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Assessment of Agronomic and Morphological characters of Somaclonal Variation in Regenerated Pearl Millet Plants

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ABSTRACT

Somaclones, regenerated from Pearl millet cultivar 5141B, were evaluated for various agronomic and morphological characters. Calli were initiated from seeds on modified Murashige and Skoog's medium containing 5 mg L^{-1} of 2, 4-D and regenerated on media supplemented with BAP (3ppm) and activated charcoal (0.3%w/v). Percentage of callus induction and embryonic calli were recorded. Calli with shoot were transferred to a hormone-free half-strength MS medium. The seedlings with well-developed roots were transplanted into pots containing a substrate and were acclimatized. After acclimation, plantlets were transplanted to field conditions. Most of the regenerated plants were phenotypically normal. However, some plantlets were albino, sterile, dwarf, with deformed leaf or with abnormalities in spike morphology. Variation was recorded in the R1 generation. Comparison were made between the somaclones and their parents (non-tissue-cultured plants) for plant height, days of flowering, total number of tillers-basal and nodal, number of fertile tillers and panicle length per plant. Comparison for agronomic and morphological characters between the parents and somaclones and among the somaclones revealed significant differences. The parameters of agronomic importance in somaclones can be further tested for heritability which would be of great importance in reviving the pearl millet cultivars withdrawn from cultivation.

***Key words**: Tissue culture, Somaclone, Pearl Millet, Plant regeneration, Agronomic characters.*

INTRODUCTION

Plant tissue culture has enormous potentials as tool in plant breeding programs. It has been considered as an efficient method of asexual rapid propagation compared to conventional methods[11, 1]. Theoretically it is necessary that all plants regenerated from tissue culture are identical with each other and with parental forms. Somaclonal variation is an important phenomenon that can be observed at varying levels in plant tissue culture in both dicot[2] and monocot families[7, 8]. However it has been observed that the phenotype or genotype variations do occur within R0 and subsequent generations which are called somaclonal variations.

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The term somaclonal variation was first used by Larkin and Scowcroft[16] to describe induced changes in plants regenerated from *in vitro* cultured somatic tissues. These variations are considered as a new source for enlarging and enriching the gene pool of improved varieties[11, 1]. Pearl millet a staple crop in drought hit region, known for its nutritional efficiency is affected due to reduction in yield and quality of agronomic traits due to various reasons[20]. Control of downy mildew disease by cultural practices, fungicides, resistant cultivars deployment represents an economic hardship to pearl millet growers. This drawback has called for the evaluation of alternate techniques to develop cultivars with beneficial agronomical traits including genetic resistance to plant pathogens[9]. Previous reports on use of somaclonal variation in Pearl millet to generate variants have been reported by several workers[19]. However, evaluation of mechanisms involved in somaclonal variation for agronomically important traits have scarcely reported. In this study, we attempted to assess the degree of variation obtained in *in vitro* regenerated plants of pearl millet with focus on phenotypic and agronomic traits to identify the possible variants that can later be integrated into a breeding program.

MATERIALS AND METHODS

Callus induction

Explant: Seeds of pearl millet genotype 5141 B line, highly susceptible to downy mildew pathogen obtained from ICRISAT, Hyderabad, India were used as explants to develop regenerants. The seeds were surface sterilized with 0.01 % w/v of mercuric chloride in distilled water and inoculated onto Murashige and Skoog's (MS) medium supplemented with 30 g of sucrose and gelled with 8 g L^{-1} agar was used throughout the study with alteration in other ingredients for various regeneration steps. The pH of the medium was adjusted to 5.6-5.8 before sterilization at 121°C temperature and 1.2 atmospheric pressure for 15 min.

Callus induction medium was basal MS media described above supplemented with 5 mg L^{-1} of 2, 4-D. Five seeds were inoculated to each flask containing 35 ml of medium in 20 replicates. All cultures were incubated under 12h photoperiod with a light intensity of 40W provided by cool fluorescent light. Temperature of 25± 2°C and humidity of 80 % were maintained. After 30 days, the calli were transferred for two 30 day subcultures on MS basal medium with step down (3 and 1mg L^{-1}) concentration of 2, 4-D for embryoid development. All operations were performed in a laminar air flow hood.

