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Morpho-physiological Performance of Selected Somaclones (SC) Which were Produced *in Vitro* Salinity Stress in the Field for Two Sequential Years

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Abstract: The experiments were carried out at the farm of Bangladesh Sugarcane Research Institute Ishurdi, Pabna, Bangladesh during 2009-2010 and 2010-2011 cropping seasons for the production of somaclones *in vitro* to select salinity tolerant lines of sugarcane. Sugarcane varieties Isd 28, Isd 35, Isd 36, Isd 37 and Isd 38 were used for selection of salinity tolerant lines. Different levels of salt were used with MS medium for *in vitro* plantlet regeneration. The *in vitro* selected somaclones were evaluated in the field for two sequential years. Somaclones SC4, SC7, SC8 of Isd 28, SC1, SC7, SC9 of Isd 35, SC4, SC6 of Isd 36, SC3, SC9 ,SC10 of Isd 37 and SC3, SC7, SC8 of Isd 38 performed better in morpho-physiological performance at field condition in two sequential years. So those lines should be considered as salinity tolerant lines for further study.

Keywords: Morpho-physiological, Somaclone, Sugarcane, Salinity Stress, Sequential Year

1. Introduction

Salinity is one of the most widespread soil degradation processes on the Earth. Soil salinisation affects an estimated 1 to 3 million hectares in Europe, mainly in the Mediterranean countries. It is regarded as a major cause of desertification and therefore is a serious form of soil degradation being salinisation and sodification among the major degradation processes endangering the potential use of European soils. For instance, in Spain 3% of the 3.5 million hectares of irrigated land is severely affected, reducing markedly its agricultural potential while another 15% is under serious risk.

Other examples of salt-affected soil in Europe are the Caspian Basin, the Ukraine, and the Carpathian Basin (Hungary) (Bruno Ladeiro, 2012). Some of the most serious problems occur in semi-arid regions associated with the great river systems of South-East Asia. In Bangladesh, over 30% of the net cultivable area lies in the coastal zone of Bay of Bengal, of which approximately 53% is affected by varying degrees of salinity. The salt affected area in the coastal zone of the country was about 0.83 million ha in 1966-76, which expanded to 3.1 million ha over the last two decades (Kader, 2006). In addition, more area in that zone is expected to become saline affected in future due to increase in sea water level as a consequence of the greenhouse effect. The other concern is that the area under irrigation is increasing worldwide day-by-day allowing more area to be affected by salinity stress. As estimated by FAO, about 20-30 million ha of irrigated lands worldwide were seriously damaged in 2002 due to the build-up of salts and every year 0.25-0.50 million ha of irrigated lands worldwide are lost from production due to salts build-up (Martinez-Beltran and Manzur, 2005).

The advantage of somaclonal variability due to tissue culture is high frequency, mostly of gene mutations in somaclones and the experimental opportunities available for selection of cells with altered biochemical features (Maralappanavar *et al.,* 2000). Somaclonal variation in combination with *in vitro* mutagenesis can be beneficial for the isolation of salinity and drought tolerant lines in a short duration employing *in vitro* selection (Samad *et al.,* 2001).

Sugarcane being a typical glycophyte, exhibits stunted growth or no growth under salinity, with its yield falling to 50% or even more of its true potential (Subbarao and Shaw, 1985). Besides this, salinity in root zone of sugarcane decreases sucrose yield through its effect on both biomass and juice quality (Lingle and Wiegand, 1996). *In vitro* selection has been used for selection of salt tolerance (Rosas *et al.,* 2003) and drought and frost tolerance (Xing and Rajashekhar, 2001). However, the several variants are often unstable or non-heritable being epigenetic changes rather than genetic changes. Therefore, a detail study is needed in this regard.

2. Materials and Methods

Five sugarcane varieties viz. Isd 28, Isd 35, Isd 36, Isd 37 and Isd 38 were used as plant material. *In vitro* healthy and rooted plantlets which were produced under salinity stress were selected for 1st and 2nd year evaluation.

