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Somaclonal Variation in Sugarcane Through Tissue Culture and Subsequent Screening for Salt Tolerance

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Abstract: Tissue culture techniques were used to develop soma-clones in sugarcane to screen salt tolerant clones subsequently. Callus masses derived from young leave of high yielding cultivar of sugarcane (CP-43/33). The plantlets were regenerated from one month old callus. The plantlets were transplanted to pots after hardening. The grown up plants were transplanted to well prepare soil. The sets of well developed plant were planted in saline sodic soil. Screening of salt tolerant soma-clones was made. The salt tolerant soma-clones performed better in the characteristics of number of tiller/plant, stem height, number of nodes/stem, root band width with variability of 20, 50, 8.33 and 14.3%, respectively. The soma-clones performed less in the characteristics of girth of stem and brix (%) of 12.5 and 5.2, respectively.

Key words: Somaclon, tissue culture, salt tolerance

INTRODUCTION

The country has total geographic land area of 79.61 m ha (Agric. Statistics, 1985-86). Out of this, only 20.12 m ha (1/4) is cultivated. Of the cultivated area, 14.36 million hectares are irrigated while the rest (5.76 m ha) depends entirely on natural precipitation for its moisture requirements. Salinity and water logging are the most serious problems effecting the agricultural production in Pakistan reducing yield by upto 25%^[1]. The pace of salinity increased at the rate of 2% per decade as the reported salt affected area in 1990 was 39.1%^[2].

Dera Ismail Khan lies to the extreme south western side of NWFP. It has a total geographic land area of 0.980 million ha. Out of which only 0.300 million ha are cultivated. About one third (0.105 m ha is irrigated while the rest of two third (0.195 million ha) depends on rainfall for its moisture requirement.

Sugarcane is one of the important cash crop of the area. A sugar mill has been installed in the area. As a result, sugarcane is being cultivated extensively. However, none of the cultivated varieties are promising enough to give good yield. The average yield in the area is about 15 ton ha⁻¹ which is much lower than the average yield of any other areas of the country. One of the reason for low yield is soil salinity. The situation demands for cultivation of salt tolerant cultivars of sugarcane. The use of tissue culture for creation of soma-clonal variation in plants for development of salt tolerant cultivars offers advantage over all other conventional methods.

This method is very efficient and has tremendous potential for producing novel and useful varieties^[3]. Taking advantage of phenomenon of occurrence of soma-clonal variation in sugarcane, the present study was initiated.

MATERIALS AND METHODS

Callus culture was initiated from young leaves of 6-12 months old field grown sugarcane var. CP-43/33. A modified MS medium^[4] was used in the experiment. The medium was supplemented with B₅ vitamins and pH was adjusted to 5.8. The supplementing of medium corresponds to the media as used especially for sugarcane^[5-7]. Young leaves were taken from the internal 10 mm whorl and were sliced into 3 mm pieces. Induced calli were maintained/proliferated on the same medium by serial transfer after 2-3 weeks. The cultures were maintained at 26±28°C under dark condition. Callus was maintained/proliferated by sub culturing after every 2-3 weeks. One month old calli were transferred to regeneration medium; MS basal supplemented with Fe-EDTA 1 mg L⁻¹, Sucrose 60 g ml⁻¹, Casein hydrolyzate 500 mg L⁻¹, Cystine free base 30 mg L⁻¹, which is often used for sugarcane^[5,6]. Four calli pieces (0.5 g) were placed in each bottle containing 50 ml of regeneration medium. Jars were placed in controlled room temperature set at 28±30°C with 16 h photoperiod. The regenerents were transplanted to sugarcane rooting medium. After development of roots the plantlets were transferred to

vermiculite under high humidity (>90%) by covering the plants with plastic envelops. Some of the regenerants were transferred to the pots after hardening. The grownup plants were transplanted from pots to fertile soil, giving all the required agricultural practices. The sets of these plants were allowed to grow in saline sodic soil. The data was recorded on important agronomic characteristic viz., number of tiller/plant and stem height etc.

RESULTS AND DISCUSSION

Selection of explant: The slices of very young leaves in a folded condition were used as ex plant. These provided the best source of ex plant which later on formed greenish white creamy mass of callus (Fig. 1A). There are reports that callus can be developed from other parts of the plant i.e. pith and inflorescence^[8].

More mature parts used as explant secrete phenolic compounds which hamper the growth of callus cells and needs frequent sub-culturing^[7]. However, the young leaves and apical meristem provide a better plant source where the formation of callus is easy to obtain^[5-7]. Their findings support the use of very young leaves as best source of explant as practiced in this study.

