Frequency distribution							
	Plant height (cm)		Number of panicle		Panicle length (cm)		Yield /plant (g)	
	No.	%	No.	%	No.	%	No.	%
Less than parental mean	180	100	72	40	126	70	180	100
Equal to parental mean	-	-	27	15	18	10	-	-
More than parental mean	-	-	81	45	36	20	-	-

might be the other plausible causes of AV in DH lines.

Variation does occur in plants development through anther culture (Raina, 1989; Mandal et al., 2000). Phenotypic variations have been reported in respect of number of tillers per plant, average panicle length, number of fertile seeds, plant height in primary regenerants of rice (Zakri, 1986). Single gene governs AV, and genes with large diagnosable effects (Ryan et al., 1987) have enormous practical implications in comparison to polygenically controlled characters. Among the quantitative characters considered under this study, superior types with increased panicle number may be developed following stringent positive selection. Decreased plant height may be achieved by selecting short statured lines without much alteration of the parental background, which is desirable for Karnal local 95 since it is very tall and found to be highly lodging susceptible and concurrently bears high quality scented grains. Interestingly panicle length did not show much variation in the androclones. Karnal local 95 possess as such long panicles. At the same time more stress may be applied to reduce the spikelet sterility (%) of the anther-derived plants to increase the net grain yield. In essence this study prospects the use of AV in rice genetic improvement.

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