2.1. Multiplication and Growth Study of Selected Somaclones (R1 Generation) in Field

Experimental field was cultivated with tractor and levelled with leveller and then trench were prepared with trencher. Then hardened plantlets were transplanted in the field on 02 February, 2010 at line to line spacing 1.0 m and plant to plant spacing 50.0 cm. Plantlets were transplanted in pits containing soils and pressmud at 3:2 ratio. The soils-pressmud mixture of each pit was fertilized at the rate of 4, 3 and 3 g of urea, TSP and MP, respectively. Full TSP, MP and 2 g urea were applied in each pit as basal dose at planting time and the rest (1.0 g +1.0 g) of urea was applied as top dressing in two equal splits at 45 and 75 days after planting. First flood irrigation was applied just after the plantation of plantlets for good establishment and second and third irrigation were applied at 30 days and 60 days after transplantation (DAT). No insecticides were applied. In the month of June-July few stem borer infestation was observed and soon preventive measures like collecting adult moths by net, destroying egg mass and cutting the top of the affected plants to kill larvae were taken Weeding were done after one month. Earthing-up were done after 2nd top dressing (60 DAT), so weeds were automatically controlled. In the month of August cross tying were applied between the tillers of two hills to protect logging.

2.2. Somaclones Selection in the Field from R1 Generation to Study Their Growth and Yield in R2 Generation (2nd Year)

Selection of plants from R1 generation was made on the basis of morphological features by maintaining somaclone number selected *in vitro*. At the end of 1st year study each somaclone produced a hill which contains several stalks. After taking the harvesting data at the end of first year the healthy stalks were used for preparation of polybag settlings for R2 generation study (in 2nd year) in field under normal condition. For polybag settlings, the cane stalks were cut into small pieces (5 cm) containing one bud each with at least 1.5 cm stalk on above the node and about 3 cm at below. All setts were treated by Bavistin solution (1:1000) for 30 minutes to prevent fungal infection. Polybags of 12.5 cm × 10.0 cm size were taken and four holes were made at the bottom of the bags to drain out the excess water. The soil for filling polybags was mixed with cowdung (1:1) and then treated with Chlorpyrifos (Regent 3 GR) @ 0.04 g bag^{-1} and Carbofuran (Furadan 5G) @ 0.05g bag^{-1} to control insect pest in the nursery. About 2/3 rds of polybag were filled with the mixed soil and a previously treated sett was placed vertically in the centre of soil of the bag keeping the bud in upward direction. Then the polybag was completely filled with the soil so that about 1.5-2.0 cm soil covered the setts. The polybags were kept in a sunny place and also covered with rice straw thinly to preserve soil moisture, and protect soil loss from heavy rain (Ali *et al.,* 1989). Watering was done at 2 days interval to the nursery beds throughout the whole period before transplantation of the settlings to the main field. Other cultural operations like weeding, rouging of diseased and pest infected settlings were done to maintain healthy settlings. Watering was done days of transplanting settlings into the main field. Land preparation, fertilization and other intercultural operations were done in 2nd year following the procedure of 1st year.

2.3. Data Collection from Field Grown Plants

From field grown plants sugarcane data on number of millable cane, stalk height, stalk diameter, total chlorophyll, chlorophyll stability index (CSI), leaf area index (LAI), brix (%) were collected, analyzed and presented in respective Tables in results section.

2.4. Millable Cane, Stalk Height and Stalk Diameter

At harvest, the total number of cane stalks from each clump was counted and expressed in number per clump. For stalk height, canes were collected from each clump and the length of individual cane was measured (m) from the bottom to the top of stalk using a measuring tape. Similarly diameter (cm) of sample cane was determined the average value of bottom, middle and top measurement by slide calipers.

2.5. Yield /Clump of Cane

The yield of sugarcane stalks was recorded at final harvest from the same sample used for millable cane count. For collection of yield data, the cane stalks were cut to the ground level by spade. Sickle was used to remove the dried old trashes and cut green top of the cane stalks. The weight of clean cane stalks was taken by a balance and expressed in kg/clump.

2.6. Estimation of Total Chlorophyll

Total chlorophyll content is a parameter for salinity tolerance measurement in sugarcane. For chlorophyll measurement, 3rd leaf from top was randomly collected

from 7-8 month- old field grown plants. The leaves were kept in a polythene bag and brought immediately after collection to the laboratory. Fresh leaf samples of 0.5 g were drawn from each sample and homogenized in a mortar by pestle with 80 % acetone. Supernatant was decanted off and filtered through a Buachner funnel using Whatman No.1 filter paper. Sufficient quantity of 80 % acetone was added with residue and the process was repeated until acetone become colourless. The volume was made to 100 ml with 80 % acetone in a volumetric flask. Optical density of the sample was measured at 645 nm and 663 nm in Spectrophotometer (Model-Thermo spectronic uv-1, England). Amount of chlorophyll a, chlorophyll b and total chlorophyll were calculated on a fresh weight basis employing the following formulae (Mahadevan and Sridhar, 1982):

$$\text{Total chlorophyll (mg g-1)} = \frac{20.2A645 + 8.02A663}{a \times 1000 \times w} \times v$$

Where,
A = Optical density in each sample
a =Length of light path in the cell (usually 1cm)
v =Volume of the extract in ml and
w =Fresh weight of the sample in gram

2.7. Estimation Chlorophyll Stability Index (CSI)

For CSI measurement sample collection and preparation was similar to that in estimation of total chlorophyll. CSI was estimated following the method described by Killen and Andrew (1969).

