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Performance of plants regenerated through somatic embryogenesis in finger millet (*Eleusine coracana* Gaertn.)

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ABSTRACT

Regenerated plants (R_1) from callus derived from seeds and their progenies (R_2) of three cultivars and R_3 progeny of one cultivar of finger millet (*Eleusine coracana* Gaertn.) were evaluated for different agronomic characters. All the R_1 plants were shorter than the control while R_2 plants were taller. Number of tillers showed an increase in R_1, R_2 and R_3 generations. In two out of three cultivars in R_2 generation, the regenerants flowered earlier compared to control. Another important trait exhibited by R_1, R_2 and R_3 plants was the branching of tillers, which was absent in the control. The range of variation was less for branches / spike, spike length, and 1000 seed weight, whereas grain yield / plant showed an extensive variation when compared with the control.

Key words: Callus, *Eleusine coracana*, finger millet, somaclonal variation.

INTRODUCTION

Heritable somaclonal variations induced by *in vitro* culture of plant cells can be used as a supplement to plant breeding programmes for crop improvement (Karp 1995). In cereals such as rice (Adkins *et al.* 1995), wheat (Chouhan and Singh 1995) and rye (Bebeli and Kaltsikes 1994), heritable variations of agronomic importance could be identified by field evaluation of the regenerants and their progenies. Although millets such as pearl millet and finger millet are of great economic importance in the arid and semi-arid regions of the tropics, there are only a few reports on somaclonal variation in these species (Morrish *et al.* 1990; Pius *et al.* 1994). In the present communication, we report the occurrence of somaclonal variation including some desirable agronomic traits in three cultivars of finger millet.

MATERIALS AND METHODS

Three cultivars of finger millet (*Eleusine coracana* Gaertn.) CO-9 (white seeded), CO-12 and CO-13 (blast tolerant and brown seeded) were used for the investigations and the dry seeds served as the source material for callus initiation. After prescribed methods of surface sterilization (Eapen and George 1989), the seeds were cultured on Murashige and Skoog (1962) basal medium supplemented with Picloram (4 mgL^{-1})[4, Amino-3,5,6-Trichloro Picolinic Acid] and Kinetin (0.5 mgL^{-1}). For somatic embryogenesis, calli obtained in the above medium were transferred to MS basal medium with Picloram (2 mgL^{-1}) and Kinetin (0.1 mgL^{-1}) and for germination of somatic embryos 500 mg of embryogenic tissue was transferred to MS basal medium lacking phytohormones. The regenerants (R_1) as well as the seed derived control plants were transplanted to the experimental field in Bhabha Atomic Research Centre, Mumbai, India to study variation in morphological and agronomic characters. The R_1 generation consisted of about 1000 plants, which were selfed to obtain R_2 and R_3 generations. In the R_1 generation, plant height, number of tillers, number of branches / tiller and 1000 seed weight were recorded on 20 seed derived plants and 35 regenerants of CO-9, 23 seed derived plants and 45 regenerants of CO-12 and 30 seed derived plants and 37 regenerants of CO-13.

The R_2 generation along with the control was grown at Punjabrao Krishi Vidyapeeth, Akola, Maharashtra, India. The progenies of 20 R_1 plants (20 lines) from each cultivar were raised and each progeny line consisted of 30 plants. The parameters studied include days to 50 % flowering, plant height, number of tillers, number of branches $tiller^{-1}$, spike length, branches $spike^{-1}$, grain colour, 1000 seed weight and grain yield $plant^{-1}$ on 5 random plants per line from 8 lines (5 X 8 = 40 plants). Field data in R_2 generation were subjected to analysis of variance using Least Significant Difference (L.S.D.) for mean separation.

Field evaluation of R_3 generation was carried out

only for the cultivar Co-12 in Mumbai. All the lines evaluated in the R_2 generation were raised as plant to row progeny in R_3 generation. Each progeny line consisted of 10 plants. All the traits studied in R_2 generation except days for 50 % flowering and spike length were evaluated in the third generation on 10 random plants per line from eleven lines which were true breeding for tillering.

RESULTS

R_1 Generation

About 95 % of the regenerated (R_1) plants survived to maturity. No visual chlorophyll variant was observed among the 1000 R_1 plants. In all the three cultivars, the R_1 plants had significantly reduced height compared to the control plants (Table 1). There was an increase in the number of tillers in the regenerants and varietal difference was observed for this trait. The regenerants of all the cultivars showed branched tillers, a character not seen in the control plants. Although seed weight was slightly higher in the regenerants, this difference was not significant (Table 1).

