Somaclonal Variation in Three Varieties of Scented Rice

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Suspended protoplast culture was made from callus culture of four scented rice varieties *viz.* Basmatibahar, Gourav, Kalimochi and Muskbudhi. A good yield of protoplast up to 0.6 x 10^6 and 4 x 10^6 protoplasts/ml were obtained from callus and cell suspension cultures respectively after 4 h of incubation. The protoplast cultured from Gourav exhibited best colony formation *i.e.* 16.2 from callus and 62.4 from cell suspension and 38.52% plant regeneration. The protoplasts derived from regenerated plants exhibited substantial magnitude of variability for five economic characters. Grain yield followed by grain number/plant and panicle number/plant showed significant variability.

Key Words: Callus/Cell Suspension Culture, Plant Regeneration, Protoplast Culture, Scented Rice

The scented rice has a premium value in international market for its aroma and slenderness. India is the second largest exporter of Basmati rice next to Thailand. It also shares a major part of total agricultural export in our country. The success of crop improvement programme largely depends upon the extent of exploitable variability in the germplasm of crop concerned. In the past, conventional breeding methods were followed in improvement of crop production. Conventional hybridization has its own limitations. Tissue and protoplast culture provides avenues to create a wide genetic variability in the improvement of export quality scented rice. The present experiment was undertaken to generate the variability in scented rice by protoplast isolation, culture and regeneration of plants from four scented rice varieties.

Materials and Methods

The protoplast isolation and culture were carried out in four scented rice varieties *viz.*, Basmatibahar, Gourav, Kalimochi and Muskbudhi. Mature seeds were used as explants for callus induction. MS medium (Murashige and Skoog, 1962) was supplemented with 2-4-D 2.0 mg/l and KIN 0.5 mg/l. The cell suspension was carried out to a 500 mg of actively growing callus into a 150 ml flask containing 30 ml of liquid R-2 (Ohira *et al.*, 1973) medium supplemented with 2-4-D 2.0 mg/l and KIN 0.5 mg/l. The protoplasts were isolated from the callus as well as from cell suspension cultures. Some 500 mg of cellulage (Onozuka R-10) was added with 50 mg pectolyase Y23 and 29 mg of EMS (buffer) in 50 ml of CPW 13 m salt solution at pH 5.5 at room temperature. The protoplasts were cultured at a density ranging from 5 x 10^5 protoplasts/ml to 2x 10^6 protoplasts/ml in Milipore filter membrane (0.2 µm) placed in feeder cells 8% PCV and are embeded in K.P.R. medium solidified with 0.8% agar. The callus were incubated at 25° ± 2 in dark till the formation of colonies. After 3-4 weeks of culturing the individual colonies were transferred to regeneration medium (MS + NAA 0.2 mg/l) to obtain regenerated plants. The plants were transferred to field, raised and grown to maturity. Variation for yields and its component characters were recorded from the individual plants. The observations from the 10 parent plants and also from all the regenerated plants were recorded. The range, mean, CV were calculated as per standard procedures.

Results and Discussion

Protoplast Isolation

The callus were incubated at 25°C and were shaken with a speed of 100 RPM. After 4 h of shaking, the protoplasts were isolated. Good protoplasts were

Table 1. Number of colonies obtained from the callus and cell suspension derived protoplast of four scented rice

Variety	I	II	III	IV	V	Mean ± SE
Callus derived-protoplast						
Basmatibahar	12	10	4	7	12	9.0 ± 1.55
Gourav	15	12	18	14	22	16.2 ± 1.74
Kalimochi	8	7	5	6	11	7.4 ± 1.03
Muskbudhi	0	5	3	2	2	2.4 ± 0.81
Cell suspension derived-protoplast						
Basmatibahar	65	53	40	32	28	43.6 ± 6.85
Gourav	82	70	43	65	52	62.4 ± 6.83
Kalimochi	45	35	30	21	25	31.2 ± 4.18
Muskbudhi	15	21	10	18	16	16.0 ± 1.82

Table 2. Shoot regeneration from protoplast derived colonies from four scented rice

Variety	Number of calli cultured	Number of calli showed shoot regeneration	Shoot regeneration (%)
Basmatibahar	102	32	31.37
Gourav	135	52	38.25
Kalimochi	65	16	24.62
Muskbudhi	28	4	14.29

collected from four scented rice varieties. Gourav gave the highest protoplast yield 0.6 x 10^6 followed by Basmatibahar 0.5 x 10^6, Kalimochi 0.4 x 10^6 and the Muskbudhi lowest 0.3 x 10^5 protoplast/ml. Similar type of work were done by Ghose and Zapata (1993) and Kaur *et al.* (1999). The protoplast yield was affected by sub-culturing of suspension cultures because at this stage the cell suspension cultures exhibited exponential growth.