Only leaf blade from middle portion was used CSI estimation. The samples of 0.25 g were placed in 125 ml conical flasks separately with 20 ml of 80 % acetone. Three samples were taken for heat treatment in water bath at 60 ±10C for 120 min. Other three samples (control) were kept at room temperature. Leaf tissues of both heated and unheated samples were removed from acetone, blotted to dry and transferred to a mortar. Both heated and unheated samples were homogenized in a mortar by pestle with 5 ml of 80 % acetone separately. Supernatant was decanted off and filtered through a Buachner funnel using Whatman No.1 filter paper. Sufficient quantity of 80 % acetone was added with the residue and the process was repeated until acetone become colorless. The volume was made to 100 ml with 80 % acetone in a volumetric flask. The optical density of heated and unheated solutions was measured in Spectrophotometer (Model-Thermo spectronic uv-1, England). The difference between the two readings (heated and unheated samples) was recorded as chlorophyll stability index. Thus CSI= OD value of heated sample at 663 nm– OD value of unheated sample at 663 nm. This test was performed for all somaclones used in the present investigation.

2.8. Leaf Area Index

The area of leaf blade was measured by a leaf area meter (ΔT Area meter MK2). Leaf area index (LAI) was calculated from leaf area of sample divided by land area following the formula mentioned below. It is area of leaf of unit land area (Watson, 1952).

$$\text{LAI} = \frac{\text{Leaf area}}{\text{Land area}}$$

2.9. Determination of Brix (%)

Sugarcane juice for Brix (%) was done at harvest of sugarcane. In the 1st year Brix (%) was collected by using hand Brix refractometer due to lack of proper cane because all canes were used for preparing polybag settlings which were used for 2nd year study and PVC pipe study and some stalks were cut into one eyed sett and directly used for germination test but in 2nd year randomly selected 5 sample cane stalks were crushed with a mini power crusher to get juice for analysis. The juice was collected in glass jars. The reading of brix (%) was recorded with Brix hydrometer. Temperature of the juice was noted. These brix reading were corrected with the help of Schmitz,s table (Spancer and Meade, 1963).

2.10. Statistical Analysis

The collected data for a character under certain treatment were calculated and statistically analyzed following Factorial Completely Randomized Design (CRD). The analysis of variance was performed and means were compared by Duncans New Multiple Range Test (DMRT) at 5% level of probability for interpretation of results.

3. Results

3.1. Millable Cane

Salinity tolerant somaclone lines in R1 generation produced 2-3 times higher millable cane than mother clones' except in few lines (Table 1). Somaclone line 4 of Isd 28 produced 15 millable canes per clump. Similarly SC1 and SC10 produced 14 millable canes per clump. The lowest millabe cane number per clump was 12 in SC3, SC6, SC7 and SC9 of Isd 28, where mother clone (control) produced only 7 millable canes per clump. Somaclones of Isd 35 produced higher number of millable cane per clump. The highest number of millable cane was 19 in SC2 of Isd 35 followed by 16 millable canes in SC5 and the lowest was in SC1, where mothers (control) had only 5 per clump (Table 1). The number of millable cane was 15, and 12 in SC6 and SC9, respectively in selected clones of Isd 36. The lowest number of millable cane in this variety was only 4 per clump where mother (control) clone produced only 5 millable cane per clump. Millable cane production per clone was comparatively less in Isd 37. Only one line SC5 produced 14 millable canes per clump and other lines produced 6-9 millable cane per clump and mother clone of Isd 37 had only 4. Mother clone of Isd 38 produced 7 millable canes per clump but selected somaclones produced 10-18 cane except SC6 where only 5 millable canes were produced (Table 1).

In the 2nd year, same somaclones of R1 generation cultivated in field showed less performance in producing millable cane compared to that produced in R1 generation. The highest number of millable cane in R2 generation of Isd 28 was 7 in SC4 SC5, and SC8 followed by other somaclones which produced 6 millable canes per clump except in SC3. SC3 of Isd 28 produced the lowest millable cane (5) which was lower than the number of cane produced by mother clone (control). Only two lines of Isd 35 SC7 and SC9 produced the significantly higher number of millable cane compared to all somaclones as well as mother clone. Other somaclone lines of Isd 35 produced statistically similar millable cane to mother clones except in SC3 and SC8 which produced lower millable cane than mother clone (control). Somaclones of other varieties produced almost similar number of millable cane to mother clone in R2 generations.

