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Agronomical and Physiochemical Characterization of Somaclonal Variants in Indica Basmati Rice

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Abstract: Seeds of three indica rice varieties B-370, B-2000 and Super basmati were sterilized and incubated on callus inducing medium (MS-Medium with 2 mgl^{-1} 2,4-D) for 2, 4, 6 and 8 weeks and calli were obtained after somatic embryogenesis. After the incubation periods, calli were transferred to the regeneration medium. Regeneration efficiencies decreased from 98.5, 98 and 95% after two weeks incubation to 30, 34 and 24% after eight weeks incubation for B-370, Super basmati and B-2000 respectively. Some interesting patterns for adventitious root formation were also observed. Significant variation was observed within and among different cultivars and incubation periods for average number of tillers, average plant height, number of panicles per plant, panicle length and average yield per plant. Seven interesting somaclones were marked and scored for agronomic and physiochemical analysis. Physiochemical studies did not reveal any significant variation. Present studies clearly indicated that somaclonal variation can successfully be used for creating genetic variation for varietal improvement.

Key words: Somaclonal variations, callus formation, indica rice, plant height

Introduction

Improvement of plant varieties through conventional or non conventional breeding methods is the main objective of the plant breeder. Genetic variation and mutation have been exploited to serve this purpose. Tissue culture can also successfully be used for creating genetic variation. The original expectation was that all plants regenerated from cell or tissue culture has a genetic constitution identical to that of the original one. However, it was soon observed that phenotypic variation may be abundant amongst regenerated plants. The advent of plant cell culture has then brought the means to increase the genetic variability even more rapidly and without any sophisticated method. Thus plants regenerated from somatic cell by tissue culture or protoplast culture often exhibit a great deal of genetic variability called somaclonal variation.

Somaclonal variation has been related to growth regulators, cultivar variability, cultivars age in culture, ploidy level, explant source and other culture conditions (Skirvin *et al.,* 1994). Larkin and Scowcroft (1981) suggested that somaclonal variation was a useful source of novel variation for plant improvement. In higher plants, phenotypic traits such as yield, growth rate, pigmentation and tolerance as well as molecular changes have frequently been described as an example of somaclonal variations (Ogura *et al.,* 1987; Muller *et al.,* 1990; Fukuoka *et al.,* 1994; Adkins *et al.,* 1995). In Pakistan, development of Rachna basmati is a successful example of genetic variation produced through tissue culture (Abbas, 2000). The aim of our studies was to observe the effect of incubation periods on regeneration efficiencies and characterization of somaclonal variation in different agronomic and physiochemical characters of three important cultivars of indica basmati rice.

Materials and Methods

One hundred and ninety eight de-hulled mature seeds of B-370, B-2000 and Super basmati were selected and sterilized with 70% ethanol followed by 50% sodium hypochloride. After sterilization these seeds were cultured on callus induction medium, MS medium supplemented with 2 mgl^{-1} 2,4-D (Murashige and Skoog, 1962) and incubated for 2, 4, 6 and 8 weeks in dark at 26±2^{0}C. Calli were then transferred to the regeneration medium [MS medium with NAA (0.1% mg ml^{-1}), BAP (0.2 mg ml^{-1}) and 30 g l^{-1} Sorbitol] and plantlets were obtained through somatic embryogenesis. Regeneration efficiencies were calculated for all treatments. After thirty five days, regenerated plants were directly transferred to the field according to split plot design. Plants were scored for different agronomic characteristics like average number of tillers, average plant height, number of panicles per plant, panicle length and average yield per plant. All these characteristics were recorded according to standards as described by IRRI (INGER, 1996). Seven interesting somaclones were marked and scored for all these characteristics.

Physiochemical analyses of six interesting somaclones were performed in order to compare the presence or absence of aroma, gelatinization temperature, kernel length, kernel width, length width ratio, %age curling, %age bursting, stickiness and elongation value. Hundred seeds from different regenerated lines were soaked in water in duplicate for thirty minutes and then kept in boiling water for 20 minutes for cooking. After this treatment, average length of selected seeds was recorded and %age elongation was determined. Similarly %age of curling and bursting was also recorded. Alkali spreading values were recorded after immersion of milled seeds in 1.7% KOH at 30 ^{0}C for 24 hours (Little *et al.*, 1958). Values were recorded by comparing the dispersion to that of the checked samples of known behavior.