R_2 Generation

The results of field evaluation are given in Table 2. No visual chlorophyll or seed colour variant was observed among the progeny. The R_2 progenies of the three cultivars showed variation in days to 50 % flowering. Significant variations were observed for all the growth parameters and yield components. The variation was very wide for plant height, number of tillers and branches/tiller^{-1}. All the R_2 plants were significantly taller than the control plants (Table 2). Number of tillers/plant^{-1}, the major yield contributing factor, showed significant difference in all cultivars. Branching of tillers was observed among the progeny in the R_2 generation also and varietal difference was evident for this trait (Table 2). Variation for branches spike^{-1} and the increase in spike length was more for the cultivar CO-13. While increase in 1000 seed weight was observed only in a few lines, vast difference in grain yield / plant was shown by all the cultivars.

R_3 Generation

Field evaluation of R_3 generation was carried out only for CO-12 and the results are presented in Table

Table 1. Results of field evaluation of regenerants in first generation (R_1) of *Eleusine coracana*.

Variety	No. of plants studied	Source material	Plant height, cm ± SE	No. of tillers plant^{-1} ± SE	No. of braches tiller^{-1} ± SE	Seed weight (1000) gm ± SE
CO-9	20	Seed-Plants	39.3±1.0	1.5±0.2	Nil	2.2±0.01
	35	Callus-Plants	21.4±0.6	3.8±1.2	3.8±0.4	2.3±0.01
CO-12	23	Seed-Plants	75.4±1.0	3.0±0.4	Nil	2.7±0.01
	45	Callus-Plants	53.4±2.6	12.4±1.1	14.2±1.6	2.7±0.01
CO-13	30	Seed-Plants	57.1±4.0	1.3±0.2	Nil	2.1±0.01
	37	Callus-Plants	45.8±1.5	1.8±0.2	2.0±0.3	2.3±0.01

SE = Standard Error

Table 2. Variation in morphological and agronomic characters among the progeny in the second generation of callus derived plants of *Eleusine coracana* cultivars CO-9, CO-12 and CO-13 compared with seed plants.

Genotype	Days to 50% flowering	Plant height, cm	No. tillers plant^{-1}	Branches tiller^{-1}	Spike length, cm	Branches spike^{-1}	1000 Seed weight, gm	Total seed weight, gm
CO-9 Control (Seed plant)	91	51.6	1.2	0	6.8	6.6	2.2	3.1
CO-9 R_2 (Progeny of callus plants)	77-99	57.6-79.8	3.2-8.8	1.8-11.0	4.1-4.9	6.8-9.4	2.2-2.6	11.7-38.4
LSD at 5%	-	**8.6**	**3.81**	**4.5**	**0.75**	**1.71**	**0.22**	**17.36**
*CO-12 Control (seed plants)	95	37.0-62.0	1.0-2.0	0	4.5-7.0	5.0-6.0	2.1-2.7	2.9-9.5
CO-12 R_2 (Progeny of callus plants)	81-94	68.0-97.9	3.8-5.6	0-3.2	5.1-6.3	5.2-6.8	2.7-3.7	9.7-18.8
LSD at 5%	-	**12.07**	**2.96**	**2.96**	**0.8**	**1.04**	**0.47**	**13.15**
CO-13 control (Seed plants)	103	32.4	1.0	0	5.5	6.4	2.1	3.6
CO-13 R_2 (Progeny of callus plants)	87	78.2-98.6	2.2-4.2	0.6-3.0	4.8-7.7	4.4-12.4	2.2-2.5	6.1-16.9
LSD at 5%	-	**14.89**	**1.84**	**2.47**	**1.74**	**2.98**	**0.36**	**8.75**

LSD - Least Significant Difference
* Data for CO-12 control taken from Pius *et al.* 1994.

Table 3. Morphological and agronomic characteristics of selected progenies in R_3 generation of *Eleusine coracana* Var. CO-12.