3.2. Leaf Area Index (LAI)

Salinity tolerant somaclone lines grew in field under R1 generation also showed more than double leaf area index compared to that in mother clone (control). In Isd 28, all selected somaclones produced significantly higher leaf area over mother clone (Table 2). The highest leaf area was in SC4, followed by SC1 and SC6 and the lowest value was in mother clone. The highest leaf area was more than three times higher over mother clone in Isd 35. SC2 produced the highest leaf area 19.2, followed by SC7 of 16.3 and the lowest leaf area of mother clone was only 5.2. In Isd 36, the highest leaf area was observed in SC10 followed by SC9 and the lowest value of 4.3 was found in SC5. Somaclones SC7 of Isd 37 showed more than four times higher leaf area over mother clone and other somaclones also produced significantly higher leaf area over mother clone except SC4 which had similar leaf area to mother clone. The highest leaf area in Isd 38 was obtained in SC9 followed by SC8 and the lowest leaf area was in SC10. Thus, it was again revealed that almost all somaclones of *in vitro* salinity stress tolerant produced significantly higher leaf area over respective mother clone in RI generation.

In R2 generation, maximum somaclones produced almost similar leaf area to respective mother clone. In Isd 28, SC3 and SC4 produced similar leaf area like their mother clone, but other somaclones produced lower leaf area than their mother clone. In Isd 35, SC5 and SC9 produced higher leaf area than their motherclone and the rest somaclones produced similar leaf area. Somaclones of Isd 36 and other varieties showed similar leaf area to that of respective mother clones.

3.3. Total Chlorophyll

In case of *in vitro* selected salinity tolerant somaclones, the total chlorophyll differed among selected somaclones of Isd 35, Isd 36 and Isd 37 in both R1 and R2 generation compared to mother clone (Table 3). In Isd 28 and Isd 38, the total chlorophyll in R1 generation did not vary significantly compared to mother clone. In Isd 35, the highest total chlorophyll was obtained in SC3 followed by SC5 and the lowest value was in mother clone (Table 3). In Isd 36, all somaclones showed significantly higher total chlorophyll except SC6. The lowest chlorophyll was in mother clone (control). Similarly in 1sd 37, somaclones SC3, SC6 and SC7 equally contained significantly higher total chlorophyll and the lowest value was in SC2 and mother clone in R1 generation. In R2 generation, all the somaclones of Isd 35 showed significantly highest total chlorophyll over mother clone (control). Other varieties like Isd 28, Isd 37 and Isd 38 showed similar chlorophyll content to mother clone. Most of the somaclones of Isd 36 showed similar chlorophyll to mother clone except SC1 and SC9 which showed lower chlorophyll content in R2 generation (Table 3).

3.4. Chlorophyll Stability Index (CSI)

Selected somaclones produced *in vitro* salinity stress, somaclones of Isd 28, Isd 35, Isd 36 and Isd 38 showed similar CSI to respective mother clone in R1 generation but somaclones of Isd 37 showed different. The highest CSI was obtained in SC6 of Isd 37 followed by SC7 and SC3. The lowest CSI was obtained in control. In R2 generation, CSI levels decreased little (Table 4). Significant variation was observed in somaclones compared to mother clone of all the varieties except in Isd 28. In Isd 35, the highest CSI was obtained in SC1 and SC2. The lowest CSI was in control. In Isd 36, mother clone and SC6 showed the highest value of CSI. In Isd 37, SC3, SC9 and SC10 showed higher CSI and in Isd 38, SC3 anSC7 showed higher CSI value (Table 4) than their mother clone.

3.5. Brix (%)

Brix per cent (%), an important factor for total sugar yield in sugarcane was varied among varieties and also between somaclones for *in vitro* salinity stress selected somaclones. Selected somaclones of *in vitro* salinity stress, all somaclones of Isd 28 in R1 generation showed higher brix % to mother clone except SC8 (Table 5). In Isd 35, SC4, SC5 and SC6 showed significantly lower brix % compared to mother clone and other selected somaclones. In Isd 36, most of the somaclones showed lower brix % than mother clone except SC5. In Isd 37, similar type of variation in brix % was observed of R1 generation except SC6 which produced similar brix per centage like their mother. In Isd 38, SC2, SC3 and SC7 showed higher brix per centage than their mother clone. In R2 generation, brix % of somaclones of Isd 28 was lower except SC7 which showed similar brix per centage like their mother clone (Table 5). In Isd 35, SC3, SC4 and SC5 had significantly lower brix % and rest of the somaclones had similar brix per cent. Most of the somaclones of Isd 36 had significantly lower brix % except SC4 and SC6 compared to mother clone. In Isd 37, SC3, SC9 and SC10 had similar brix % to mother clone and other somaclones produced significantly lower brix per cent. Similar brix %

was observed in Isd 38, where only SC7 and SC8 had higher brix % than other somaclones.

