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A COMPARATIVE STUDY OF VARIATION IN RICE PLANTS REGENERATED FROM DIFFERENT SOURCES OF PROTOPLASTS

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ABSTRACT

Plant regeneration from protoplasts is always associated with protoclonal variation. Protoclones were obtained from immature embryo, mature embryo and anther-derived calli. Significant protoclonal variations, across genotypes, were seen in the R0. Plant height, panicle length, spikelets/ panicle and spikelet fertility showed significant reduction in R0. 1000-grain weight did not show any significant change. In the R1 generation no significant difference from seed-derived control was seen in the number of panicle bearing tillers (PBT) and 1000 grain weight. Panicle length and yield /plant did not show much variation across the genotypes. Spikelets /panicle and spikelet fertility percentage were most affected and showed negative shift. Panicle length remained largely unaffected but spikelets/ panicle and spikelet fertility showed negative shift. Uniform reduction in the plant height, panicle length and yield/plant along with spikelets/panicle and spikelet fertility across the generations, indicated additive expression of these characters and stable inheritance of this trait. Although immature embryo-derived calli are good source of cell suspension and protoplasts, variations in immature embryo-derived protoclones were higher than those seen in protoclones derived from seeds. Anther - derived calli showed a much higher frequency of variation in their protoclones. From the results of the present investigation it can be concluded that suspension cells derived from seeds may be a better source of protoplasts if somaclonal variations are not desirable.

INTRODUCTION

In studies relating to rice tissue culture, several authors reported the presence of variability among the regenerated plants and their progenies (Chu et al., 1985; Fukui, 1986), whereas others emphasized the stability and uniformity of the regenerates through anther culture and their progenies (Niizeiki 1985; Shen et al., 1983). The development of protoplast to plant system in rice has also led to generations of culture induced variations which are known as protoclonal variation. These variations are seen in both protoplast-derived plants and their progenies (Lynch et al. 1991). In rice, protoclonal variations were first reported in Japonica rice (Abdullah et al., 1989, Ogura et al., 1989). Variations in several agronomic characters like days to flowering, maturity, fertile tiller number, spikelet number, fertility, grain weight, albinism etc. have been reported in the protoplast-derived plants of rice (Abdullah et al., 1989; Ogura et al., 1989; Kawata et al., 1992) and DNA polymorphism in regenerated plants (Brown et al., 1990). In this paper we report a comparison of variation in rice plants regenerated from different sources of protoplasts.

MATERIALS AND METHODS

Four genotypes viz. Megha Rice 1, Megha Rice 2 (both Japonica), IR64 and IR72 (both Indica) were used in this experiment. Callus induction, cell suspension culture, protoplast culture and plant

regeneration have been described previously. Agronomic characters of protoplast-derived plants maintained in pot (R0) were compared with seed-derived plants. In this generation plant height, panicle length, splikelets/panicle, splikelet fertility (%) and 1000 grain weight were compared. For proper comparison, 20 tubes from each genotype were marked and control seeds were sown when plantlets were formed in these tubes. Seeds harvested from the R0 plants (R1 generation) were sown in the field in the next season and compared again with seed-derived control plants. R1 seeds were sown in a plant to row manner and transplanted using a Randomized Block Design (RBD). Seven agronomic characters viz. plant height at maturity, number of fertile tillers, panicle length, number of spikelets/panicle, spikelet fertility (%), yield/ plant and 1000 grain weight were compared.. Spikelets/panicle was counted manually and unfilled grains were separated and counted to calculate fertility percentage. For assessment variations in agro-botanic characters Duncan's Multiple Range Test - DMRT (Gomez and Gomez 1984) was used.

RESULTS AND DISCUSSION

In Megha Rice 1, plant height, spikelets/panicle and spikelet fertility percentage of R0 plants were significantly lower than control plants. However, in the R1 generation, the difference was not significant as revealed by DMRT.. Observations on number of panicle bearing tillers (PBT) and yield/plant were not taken in R0. In R1 plants, PBT as well as yield/plant was comparable to control plants (Table 1). Among the R0 plants obtained from different explant sources, porotoclones derived from anthers showed highest variations.

In Megha Rice 2, R0 plants obtained from mature seed-derived and anther-derived suspensions were significantly dwarf than control plants but protoclones obtained from immature embryo-derived suspensions showed no difference in height in the R0 generation. In R1 generation, however, plant height was not significantly different from controls. Grain weight (1000 grains) also did not show any significant difference from the control in either R0 or R1 generation. Number of spikelets/panicle was significantly higher than control in the protoclones (R1) derived from mature seed (108.6 compared to 103.3 in control). On the other hand it was significantly lower than control in all protoclones in R0 and in the protoclones derived from immature embryo and anther in R1 generation. Spikelet fertility in the protoclones was significantly lower than controls. PBT, panicle length, yield/plant and 1000 grain weight did not show any significant difference from the control plants either in RO and R1 generation in both Megha Rice 1 and Megha Rice 2. (Table 2).