Results

Three different varieties of rice B-370, B-2000 and Super basmati were tested for their regeneration efficiency. The calli in dark at 26±2^{0}C were incubated for 2, 4, 6 and 8 weeks. A significant difference for regeneration efficiency was observed in 2, 4, 6 and 8 weeks incubations. The highest regeneration efficiency of 98.5, 98 and 95% was found for B-370, Super basmati and B-2000 respectively after two weeks incubation on callus induction medium. Lowest regeneration percentage was observed for eight weeks incubation period. Regeneration percentage ranged between 24 and 34% for B-2000 and B-370 respectively. It was also observed that in general highest regeneration percentage was recorded for B-370 followed by super basmati and B-2000 (Fig. 1). These results also showed that variation existed for both factors i.e. incubation period and varietal differences. Another significant difference was observed for adventitious root formation during regeneration of plantlets among all three

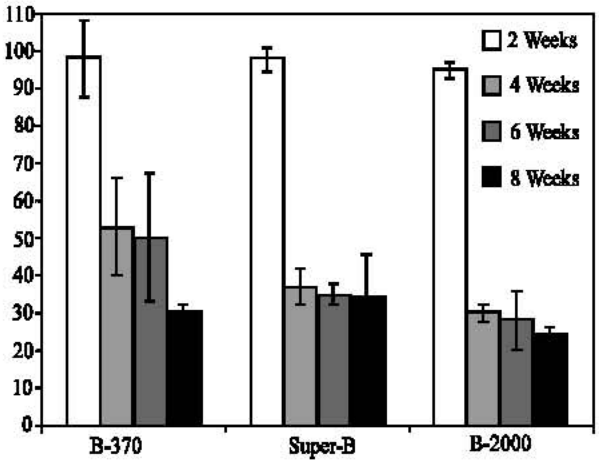

Fig. 1: Regeneration response of different varieties of basmati rice after different incubation periods

Fig. 2: Varietal response to the root formation in three varieties
(a): Super basmati showed less adventitious root formation
(b): B-2000 showed extensive root formation
(c): B-370 showed intermediate root formation

Fig. 3: (a): Weak and diseased plant of B-370 regenerated after tow weeks of incubation periods
(b): Weak and diseased plant of super basmati regenerated after two weeks of incubation periods
(c): Dwarf plants of B-2000 regenerated after four weeks incubation period
(d): Purplish strow color and bushy plant of B-2000 regenerated after eight weeks incubation

varieties. These adventitious roots appeared to be extensive in B-2000, intermediate in B-370 and very less in Super basmati (Fig. 2). Healthy rooting also enhanced the chances of survival when directly transferred to the soil. These plants were directly transferred into soil according to split plot design and different parameters such as average number of tillers, plant height, number of panicles, panicle length were compared with respect to the effect of incubation periods. Significant differences in three varieties B-2000, B-370 and Super basmati for total number of tillers were observed. Highest number of tillers were present in eight weeks incubated plant with 35, 39 and 39 tillers per plant for B-370, Super basmati and B-2000 respectively. Lowest number of tillers were recorded for six weeks incubated plants with 15, 27 and 19 tillers per plant for B-370, Super basmati and B-2000 respectively. Significant differences for plant height were also observed for all the varieties and among all incubation periods (Table 1). Highest value of 142 cm was recorded for B-370 after four weeks of incubation and lowest value of 84 cm was recorded for B-2000 after six weeks of incubation. In general plants were dwarf and it might be due to late transplanting of plants to the field. Average number of panicles ranged between 21 and 32 for Super basmati after four weeks of incubation and B-370 after eight weeks of incubation period respectively. No statistical variation was observed for panicle length while significant variation was observed for average yield per plant. Highest yield was recorded for B-370 after eight weeks of incubation period while lowest yield was recorded for B-2000 after six weeks of incubation period (Table 1).

Typical Somaclonal variation was observed for some plants and these plants were marked (Table 2). Only the plants of B-370 showed variegated conditions of few tillers (leaves) in the case of two weeks incubation period. Similarly the plants of Super basmati showed extensive panicle formation than other plants in two weeks incubation period. On the other hand, B-2000 after four weeks incubation period exhibited three short statured plants than the other plants. In case of eight weeks incubation period, Super basmati appeared to be short statured as compared to control and early panicle

Table 1: Effect of incubation on important agronomic characteristics

Incubations	Average No. of Tillers	Plant Height (Cm)	No. of Panicles	Panicle Length (Cm)	Yield (g/plant)
8 Weeks					
B-370	35±4	124±5	32±1	27±1	59±09
Super Bas.	39±5	093±6	31±3	26±1	55±08
B-2000	39±2	116±8	29±6	28±2	35±13
6 Weeks					
B-370	15±5	122±7	14±8	28±3	37±03
Super Bas.	27±4	86±5	19±2	25±1	32±06
B-2000	19±7	84±4	15±10	24±1	18±11
4 Weeks					
B-370	25±6	142±7	29±4	29±1	45±09
Super Bas.	30±6	095±6	21±2	27±1	35±10
B-2000	14±3	126±7	14±4	29±1	17±10
2 Weeks					
B-370	23±5	131±4	22±6	29±0	37±11
Super Bas.	29±3	099±8	21±6	28±1	27±16
B-2000	23±2	118±6	21±4	29±1	28±07