Parameters recorded:

1. Percentage of callus induction (PCI) was calculated by using the formula

$$PCI = \frac{\text{Number of explants that callused}}{\text{Total number of explants}} \times 100$$

2. The callus growth was measured in terms of weight as on 60th day.
3. At the end of third passage (90 days), the percentage of embryogenic calli were calculated

Shoot induction:

The embryos developed were later transferred into shoot induction medium containing MS medium supplemented with 3ppm of benzyl amino purine for 30 days. The shoots developed were subjected to root induction.

Root induction:

For root induction, the plantlets with well developed shoots were transferred to root induction medium containing half strength MS medium with 0.3% w/v of activated charcoal. The regenerants with well developed root and shoot were then subjected to hardening.

Hardening of Regenerants:

The plantlets with well developed roots and shoots were hardened to acclimatize the natural environment, under green house condition by transplanting into earthen pots containing sterile soil:sand mixture at 1:1 ratio. After the process of hardening, the plants were transferred to pots containing soil: sand mixture supplemented with fertilizers.

Raising of Control plants:

Non tissue cultured controls -5141 B line highly susceptible to downy mildew were obtained by germinating seed in earthen pots

containing 1: 1: 1 of soil:sand:manure mixture under greenhouse conditions.

Collection of seeds:

The regenerated plants were grown to maturity in the green house. The regenerated plants were selfed by bagging the panicle, using transparent paper bags before anthesis. The seeds (Rl) were collected separately from each somaclone. Plants from each somaclone were considered as a separate somaclonal family.

Nomenclature system followed:

The nomenclature system followed was that proposed by Chaleff (1981). The regenerants obtained were referred to as R0 generation.

Phenotypic analysis for variation in somaclones of pearl millet:

The regenerants (R0) obtained were evaluated for the following traits such as: plant height (distance from ground level to the base of the panicle), days of flowering, total number of tillers-basal and nodal, number of fertile tillers, panicle length

All the characters considered for evaluation of somaclones were recorded for the non tissue cultured control plants as well.

Statistical analysis:

Data on callus induction, embryogenic calli development were examined statistically by analysis of variance (ANOVA). For each character compared, among the regenerants and with that of control the value was analyzed by DMRT. Percentages were transformed into *arc sine* and analyzed using Duncan's multiple range test. Significant difference was determined at 5% level of F value.

RESULTS

Development of Regenerants from Pearl Millet

Seeds of pearl millet genotype 5141 B cultured on MS basal medium (Fig 1a) started germinating from the second day onwards and the callus formation (Fig 1b), was visible on the fifth day from the mesocotyl region. Soon after, the growth of the seedlings stopped. All the seeds showed callusing (100%) to equal extent. At the end of 60 days of culture, a total of 600 mg weight of calli was obtained. The calli was fresh, fleshy and soft (Fig 1c). On the third passage, *i.e*. after 90 days of culture, smooth, globular structures that resembled somatic embryos developed. These were recorded as embryogenic calli (Fig 1d) and those without the globular nature were recorded as non-embryogenic calli. Thirty nine per cent of total embryogenic calli were observed. The shoots regenerated were green, sturdy and fresh (Fig 1e). However, a few albinos also were obtained during shoot induction (Fig 1f). The well developed shoots were transferred for rooting (Fig 1g) and then hardened.

Initial number of regenerated plants was 70 but 10 plants died during hardening while 30 were albinos. Among the 25 plants that were grown to maturity, 11of them were sterile and five were dwarf.