3.6. Stalk Height

Somaclones selected as salinity stress tolerant of all varieties also showed vigorous in R1 generation. In Isd 28, all somaclones produced significantly higher stalk height than mother clone (control). The highest and lowest stalk height was found in SC4, SC5 and SC3 respectively, was observed in the variety Isd 28. Similarly all somaclones of Isd 35 produced higher stalk height over control (Table 6). The lowest stalk height was observed in SC1 and the highest was in SC6 and SC10 of Isd 35. In Isd 36, SC5, SC7, SC9 and SC10 produced the highest stalk height and the lowest value was for SC1. Similar stalk heights were obtained by all somaclones which was significantly higher over control except SC3 in Isd 37. In Isd 38 the highest and lowest stalk height was recorded in SC5 and SC6, respectively during R1 generation (Table 6).

Stalk height of selected somaclones did not differ significantly to that of mother clone (control) in R2 generation in all varieties.

3.7. Stalk Diameter

Similarly selected somaclones of *in vitro* salinity stress stalk diameter also varied only in Isd 36 in R1 generation (Table 7). Most of the somaclones of Isd 36 produced significantly higher stalk diameter over mother clone. The highest stalk diameter was in SC10 followed by SC1, SC4, SC5, SC7, SC8 and SC9. The lowest diameter was observed in plants under control. Stalk diameter of all the somaclones of all varieties did not differ significantly to mother clone in R2 generation (Table 7).

Table 1. *Millable cane of in vitro salinity tolerant somaclones of sugarcane varieties in field in two sequential years.*

Somaclones (SC)	Millable cane in R1 generation					Millable cane in R2 generation				
	Isd 28	Isd 35	Isd 36	Isd 37	Isd38	Isd 28	Isd 35	Isd 36	Isd 37	Isd38
Mother clone	7	5	5	4	7	7 a	6 bc	6 a	4	7
SC1	14	10	11	9	12	6 ab	7 ab	6 a	5	6
SC2	13	19	11	6	11	6 ab	7 ab	6 a	5	7
SC3	12	12	7	7	13	5 b	5 c	5 ab	4	7
SC4	15	13	10	4	11	7 a	7 ab	6 a	5	6
SC5	13	16	9	14	11	7 a	7 ab	5 ab	4	7
SC6	12	15	15	7	5	6 ab	7 ab	5 ab	5	7
SC7	12	13	4	6	10	6 ab	8 a	6 a	5	6
SC8	13	11	10	7	12	7 a	5 c	6 a	4	6
SC9	12	12	12	8	18	6 ab	8 a	5 ab	5	7
SC10	14	15	7	8	12	6 ab	6 bc	4 b	5	7
Lsd (0.05)						1.663	1.663	1.663	NS	NS

Figures with similar letter (s) don't differ significantly at 5.0% probability by DMRT

Table 2. *Leaf Area Index (LAI) of in vitro salinity tolerant somaclones of sugarcane varieties in field in two sequential years.*