Protoplast Culture

The isolated protoplasts were cultured at a density of 5 x 10^5 to 2 x 10^6 protoplasts/ml when placed under milipore filter paper. The cultured protoplasts were examined at different periodic interval during incubation period. The cell wall formation and cell divisions occurred 2 and 5 days after incubation, respectively. The colonies appeared 4 weeks after incubation. The protoplast with plating efficiency were compared with callus-derived protoplast. From callus-derived protoplasts, best colony formation was observed for Gourav (16.2) followed by Basmati bahar (9.0), Kalimochi (7.4) and lowest for Muskbudhi (2.4). In cell suspension protoplast, colony formation was maximum in Gourav (62.4) followed by Basmatibahar (43.6), Kalimochi (3.12) and minimum in Muskbuhi (16.0) (Table 1).

Plant Regeneration from the Protoplast-derived Calli

After 5 weeks, the protoplast derived calli were transferred to regeneration medium (MS + 0.5 mg/l NAA + 2 mg/l KIN) and were incubated under high light intensity 5000 lux with 16 h light and 8 h dark. After 2 weeks of incubation, shoots and roots came out and after 5 weeks of incubation complete plants were obtained. The regenerated shoots were maximum in Gourav (38.25%) and the lowest in Muskbudhi (14.29%) (Table 2).

Table 3. Variability in regenerated plants in four scented rice

Variety	Parents			Regenerated Plants		
	Range	Mean	CV	Range	Mean	CV
Days to 50% flowering						
Basmatibahar	83-92	88.0	3.71	75-95	86.5	7.16
Gourav	93-100	95.5	2.85	85-106	95.0	6.25
Kalimochi	96-105	102.0	3.55	84.108	98.13	6.16
Muskbudhi	79-96	83.0	3.06	78-92	86.25	7.23
Plant height (cm)						
Basmatibahar	98-115	110.0	5.72	94-129	109.0	8.70
Gourav	85-102	95.3	6.32	82-108	93.88	10.07
Kalimochi	118-132	125.0	3.71	110-135	122.81	6.09
Muskbudhi	75-87	81.1	6.51	78-95	87.25	8.25
Panicle number/plant						
Basmatibahar	5-9	7.33	18.32	4-15	9.45	37.51
Gourav	6-10	7.70	18.41	4-12	9.31	23.10
Kalimochi	5-9	6.70	21.17	4-12	8.0	31.75
Muskbudhi	4-7	5.00	21.08	6-8	6.75	14.18
No. of grains/panicle						
Basmatibahar	80-116	101.2	12.07	75-140	111.63	17.56
Gourav	60-92	78.0	14.72	75-120	99.69	14.66
Kalimochi	50-85	67.7	17.71	45-115	84.13	21.23
Muskbudhi	45-65	55.3	12.11	55-70	62.5	10.33
1000-grain weight (g)						
Basmatibahar	11.95-15.00	13.48	7.84	12.25-19.65	15.18	14.59
Gourav	18.75-22.50	20.93	6.71	18.65-24.35	21.31	8.34
Kalimochi	15.75-18.65	17.27	5.94	14.50-20.00	17.73	10.23
Muskbudhi	17.80-21.15	19.23	6.18	18.50-22.10	20.53	7.65
Grain yield/plant (g)						
Basmatibahar	6.45-8.15	7.15	8.16	6.00-9.80	7.93	14.85
Gourav	7.65-9.15	8.32	7.22	8.35-12.15	10.11	11.77
Kalimochi	6.55-9.00	7.66	12.36	6.30-10.60	8.34	15.72
Muskbudhi	7.55-10.95	9.15	12.92	6.50-9.15	8.35	14.90

The protoplast-derived plants were transferred to soil for normal growth till maturity. Normal package and practices were followed. The magnitude for genetic variability for all the economic characters were studied. There was no consistency in economic characters between normal parents and with the regenerated plants obtained from protoplast culture. The range mean and CV fluctuates on both the side of the parents for all the characters studied (Table 3).

The regenerated population except in Muskbudhi indicated their earliness for days to 50% flowering. Early flowering provides scope for early maturity in regenerated plants. The mean plant height in regenerated plants varied in all the four scented rice. Significant higher population mean were noticed in all the four scented varieties as compared to the parents. Number of grains/panicle and 1000-grain weight in regenerated plants were higher in comparison to that of the parents. There was change in shape (long slender) than the parents in Basmatibahar and Gourav, which is a desired character in scented rice.

The mean and CV for grain yield/plant was higher in Basmatibahar, Gourav and Kalimochi. This variation provides a scope for grain yield in above three varieties.

From comparison of normal parents with that of the regenerated plants (somaclonal variants) the later showed a wide range of variability for different economic characters. There was no consistency in behaviour and mean of different characters of parents with that in the regenerated plants of all the scented rice. The yield is as such a complex character which depends upon several combining factors in physiological process of scented rice. So the protoplast-derived plants contributed better physiological efficiency for grain/plant. The seeds obtained from the protoplast-derived plants need to be grown further to test the stability in somaclonal variation induced through protoplast culture.

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