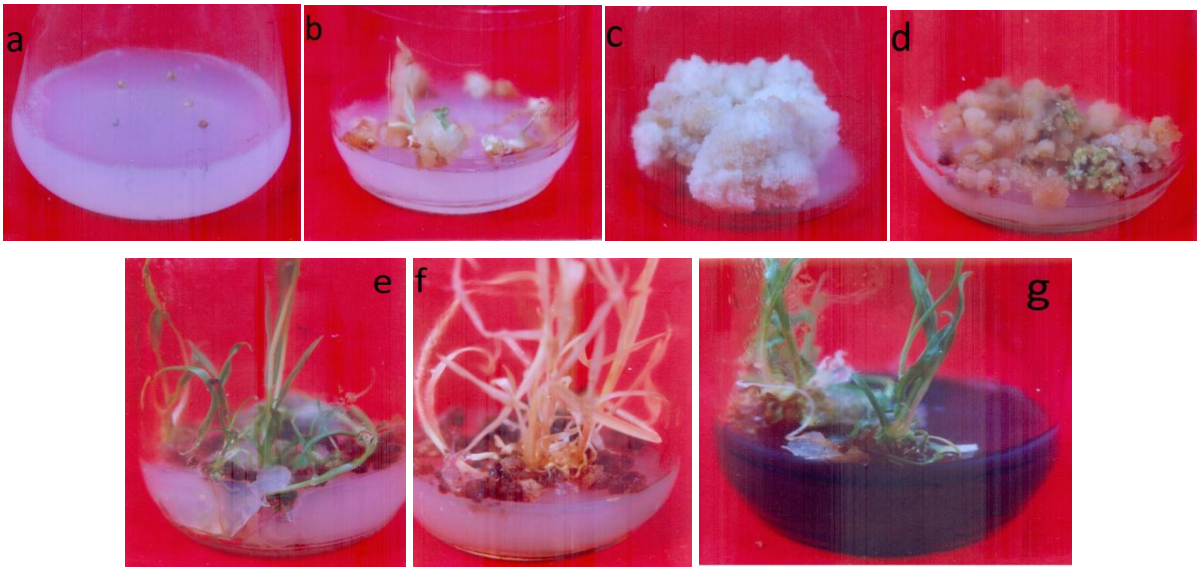

Figure 1: a. Explant - Seed of 5141B. b. Callusing in explants c. Friable callus d. Embryogenic callus e. Shoot formation from Somatic embryos f. Albino Shoots from Somatic embryos g. Root formation

Phenotypic Analysis for Variation in Somaclones of Pearl Millet

In the regenerants, the phenotypic characters such as plant height, panicle length, total number of tillers and days for 50% flowering were recorded (Table 1). Significant variation in all the characters were observed among the clones and also when compared with that of control. The height of plants as recorded after the ear head formation varied from 5 to 150 cm (Fig 2a to c). Height in control plants varied from 125-128 cm. No nodal tillers were recorded in any of the regenerants whereas 5 were observed in control plants. Basal tillers were observed in 7 of regenerants (Fig 2b and Fig 2d). While in control plants only 2 showed basal tillering. All the basal tillers observed in the regenerants and control plants were fertile. The timing of flowering varied between 15 to 55 days in regenerants, whereas in control plants the flowering started between 35-40 days of planting (Fig 3a). Those regenerants which showed early flowering (within 15 to 20 days of transplantation of regenerants) were dwarf and sterile with no seed setting (Fig 2c). Those which showed flowering between 30 to 35 days of planting were fertile, with plants showing sturdy and healthier growth (Fig 3a). The panicle length and shape varied significantly among the regenerants (Fig 3b to Fig d). The shape was round and sturdy resembling healthy control in 5 regenerants with a length of 13 to 17 cm (Fig 3b). Eleven regenerants showed slender panicle with a length of 3 to 8 cm and were weak and bushy in appearance (Fig c and Fig d). Seed setting also varied with the regenerants. The seed setting in a few regenerants were uniform and throughout the panicle length (Fig 3b) where as in few other it was sparce. Few regenerants were sterile with no seed setting (Fig 3c and d).

In order to maintain uniformity in sample preparation the boot leaf of all the regenerants which reached maturity were selected for biochemical and molecular studies.

Table 1: Phenotypic analysis of Somaclones

Somaclone No.	Height (cm)	Basal Tiller		Nodal Tiller		Time of flowering	Panicle		Seed setting
		No	No Fertile	No	No Fertile		Length (cm)	Shape	
C	125-128	2	2	5	-	35-40	12	R,S	Uniform
1	5	-	-	-	-	15	3	S	Sparce
2	125	2	2	-	-	30	11	S	Sparce
3	120	-	-	-	-	30	12	S R	Sparce
5	115	2	2	-	-	32	15	S	Uniform
6	128	-	-	-	-	35	14	R	Sparce
7	136	2	2	-	-	33	16	S	Uniform
8	147	-	-	-	-	31	13	W,B	Sparce
9	8	-	-	-	-	15	8	W,B	Sterile
10	10	-	-	-	-	17	7	W,B	Sterile
12	15	-	-	-	-	16	5	W,B	Sterile
14	25	-	-	-	-	16	3	W,B	Sterile
15	45	-	-	-	-	17	4	W,B	Sterile
16	39	-	-	-	-	20	7	W,B	Sterile
17	51	-	-	-	-	20	6	W,B	Sterile
18	56	-	-	-	-	15	3	W,B	Sterile
19	70	-	-	-	-	17	4	W,B	Sterile
20	120	1	1	-	-	31	13	R	Uniform
21	31	-	-	-	-	18	4	W,B	Sterile
22	32	-	-	-	-	19	3	W,B	Sterile
23	123	2	2	-	-	33	10	S	Sparce
24	121	1	1	-	-	32	9	S	Sparce
25	8	-	-	-	-	20	5	W,B	Sterile
26	9	-	-	-	-	16	6	W,B	Sterile
28	150	1	1	-	-	35	17	R	Uniform
29	136	-	-	-	-	34	15	R	Uniform