Callus induction, formation and development of plantlets:

Formation of callus was initiated after 12-16 days of explanting. A large amount of greenish white and cream coloured mass was formed after 5-6 weeks (Fig. 1 A). This practice of maintaining the calli through serial transfers in basal medium has also been reported by Shahid *et al.*^[6].

On the transference of cut pieces (each of 0.5 mg) of calli to regeneration medium, green spots representing the shoot primordia appeared on the calli (Fig. 1B). The regenerating medium did not contain 2, 4-D. In other words the appearance of shoot primordia is linked with the removal of influence of 2, 4-D from the medium. Same

Table 1: Physio-chemical properties of soil

Elements/Property	Value	Remarks
Silt electrical conductivity	4.00 dSm ⁻¹	The soil
pH	8.50	is marginally
Ca ⁺⁺ Mg ⁺	8.30 mg L ⁻¹	saline and
K ⁺	180.00 mg Kg ⁻¹	sodic
P	7.00 mg Kg ⁻¹	
CO ₃ ⁻	Nil	
HCO ₃ ⁻	1.60 mg L ⁻¹	
Cl ⁻	1.84 mg L ⁻¹	
N (%)	0.03	
Lime (%)	7.20	
Texture	Silty clay loam	
Gypsum requirement	4.20 ton acre ⁻¹	

kind of observations have been made by Siddiqui *et al.*^[7] and Shahid *et al.*^[6]. The shoot primordia developed into shoot which started bearing young leaves after ten days of culturing in regeneration medium (Fig. 1C). The rate of regeneration and formation of young shoots was about 60%. The rate of survival though is higher than the mortality rate but still is a cause of concern. The reasons could be attributed to the presence of non viable cell in the callus which later on affected the formation of shoots.

On the transference of regenerants to rooting medium, the plantlets developed tiny roots which later on were the cause of formation of root system of the plants (Fig. 1D). At this stage the plantlets with well developed roots had three states to exhibit. Some of these plantlets were dark green, some light green and some almost white coloured. Similar findings were reported by Siddiqui *et al.*^[7]. The development of roots was achieved in two weeks time with the use of 7% of sucrose in the medium. A dose of 6-9% of sucrose has been recommended by many workers for successful formation of roots for various cultivars of sugarcane^[6,7] linked the vigorous growth of roots with the separation of plantlets from one another, trimming of leaves and placement of it in MS medium with 6% sucrose at 28±30°C with 16 h photoperiod.

Table 2: Variability observed in the characteristics of salt tolerant soma-clones of sugarcane developed through tissue culture technology

Characteristics	Soma-clones		Source plants (Genotype CP-43/33)		Variability * (%)
Plant scored	110		100		
	Range	Average	Range	Average	
Sett germination (%)	60	50			
Number of tillers/plant	2-12	6	2-7	4.00	50.0
Stem height (cm)	131-302	201	136-261	186.00	8.0
Number of nodes/stem	10-20	16	10-16	12.00	33.0
Girth of stem (Commerce and Mgt.)	6-8	7	7-9	8.00	12.5
Root band width (cm)	0.5-1.2	0.8	0.3-1	0.70	14.3
Brix (%)	14.70-17.75	16.92	15.9-18.09	17.85	4.7

Difference between the average values of plants and soma-clones

*. Variability (%) = $\frac{\text{Difference between the average values of plants and soma-clones}}{\text{Average value of source plants}} \times 100$