Progeny number	Plant height, cm ±S.E.	No. tillers plant^{-1} ±S.E	Branches tiller^{-1} ±S.E.	Branches spike^{-1} ±S.E.	Grain yield plant^{-1}, gm ± S.E.
Control	92.1 ± 1.5	9.5 ± 1.0	0	7.3 ± 0.2	20.5 ± 4.1
R_3 - 7	94.1 ± 3.9	13.3 ± 0.6	2.2 ± 0.5	7.8 ± 0.2	44.9 ± 8.1
R_3 8	100.2 ± 1.9	14.0 ± 1.5	2.2 ± 0.3	8.2 ± 0.3	21.3 ± 2.0
R_3 12	98.4 ± 5.9	15.9 ± 1.2	4.2 ± 1.6	6.9 ± 1.8	36.8 ± 3.5
R_3 14	100.4 ± 4.3	15.7 ± 0.6	1.1 ± 0.3	7.9 ± 0.2	34.4 ± 3.9
R_3 21	95.9 ± 2.2	15.0 ± 1.9	1.8 ± 0.4	8.8 ± 0.4	41.7 ± 10.4
R_3 22	99.8 ± 4.4	14.3 ± 2.1	1.9 ± 0.7	6.9 ± 1.3	29.1 ± 6.3
R_3 27	101.2 ± 3.8	17.3 ± 2.4	2.3 ± 0.3	8.1 ± 0.5	31.4 ± 1.6
R_3 29	98.6 ± 0.8	14.8 ± 0.8	1.8 ± 0.2	8.3 ± 0.4	26.8 ± 3.3
R_3 33	96.3 ± 0.6	13.9 ± 1.5	1.4 ± 0.5	7.5 ± 0.1	33.7 ± 3.3
R_3 35	100.3 ± 1.3	14.5 ± 1.7	1.9 ± 0.5	8.1 ± 0.5	38.6 ± 5.8
R_3 36	103.1 ± 4.8	13.9 ± 0.7	2.2 ± 0.3	7.9 ± 0.3	40.7 ± 8.6

S.E. = Standard Error

3. In general, there was no significant difference in plant height and branches spike^{-1} between the regenerants and seed-derived control plants. However, wide variation was observed in total yield plant^{-1}. Enhanced tillering observed among the regenerants in R_1 and R_2 was also observed in the R_3 progeny (Table 3). Similarly, branching of tillers was observed only in the regenerants (Table 3). Furthermore, visual chlorophyll variants or seed colour variants were not observed in R_3 generation.

DISCUSSION

In the present study using three cultivars of finger millet, variations were observed between control (seed derived plants) and the regenerants for most of the characters evaluated in the R_1, R_2 and R_3 generations. Variations obtained by tissue culture were comparable to those induced by irradiation of the callus or seeds (Pius *et al.* 1994). Phenotypic variations in tissue culture derived plants have been reported in millets (Morrish *et al.* 1990; Pius *et al.* 1994). No visual chlorophyll variant was obtained in the R_1, R_2 and R_3 population of finger millet, although such variants were of frequent occurrence among regenerants of pearl millet (Morrish *et al.* 1990). The variations observed in R_1 plants included reduced plant height, increased tillering and branching of the tillers. As in pearl millet (Morrish *et al.* 1990) and wheat (Hashim *et al.* 1990), the reduced plant height observed in the R_1 progeny could be due to physiological effect induced by tissue culture. Although a significant increase in height among the regenerants of finger millet was observed in R_2 generation, this change was not observed in the R_3 generation. Increased tillering observed in the R_1, R_2 and R_3 generations was in conformity with the observations in other species such as wheat (Hashim *et al.* 1990) and pearl millet (Morrish *et al.* 1990). In finger millet, enhanced tillering appears to be a true breeding trait and has been observed even in the 3rd generation. Since tillering is an important yield contributing factor in cereals including millets, the trait if proved stable could be of significance in the improvement of finger millet.

Another important character observed only in the regenerants but not in the control was the branching of tillers, an important yield contributing factor reported only in finger millet (Pius *et al.* 1994). This character was observed in the R_1, R_2 and R_3 progenies thus indicating a possible genetic stability.

Other variations in the R_2 progeny included difference in the days to flowering, spike length, branches per spike, 1000 seed weight and yield per plant. The significance of these observations in finger millet breeding programmes needs to be ascertained.

In finger millet, the genotypic effect of source material on somaclonal variation was evident. Inter-varietal difference was more prominent in certain agronomic traits such as days to flowering, tillering, branching of tillers and yield per plant. Genotypic effects on somaclonal variations have been reported in several species including pearl millet (Morrish *et al.* 1990) and rye (Bebeli and Kaltsikes 1994).

In conclusion, the results obtained from the present study indicate that a wide range of somaclonal variations occur in finger millet genotypes. Some of these variations such as increased tillering and branching of the tillers are potent yield contributing factors which could find use in the breeding programmes. Besides, the novel trait, branching of the tillers, adds to the biomass when the plant is used as a fodder. Further studies are being carried out for assessing the stability of these beneficial traits for incorporation into the conventional breeding programmes.

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