- Not observed, R-Round, S-sturdy, W-Weak, B-Bushy

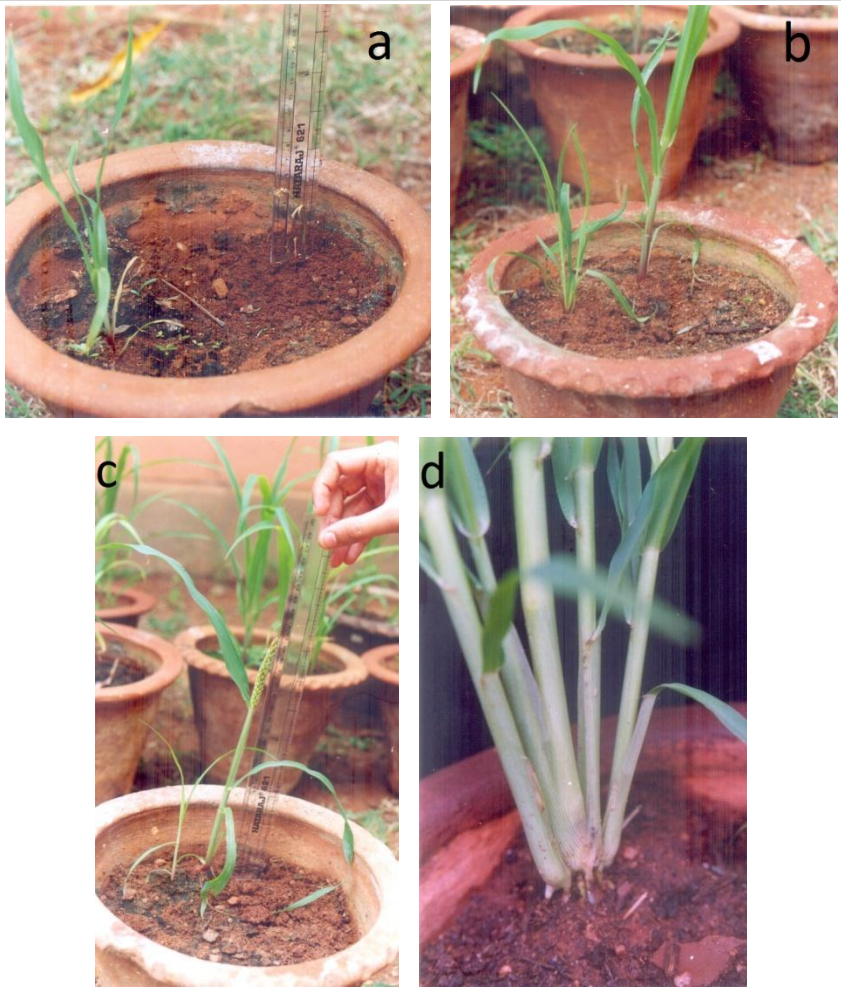

Figure 2: a. Dwarf regenerant (R0); b. Dwarf regenerant (R0) showing basal tillering; c. Early ear head formation in dwarf regenerant (R0) d. Regenerant (R0) showing basal tillers.