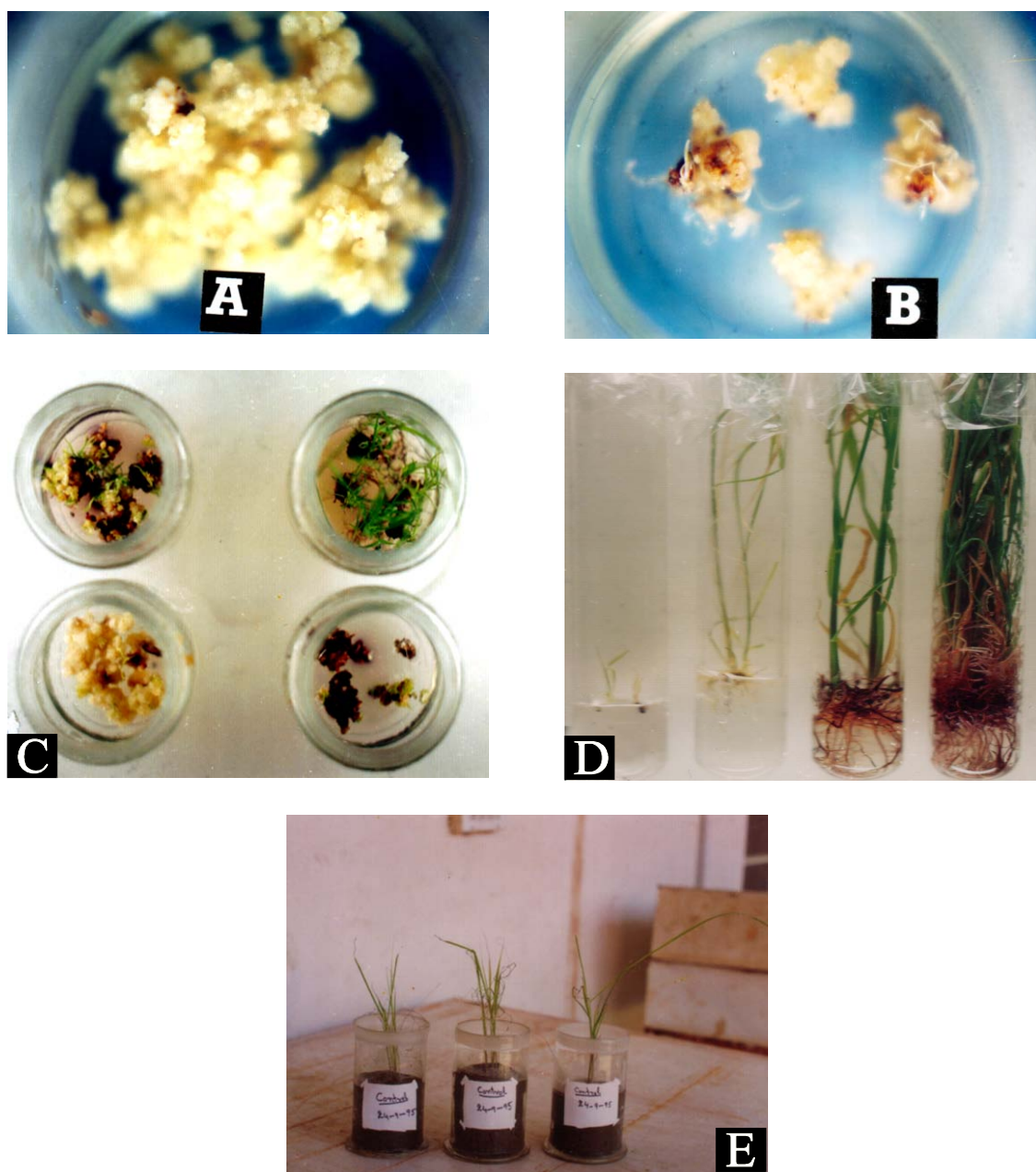


Fig. 1A: Callus mass, B: Shoot primordia, C: Formation of plant let, D: Development of plantlet in rooting media, E: Hardening of plantlets in Sand Jars

They also emphasized the usefulness of leaf trimming which resulted in stronger, greener, more luxuriant growth and better rooting in developed plantlets. However, no such results, were attained in this study as the plantlets grew and showed healthier sign of growth without the act of trimming of leaves. The difference of behavior could be due to different base material. The well developed plantlets with dark green colour and showing luxuriant growth were

selected for further treatment. The selected plantlets were passed through a process of hardening in Sand jars (Fig. 1E). The hardened plants were transferred to pots containing mixed soil (Fig. 2A). The well grown plants or soma-clones so developed were transplanted to field in fertile soils (Fig. 2B). The sets of fully grown soma-clones were planted in saline sodic soils (Table 1) which were grown to full size plants (Fig. 3A and B).



Fig. 2A: Development of plantlets in pots, B: Development of somaclones in the field