Table 2: Summary of morphological variation

Varieties	Plant No.	Agronomic characters
B-2000	1-2-1	Healthy bushy plant with purplish straws and higher tiller number
	1-2-5	Short and stunted plant with bushy and purplish straws
	1-2-6	Increased tiller number. Bushy nature and purplish straws
	8-2-1	Dwarf and healthy plant
	8-2-4	Dwarf and healthy plant
Super Basmati	3-SB-2	Early panicle initiation and stunted growth
B-370	10-3-3	Healthy plant showing few variegated leaves

Table 3: Physiochemical analysis of different clones

Serial No.	Plant No.	Alkali spreading value	Average length (mm)	Average breadth (mm)	Length breadth ratio	% age Bursting	% age curling	Elongation ratio	Aroma
1	1-2-1	4-5	6.56	2.06	3.18	10	6.12	1.68	Present
2	1-2-5	4-5	7.28	2.16	3.37	3.5	6.35	1.51	Present
3	1-2-6	4-5	6.78	2.00	3.39	4.0	5.22	1.62	Present
4	8-2-1	4-5	7.30	2.10	3.48	4.0	5.17	1.64	Present
5	8-2-4	4-5	7.18	2.18	3.29	5.0	5.83	1.53	Present
6	B-2000	4-5	8.68	2.26	3.84	4.0	3.08	1.38	Present
7	10-3-3	4-5	7.54	2.16	3.49	4.8	7.81	1.46	Present
8	B-370	4-5	6.33	2.06	3.18	3.3	5.5	1.68	Present

formation was also observed. Similarly, B-2000 exhibited few plants of quite different nature (Fig. 3). These plants had the appearance with purple leaves.

Six interesting clones along with two controls of B-2000, B-370 were used for physiochemical studies. Eight parameters i.e. alkali spreading value, kernel length, kernel width, length width ratio, %age bursting, %age curling, elongation ratio and presence of aroma were studied. In case of two varieties B-2000 and B-370, alkali spreading value was same in all the clones and their respective parental lines. Average length breadth ratio decreased for different clones as compared to parental lines. The average length breadth ratio ranged between 3.1-3.8 mm. Whereas in B-370, the length breadth ratio was increased. Clone 1-2-1 had the highest bursting %age i.e. 10 when compared with control of B-2000 i.e. 4. Interestingly, 1-2-1 had given highest elongation ratio as compared to control. The %age of curling was also significantly increased in case of B-370 clone 10-3-3 (Table 3). All the clone of aromatic varieties retained their aroma when smelled by a panel of three scientists.

Discussion

The *in vitro* culture response of the three rice varieties was analyzed for regeneration efficiency, different agronomic characters and physiochemical analysis. Significant variation in terms of regeneration frequency for different varieties and for different incubation period was recorded. It was observed that different varieties of rice responded differentially in their inheritance ability of embryogenesis and regeneration. B-370 showed highest regeneration efficiency of 98% followed by Super basmati and B-2000. Abe and Futsuhara (1986) also observed the varietal and genotypic variability of callus formation and regeneration efficiency. It was also observed that regeneration efficiency gradually decreased with increasing age of the culture. Highest regeneration efficiency of 98.5% was observed for B-370 after two week incubation period then declining to 53, 50 and 30% after 4, 6 and 8 week incubation periods respectively. Same pattern was observed for Super basmati and B-2000. Larkin and Scowcroft (1981) proposed that the progressive loss of totipotency in long term plant tissue

culture is a common event, which is typical trait in plant neoplastic progression.

Somaclonal variation was also observed for agronomic characteristics including plant height, tillers per plant, number of panicles, panicle length and grain yield. Tiller number of three varieties varied significantly for different incubation periods. All the three varieties showed characteristics difference in plant height, number of panicles, length of the panicle and yield. However the pattern of somaclonal variation was not directional and results were difficult to explain. Seven different somaclones were marked with either bushy nature, short and stunted growth, increased tiller number, purplish straws, early panicle formation or variegated leaves. The cause of somaclonal variation was not fully understood (Phillips et al., 1990), thus it cannot be controlled and because changes can be epigenetic (Maretzki ,1987). It was already documented that the genome of a somaclone could be variant at different location and the changes accumulated in complex characters during callus culture. So a single somaclone may be variant in several traits and in progeny analysis, appear to assort independently (Chaleff R.S, 1983).

These clones were also checked for physiochemical analysis and results showed that length width ratio, %age bursting, %age curling and elongation ratio was not significantly different than parental lines. However, there were few exceptions of two clones for highest %age bursting and elongation ratio. Genetic variations in these clones caused no significant physiochemical changes. Somaclonal variation has a vast potential for inducing genetic variation but there is need to incorporate it in crop improvement. To select desirable somaclones, it is essential to produce large populations of plants. It is highly desirable to understand the molecular bases of somaclonal variation. So that it can be used without fear of loss of genetic trait. Plant breeders need to be convinced that the stable somaclones are safer to use in breeding new varieties.

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