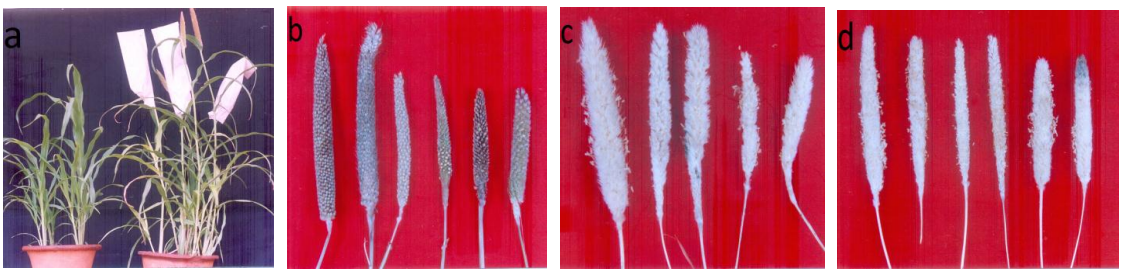

Figure 3: a. Early ear head formation and basal tillering (right) delayed ear head formation and basal tillering in control. b. Panicle -sturdy and round in shape, uniform and sparce seed setting c. Panicle - weak and bushy, no seed setting; d. Panicle - weak and slender, no seed setting.

DISCUSSION

The effective use of tissue culture techniques, such as *in vitro* selection, exploitation of somaclonal variation and the possibility of gene introgression depends upon the ability to initiate and establish stable callus cultures capable of plant regeneration. Once the genotypes for desirable characters are screened by preliminary evaluation of culture response, transfer of such elite germplasm can be carried out[14]. Focus of study is in development of somaclones resistant to downy mildew from highly susceptible cultivar of pearl millet. The preliminary screening of genotypes for efficient callusing has been carried out[19]. However the changes associated with somaclonal variation have not been evaluated till date. Hence in the present study, a highly callusing genotype of

pearl millet was selected and the changes associated morphological, agronomically significant were evaluated in the regenerants developed from it.

In the present study regenerants were obtained from 5141 B cultivar of pearl millet highly susceptible to downy mildew. Though initially the number of regenerants were more, few were lost during hardening. After 15 days of hardening the plants were transferred to the green house. Direct screening of regenerants under field conditions were not carried out. The significance of the present work is to standardize protocol to induce callusing and to develop regenerants from pearl millet genotype 5141B. The Rl seeds collected shall be subjected to further studies with emphasis on screening for downy mildew disease resistance.

A full-fledged screening system forms the basic necessity for successful evaluation of somaclonal variation in tissue culture derived plants. Significant variations were observed in the R0 generation for plant height, panicle length, total number of tillers, number of fertile tillers, panicle length and shape and days to 50% flowering (Table 1). Such variation in agronomic characters has been reported in height and maturity[4, 12], tillering, biomass and grain yield[17]. Sterility is one of the negative variations observed among the plants regenerated from cell culture[4].

During our study around 50% of the surviving regenerants were sterile. Plant vigor was reduced in some of the regenerants. Such variation due to enhanced repressive physiological effects on plant vigor resulting from the in vitro process is not uncommon. Similar results were recorded with maize and sorghum and few other crops[14]. Some of these traits might be non transferable to next generation whereas a few might be heritable. Thus the differential expression of morphogenic response to tissue culture among regenerants provides the potential for exploiting the genetically heritable variation to develop novel breeding material, particularly for single gene variants[12].

After transfer of plantlets to soil and their follow up to maturity, we noted a few cases of sterility and abnormalities in panicle length and morphology. Similar results have been obtained in Wheat[3] Chlorophyll deficiency or albinism is a standard marker of variation in the cytoplasmic genome[13].

Somaclonal variation can be epigenetic type, not inherited, often resulting from a change in gene expression originating from DNA methylation[15] or may involve gene type, and therefore may be heritable through generations and transmitted by crossing. Agronomic trait number of grains per panicle, somaclones did not show better performance compared to parents. On the contrary, some somaclones were better than parents for traits such as number of fertile tillers per plant, plant height or number of days to ear head formation. Our results have also recorded significant duration reductions in the number of days to heading in somaclones. According to Carver and Johnson[5], obtaining somaclonal variants with superior crop yields compared to parents is difficult. The work of Cheng *et al.* [7] on winter wheat somaclones showed that majority of agronomic characteristics in somaclones and their progeny were low as compared to parents. However, variants were selected having improved earliness.

The study concluded that many somaclones appeared to display superiority compared to their parent. Further studies of biochemical and molecular are needed for a complete evaluation of these variations for use in breeding programs. For proper evaluation of variation stability, the plants should be grown for further generation and evaluated for the persistence of studied characters.

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