Somaclones (SC)	Leaf Area Index (LAI) in R1 generation					Leaf Area Index (LAI) in R2 generation				
	Isd 28	Isd 35	Isd 36	Isd 37	Isd38	Isd 28	Isd 35	Isd 36	Isd 37	Isd38
Mother clone	7.1 d	5.2 f	5.3 f	5.5 f	7.1 d	7.2 a	6.3 abc	6.1	5.5	7.2
SC1	14.1 ab	10.5 e	11.6 bc	12.3 b	12.3 bc	6.2 ab	7.1 abc	6.1	6.6	6.1
SC2	13.1 bc	19.2 a	11.7 bc	7.4 e	11.1 c	6.2 ab	7.2 ab	6.1	6.6	7.2
SC3	13.3 bc	11.4 de	10.6 cd	10.1 cd	12.1 c	7.2 a	5.2 c	6.1	5.5	6.2
SC4	15.1 a	13.4 c	10.6 cd	5.5 f	11.1 c	7.1 a	7.1 abc	6.3	6.6	6.2
SC5	12.2 bc	13.5 c	4.3 f	8.6 de	11.0 c	6.0 ab	8.2 a	6.1	6.6	6.2
SC6	14.1 ab	15.5 b	7.1 e	11.1 bc	12.3 bc	6.3 ab	7.1 abc	5.3	6.6	7.1
SC7	13.1 bc	16.3 b	9.6 d	20.8 a	11.2 c	6.2 ab	6.2 bc	4.5	6.6	7.2
SC8	12.2 bc	12.6 cd	7.1 e	8.6 de	13.2 b	5.1 b	5.2 c	5.3	5.5	7.2
SC9	12.1 bc	12.6 cd	12.7 b	11.2 bc	18.6 a	6.3 ab	8.1 ab	5.3	6.6	7.2
SC10	12.2 c	15.6 b	15.9 a	8.6 de	5.2 e	5.1 b	7.1 abc	5.3	5.5	7.1
Lsd (0.05)	1.663	1.663	1.663	1.663	1.663	1.663	1.663	ns	ns	ns

Figures with similar letter (s) of a column don't differ significantly at 5.0% probability by DMRT

Table 3. *Chlorophyll level (mgg^{-1}) of in vitro salinity tolerant somaclones of sugarcane varieties in field in two sequential years.*

Somaclones (SC)	Chlorophyll level (mgg^{-1}) in R1 generation					Chlorophyll level (mgg^{-1}) in R2 generation				
	Isd 28	Isd 35	Isd 36	Isd 37	Isd38	Isd 28	Isd 35	Isd 36	Isd 37	Isd38
Mother clone	1.94	2.04d	1.97b	2.31d	1.96	1.96	2.00b	2.35 a	2.31	1.92
SC1	2.84	2.18cd	2.77a	2.71bc	2.20	1.91	2.31 a	2.15 b	2.30	1.93
SC2	2.64	2.12cd	3.01a	2.33d	2.19	1.96	2.23 a	2.30 ab	2.31	1.93
SC3	2.53	2.67a	2.64a	3.08a	2.15	1.93	2.36 a	2.28 ab	2.30	1.98
SC4	2.46	2.09d	2.81a	2.36d	2.10	1.90	2.21 a	2.21 ab	2.35	1.95
SC5	2.66	2.56ab	2.91a	2.43cd	2.06	1.88	2.20 a	2.17 ab	2.29	1.96
SC6	2.72	2.10d	2.00b	3.10a	2.17	1.98	2.27 a	2.25 ab	2.34	1.97
SC7	2.67	2.28cd	2.74a	3.05a	2.07	1.95	2.30 a	2.21 ab	2.31	1.97
SC8	2.48	2.14cd	2.93a	2.83ab	2.12	2.01	2.25 a	2.19 ab	2.28	1.96
SC9	2.32	2.36bc	2.67a	2.91ab	2.14	1.99	2.24 a	2.13 b	2.35	1.93
SC10	2.57	2.07d	2.68a	2.87ab	2.11	2.00	2.31 a	2.16 ab	2.31	1.93
Lsd (0.05)	NS	0.2414	0.4603	0.3172	NS	NS	.1663	.1663	NS	NS

Figures with similar letter (s) of a column don't differ significantly at 5.0% probability by DMRT

Table 4. *Chlorophyll Stability Index (CSI) of in vitro salinity tolerant somaclones of sugarcane varieties in field in two sequential years.*

Somaclones (SC)	Chlorophyll Stability Index (CSI) in R1 generation					Chlorophyll Stability Index (CSI) in R2 generation				
	Isd 28	Isd 35	Isd 36	Isd 37	Isd38	Isd 28	Isd 35	Isd 36	Isd 37	Isd38
Mother clone	80.00	66.50	78.00	80.8c	70.3	76.60	69.63 c	77.60 a	80.40 a	72.20a-c
SC1	82.30	72.50	83.00	83.3abc	73.3	76.10	73.00 a	75.20 bc	74.30 f	71.20 c
SC2	84.30	7140	85.00	81.0c	73.1	75.30	73.40 a	74.10 c	78.10 bc	70.70 c
SC3	84.30	73.30	83.00	87.4ab	73.1	76.30	71.10 bc	76.40 ab	80.50 a	73.30 a
SC4	83.20	69.10	84.00	82.1bc	72.3	75.20	71.60 bc	75.30 bc	76.20 de	73.10 ab
SC5	82.63	70.80	85.00	82.4bc	71.8	76.20	71.00 bc	74.20 c	75.10 ef	72.40a-c
SC6	84.20	72.50	82.96	88.5a	72.7	75.40	72.00 ab	77.60 a	75.30 ef	73.10ab
SC7	83.30	71.60	84.00	88.3a	72.4	76.20	72.30 ab	75.30 bc	74.10 f	73.40 a
SC8	82.40	72.30	85.00	84.1abc	71.4	75.10	72.10 ab	75.20 bc	74.50 ef	72.30a-c
SC9	83.30	70.50	82.00	84.3abc	72.5	76.70	71.70 ab	75.20 bc	80.50 a	71.30 bc
SC10	83.40	71.80	83.00	84.2abc	71.6	76.30	72.50 ab	74.10 c	80.30 a	71.10 c
Lsd (0.05)	NS	NS	NS	5.306	NS	NS	1.618	1.663	1.663	1.663