Significant differences in all characters except panicle length and grain weight were observed in the protoclones of IR64 in R0 (Table 3). In the R1 generation, plant height, number of PBT, panicle length, spikelet fertility, yield/plant and grain weight did not show significant difference from control plants. Spikelet fertility percentage was significantly lower in protoclones obtained from immature embryo and anther-derived suspensions. On the contrary, spikelets/panicle in protoclones of mature seed-derived suspensions was comparable to that of control plants (Table 3).

In IR72, protoclones showed significant differences from control in R0 in plant height and spikelets/ panicle. In the R1, however, protoclones did not show significant difference from control in any of the characters studied (Table 4).

Among the protoclones obtained from different suspension sources, highest variation was observed in protoclones obtained from anther callus-derived suspensions followed by immature embryo and mature seed-derived suspensions. Protoclones obtained from anthers showed variations in plant height (IR72), number of spikelets per panicle (Megha Rice 1, IR64, and IR72). Immature embryo-derived protoclones showed variations in plant height (IR72) and spkielets/panicle (Megha Rice 1, IR64 and IR72). Protoclones developed from protoplasts of mature seed-derived suspensions showed variations only in plant height

(IR72) and spikelet fertility percentage (IR72). Number of panicle bearing tiller and yield/plant did not show any variation. Among the protoclones of different genotypes, highest stable variation (variations in R1) was seen in IR72 followed by Megha Rice 1 and IR64.

Significant protoclonal variations, across genotypes, were seen in the R0. Plant height, panicle length, spikelets/ panicle and spikelet fertility showed significant reduction in R0. Reduction in the number of spikelets / panicle has also been reported in R0 plants of Niponbare, Fujisaka 5 and Iwaimochi, which was accompanied by reduction (53.9 compared to 75.4% of control) in spikelet fertility (Ogura et al.1987). Other researchers also have reported reduction in spikelet fertility ranging from 3.4 - 93% in Nipponbare (Li and Murai 1990) and 10-80% in Tepi Boro (Alam et al. 1994). Some characters like number of PBT (Ogura et al. 1987) and 1000-grain weight (Alam et al. 1994) have been reported to show positive shift in R0. In the present experiment although 1000-grain weight did not show any significant change, panicle bearing tillers were not counted, as most of the R0 plants were clumps of more than one plant. R0 plants are always aberrant in their physiology, consequently protoclonal variation in R0 is not of much importance. R1 generation, nevertheless, is expected to reflect the protoclonal variation more precisely.

In the R1 generation no significant difference from seed-derived control was seen in the number of panicle bearing tillers (PBT) and 1000 grain weight. Panicle length and yield /plant did not show much variation across the genotypes. Spikelets /panicle and spikelet fertility percentage were most affected and showed negative shift. Abdullah et al. (1989) studied protoclonal variation in R1 progenies of Taipei 309 and reported unidirectional positive shift for spikelets/ panicle and negative shift for panicle length. In the present study, however, panicle length remained largely unaffected but spikelets/ panicle and spikelet fertility showed negative shift. Uniform reduction in the plant height (IR72), along with spikelets/panicle and spikelet fertility across the generations, indicated additive expression of these characters and stable inheritance of this trait. The outcome of the present investigation is broadly in agreement with the findings of Ogura et al. (1989) and Ramaswamy et al. (1996).

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Table 1. Comparison of agrobotanic characters of protoplast-derived R0, R1 and seed-derived control plants of Megha Rice 1*.

Plants	Plant height(cm)	PBT	Panicle Length(cm)	Spikelets/ Panicle	Spikelet ferrtility(%)	Yeild/ plant(gm)	1000 grain wt.(gm)
Control	112.8^{a}	3.3^{a}	21.23^{a}	97.8^{a}	82.5^{a}	2.15^{a}	23.5^{a}
R0 (IM)	95.3^{b}	xx	17.85^{a}	85.3^{b}	56.2^{bc}	xx	22.8^{a}
R1 (IM)	110.8^{a}	3.2^{a}	20.62^{a}	98.2^{a}	83.8^{a}	1.95^{a}	23.2^{a}
R0 (M)	97.5^{b}	xx	18.93^{a}	84.3^{b}	59.7^{b}	xx	23.1^{a}
R1 (M)	111.2^{a}	3.3^{a}	21.33^{a}	97.5^{a}	81.3^{a}	2.08^{a}	23.3^{a}
R0 (AN)	88.3^{b}	xx	18.92^{a}	89.3^{b}	53.8^{c}	xx	21.8^{a}
R1 (AN)	103.8 ^{ab}	3.3^{a}	20.93^{a}	95.3^{a}	81.3^{a}	2.05^{a}	22.9^{a}
CD 5%	13.80	3.25	3.57	6.82	7.89	1.86	5.89

*Average of 20 plants in R0 and 3 replications with 50 plants / replication in R1. Within each column means followed by a same letter are not significantly different at 5% level by Duncan's Multiple Range Test. xx Data were not recorded because protoplast-derived plants are generally a cluster of multiple shoots. IM = immature embryo-derived; M = mature embryo-derived; AN = anther-derived.