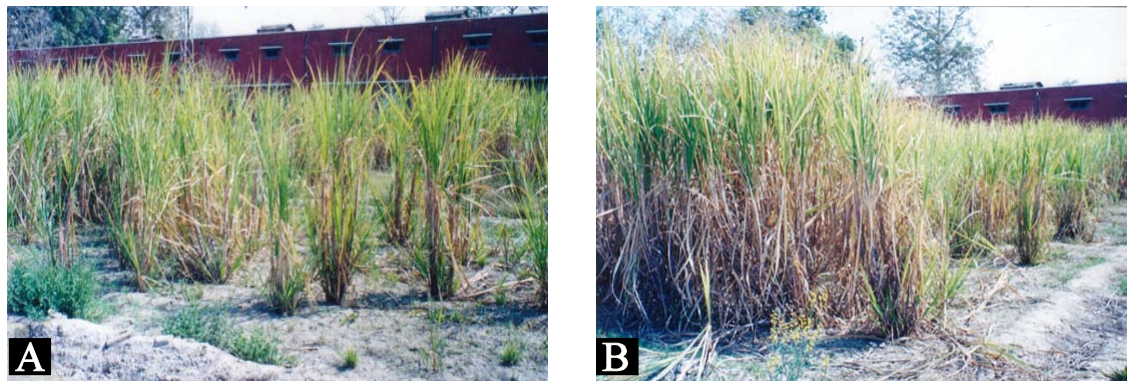


Fig. 3A: Somaclones growing in saline sodic soil B: Fully developed somaclones in saline sodic soil

Number of tillers per plant: The number of tillers scored in soma-clones were found to be higher than those of source plant. A range of 2-12 with an average of 6 was found in soma-clones while this range was 2-7 with an average of 4 tillers of the source plant (Table 2). Such kind of variation from parent plants has also been observed in soma-clones of other cultivars of sugarcane^[7].

Stem height: There is much variation in the height of stem within a plant. The height of stem recorded in soma-clones was more compared to the stem of its source plants. A range of 131-302 cm with an average of 201 cm was found in the stems of soma-clones while this range was 136-260 cm with an average of 186 cm in the stems of the source plants (Table 2).

Number of nodes per stem: The nodes in sugarcane are smaller than the internodes. It exhibit root bands which contain the lateral buds, one to several rows of root primordia and leaf sheath scar. The number of nodes also indicate the size of stem. The tall stem contain many nodes then the shorter ones. The number of nodes/stem was quite higher in soma-clones than its source plants. The number of nodes/stem in soma-clones had a

range of 10-20 with an average of 16, while this range was 10-6 with an average of 12 in the source plants (Table 2). The number of nodes correlate with the size of stem.

Girth of stem: The girth of stem is represented by measuring the girth of its internodes. The girth of internodes at the base of stem is short. The girth of internodes increases gradually in the upper internodes. The increase in girth with successively higher internodes causes an abrupt change in the shape of internodes. The increase in girth of internodes is associated with the rate of growth^[9]. Some kind of situation was observed in this study while measuring the girth of stem measured in this study refer to the 5th to 8th internodes from the base of stem.

The girth of stem in the internodes of soma-clones had a range of 6-8 cm with an average of 7 cm, while this range was 7-9 cm with an average of 8 cm in the internodes of its source plants (Table 2).

Root band width: The root band is the basal region of the internodes and is limited by growth ring and the leaf scar below the root band, containing root primordia and the bud,

varies in width among clones and frequently within clones from widest behind the bud to narrowest on the opposite side of the stalk^[10]. The width of root band had a range of 0.5-1.2 cm with an average of 0.8 cm in soma-clones while the range of width was 0.3-1 cm with an average of 0.7 cm in the source plants (Table 2). Siddiqui *et al.*^[7] also reported 100% variation among the parents and their soma-clones in this character. They used a different parameter for comparison.

Brix percentage: The brix percentage in the sugarcane represent the sugar content in the cane. A range of 14.70 to 17.75% with an average of 16.92% of brix was found in the canes of soma-clones while this range was 15.90 to 18.09% with an average of 17.85% in the canes of its source plants (Table 2). The result of this study do not agree with the findings of Siddiqui *et al.*^[7]. They compared the brix percentage of canes of soma-clones with those of their parents and found the soma-clones better than their parents in this character. The variations were noted in almost all the characters under study. The soma-clones were found better in the characters, of Number of tillers/plant, Stem height, Number of nodes/stem and Root band width. There was no difference in the length of internodes of soma-clones and source plants. The cause of variation, though not well defined, have been linked with the inherent chromosomal instability in a callus culture.

Larkin *et al.*^[11] evidenced that a major source of soma-clonal variation was due to activation of transposable elements. Whereas Siddiqui *et al.*^[7] mentioned that one did not know the phase at which the variation arise. According to them the variations were caused by a combination of physical and chemical phenomenon. A physical factor was the environments suitable for un-inhibited growth while the chemicals in the nutrients such as 2, 4-D might induce abnormal division. The present studies proved that salt tolerant soma-clone of sugarcane (cultivar CP-43/33) can be raised through tissue culture. The variations noted in the soma-clones can be favourable or otherwise has yet to be seen from the inheritance point of view. These soma-clones can be acceptable commercially if the good characters so exhibited are transmitted through the generations.

However, decrease in the brix percentage in soma-clones is a cause of concern and needs further study for rectification.

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