Figures with similar letter (s) of a column don't differ significantly at 5.0% probability by DMRT

Table 5. *Brix per centage of in vitro salinity tolerant somaclones of sugarcane varieties in field in two sequential years.*

Somaclones (SC)	Brix per centage in R1 generation					Brix per centage in R2 generation				
	Isd 28	Isd 35	Isd 36	Isd 37	Isd38	Isd 28	Isd 35	Isd 36	Isd 37	Isd38
Mother clone	18.30ab	19.10a	19.30a	20.5a	19.1bc	19.30 a	21.50 abc	20.50 a	20.30 a	21.60 a
SC1	20.40a	19.40a	16.40cd	17.5cd	18.7bc	15.10 c	22.60 ab	17.30 c	16.20 cd	18.00 c
SC2	20.00a	19.00a	16.30cd	19.3abc	19.6abc	16.70 bc	22.90 a	18.50 bc	19.70 ab	18.70 bc
SC3	19.30a	20.27a	17.50bc	17.7cd	23.0a	17.30 b	20.80 bc	17.70 c	20.10 a	19.10 bc
SC4	18.50ab	17.00b	15.77cd	17.1d	17.6bc	17.00 b	20.40 c	20.40 a	16.30 cd	18.70 bc
SC5	19.60a	17.00b	19.10ab	18.3bcd	19.5bc	17.50 b	20.30 c	17.23 c	16.00 d	18.90 bc
SC6	20.10a	16.30b	15.30d	19.6ab	18.4bc	17.10 b	21.40 abc	19.70 ab	16.60 cd	18.20 c
SC7	19.60a	19.30a	16.70cd	19.1abc	20.5ab	19.70 a	21.40 abc	17.40 c	16.20 cd	21.70 a
SC8	16.30b	20.00a	16.20cd	18.4bcd	19.3bc	16.50 bc	21.00 bc	17.10 c	16.40 cd	20.40 ab
SC9	20.10a	19.50a	16.60cd	16.8d	16.6c	16.30 bc	21.80 bc	17.30 c	20.60 a	18.20 c
SC10	20.20a	19.00a	16.60cd	17.6cd	18.5bc	16.20 bc	21.60 abc	19.00 b	20.50 a	18.80 bc
Lsd (0.05)	1.312	1.312	1.747	1.891	3.406	1.663	1.663	1.697	1.663	1.663

Figures with similar letter (s) of a column don't differ significantly at 5.0% probability by DMRT

Table 6. *Stalk height (m) of in vitro salinity tolerant somaclones of sugarcane varieties in field in two sequential years.*

Somaclones (SC)	Stalk height (m) in R1 generation					Stalk height (m) in R2 generation				
	Isd 28	Isd 35	Isd 36	Isd 37	Isd38	Isd 28	Isd 35	Isd 36	Isd 37	Isd38
Mother clone	2.69 c	2.67 c	2.70 d	2.77 b	2.71 d	2.75	2.66 a	2.70	2.78	2.64
SC1	3.04 ab	2.96 b	2.96 c	3.35 a	3.02 bc	2.71	2.69 a	2.69	2.82	2.63
SC2	3.05 ab	3.03 ab	3.07 abc	3.32 a	3.07 abc	2.68	2.68 a	2.71	2.80	2.66
SC3	2.96 b	3.05 ab	3.04 abc	2.94 b	3.08 abc	2.70	2.71 a	2.70	2.80	2.64
SC4	3.15 a	3.05 ab	3.05 abc	3.35 a	3.05 abc	2.69	2.71 a	2.74	2.86	2.66
SC5	3.15 a	3.05 ab	3.12 a	3.35 a	3.15 a	2.67	2.70 a	2.72	2.87	2.67
SC6	3.04 ab	3.08 a	2.97 bc	3.36 a	2.97 c	2.68	2.70 a	2.72	2.78	2.64
SC7	3.08 ab	3.04 ab	3.09 a	3.28 a	3.09 abc	2.67	2.68 a	2.68	2.84	2.62
SC8	3.02 ab	3.02 ab	3.08 ab	3.28 a	3.08 abc	2.69	2.67 a	2.70	2.87	2.64
SC9	3.07 ab	3.03 ab	3.09 a	3.29 a	3.09 abc	2.71	2.69 a	2.66	2.79	2.66
SC10	3.10 ab	3.10 a	3.10 a	3.26 a	3.13 ab	2.71	2.70 a	2.66	2.77	2.63
Lsd (0.05)	0.1288	0.09109	0.1052	0.3111	0.1052	NS	.1663	.1663	.1744	NS