Table 2. Comparison of agrobotanic characters of protoplast-derived R0, R1 and seed-derived control plants of RCPL1-1C (Meghalaya 2)

Plants	Plant height(cm)	PBT	Panicle Length(cm)	Spikelets/ Panicle	Spikelet ferrtility(%)	Yeild/ plant(gm)	1000 grain wt.(gm)
Control	100.0^{a}	4.2^{a}	23.0^{a}	103.3^{b}	85.0^{a}	2.2^{a}	23.0^{a}
R0 (Im)	87.5^{ab}	xx	22.6^{a}	95.7^{c}	55.6^{c}	xx	21.9^{a}
R1 (IM)	98.3^{ab}	4.3^{a}	22.4^{a}	98.3^{c}	80.3^{b}	2.13^{a}	22.3^{a}
R0 (M)	85.2b	xx	22.8^{a}	98.3^{c}	54.2^{c}	xx	22.6^{a}
R1 (M)	97.5^{ab}	4.2^{a}	23.3^{a}	108.6^{a}	80.9^{b}	2.35^{a}	22.3^{a}
R0 (AN)	85.3^{b}	xx	20.3^{a}	95.3^{c}	53.8^{c}	xx	22.3^{a}
R1 (AN)	97.3^{ab}	4.2^{a}	22.5^{a}	98.7^{c}	80.2^{b}	2.15^{a}	22.3^{a}
CD 5%	14.62	3.86	4.71	4.15	4.26	1.74	4.95

*Average of 20 plants in R0 and 3 replications with 50 plants / replication in R1. Within each column means followed by a same letter are not significantly different at 5% level by Duncan's Multiple Range Test. xx Data were not recorded because protoplast-derived plants are generally a cluster of multiple shoots. IM = immature embryo-derived; M = mature embryo-derived; AN = anther-derived.

Table 3. Comparison of agrobotanic characters of protoplast-derived R0, R1 and seed-derived control plants of IR 64*.

Plants	Plant height(cm)	PBT	Panicle Length(cm)	Spikelets/ Panicle	Spikelet ferrtility(%)	Yeild/ plant(gm)	1000 grain wt.(gm)
Control	67.4[a]	6.5[a]	20.2[a]	82.2[a]	64.0[a]	6.43[a]	18.82[a]
R0 (Im)	58.9[ab]	xx	19.7[a]	60.7[c]	51.8[b]	xx	17.05[a]
R1 (IM)	65.6[ab]	5.8[a]	19.5[a]	77.8[c]	58.8[ab]	4.42[a]	17.55[c]
R0 (M)	55.8[b]	xx	19.2[a]	61.2[c]	55.6[b]	xx	17.33[d]
R1 (M)	65.2[ab]	5.6[a]	19.6[a]	78.3[ab]	58.6[ab]	4.58[a]	17.83[a]
R0 (AN)	55.7[b]	xx	19.2[a]	65.8[c]	53.2[b]	xx	17.62[a]
R1 (AN)	64.8[ab]	6.0[a]	19.4[a]	72.5[bc]	57.6[ab]	4.42[a]	17.66[a]
CD 5%	7.70	2.85	3.80	8.70	6.50	2.60	4.12

*Average of 20 plants in R0 and 3 replications with 50 plants / replication in R1. Within each column means followed by a same letter are not significantly different at 5% level by Duncan's Multiple Range Test. xx Data were not recorded because protoplast-derived plants are generally a cluster of multiple shoots. IM = immature embryo-derived; M = mature embryo-derived; AN = anther-derived.

Table 4. Comparison of agrobotanic characters of protoplast-derived R0, R1 and seed-derived control plants of IR 72*.

Plants	Plant height(cm)	PBT	Panicle Length(cm)	Spikelets/ Panicle	Spikelet ferrtility(%)	Yeild/ plant(gm)	1000 grain wt.(gm)
Control	58.8[a]	7.6[a]	19.4[a]	82.4[ab]	63.0[a]b	4.81[a]	12.20[a]
R0 (Im)	51.6[b]	xx	19.6[a]	75.8[c]	65.7[a]	xx	12.50[a]
R1 (IM)	53.8[b]	7.3[a]	19.3[a]	79.6[b]	65.8[a]	4.58[a]	11.98[a]
R0 (M)	52.8[b]	xx	18.5[a]	77.3[bc]	61.3[b]	xx	12.36[a]
R1 (M)	54.3[b]	6.6[a]	18.9[a]	85.1[a]	61.8[b]	4.40[a]	12.55[a]
R0 (AN)	52.6[b]	xx	19.4[a]	79.3[b]	61.5[b]	xx	12.10[a]
R1 (AN)	54.5[b]	6.8[a]	19.5[a]	79.6[b]	63.5[ab]	4.20[a]	12.22[a]
CD 5%	4.27	3.89	4.52	3.19	4.04	3.65	3.01

*Average of 20 plants in R0 and 3 replications with 50 plants / replication in R1. Within each column means followed by a same letter are not significantly different at 5% level by Duncan's Multiple Range Test. xx Data were not recorded because protoplast-derived plants are generally a cluster of multiple shoots. IM = immature embryo-derived; M = mature embryo-derived; AN = anther-derived.