Figures with similar letter (s) of a column don't differ significantly at 5.0% probability by DMRT

Table 7. *Stalk diameter (cm) of in vitro salinity tolerant somaclones of sugarcane varieties in field in two sequential years.*

Somaclones (SC)	Stalk diameter (cm) in R1 generation					Stalk diameter (cm) in R2 generation				
	Isd 28	Isd 35	Isd 36	Isd 37	Isd38	Isd 28	Isd 35	Isd 36	Isd 37	Isd38
Mother clone	1.89	1.91	2.01d	2.15	2.22	1.94	1.92	2.24	2.17	2.20
SC1	1.92	1.97	2.40ab	2.31	2.34	1.94	1.91	2.23	2.14	2.20
SC2	1.98	1.95	2.17bcd	2.31	2.28	1.92	1.96	2.35	2.17	2.23
SC3	1.94	1.95	2.20bcd	2.35	2.31	1.91	1.93	2.16	2.16	2.21
SC4	1.95	1.93	2.30abc	2.32	2.32	1.90	1.91	2.27	2.17	2.23
SC5	1.96	1.94	2.30abc	2.33	2.35	1.95	1.92	2.20	2.17	2.24
SC6	1.93	1.95	2.14cd	2.37	2.37	1.92	1.93	2.25	2.21	2.24
SC7	1.92	1.96	2.30abc	2.30	2.30	1.92	1.93	2.28	2.11	2.23
SC8	1.94	1.96	2.27abc	2.33	2.33	1.90	1.96	2.24	2.13	2.21
SC9	1.96	1.97	2.40ab	2.23	2.35	1.91	1.92	2.17	2.14	2.21
SC10	1.93	1.96	2.50a	2.34	2.32	1.94	1.91	2.30	2.16	2.23
Lsd (0.05)	NS	NS	0.2521	NS	NS	.1968	NS	.1663	.1663	NS

Figures with similar letter (s) of a column don't differ significantly at 5.0% probability by DMRT

Table 8. *Cane Yield per clump (kg) of in vitro salinity tolerant somaclones of sugarcane varieties in field in two sequential years.*

Somaclones (SC)	Cane Yield per clump (kg) in R1 generation					Cane Yield per clump (kg) in R2 generation				
	Isd 28	Isd 35	Isd 36	Isd 37	Isd38	Isd 28	Isd 35	Isd 36	Isd 37	Isd38
Mother clone	5.0	7.1	6.8	4.6	7.1	5.0 c	7.8 bc	7.0 ab	4.3 b	7.0 b
SC1	14.5	14.5	9.3	8.1	12.3	7.8 ab	10.4 a	6.5 ab	5.2 ab	7.8 ab
SC2	16.9	22.8	14.3	7.7	14.4	9.6 a	9.1 ab	7.8 a	6.5 a	7.8 ab
SC3	14.7	14.5	10.1	8.2	15.6	9.1 a	6.5 c	6.5 ab	5.2 ab	9.1 a
SC4	18.4	15.8	12.4	5.4	13.2	6.5 bc	9.1 ab	7.8 a	6.5 a	7.8 ab
SC5	15.3	19.2	10.8	16.8	13.3	7.8 ab	7.8 bc	5.2 b	6.5 a	9.1 a
SC6	16.7	18.3	18.4	10.1	14.2	7.8 ab	9.1 ab	6.5 ab	6.5 a	9.1 a
SC7	14.6	15.8	6.9	8.8	6.4	7.8 ab	9.1 ab	7.8 a	6.5 a	9.1 a
SC8	15.5	13.4	12.4	9.3	13.2	8.2 ab	6.5 c	7.8 a	6.5 a	9.1 a
SC9	14.8	18.2	14.4	8.9	20.1	7.8 ab	9.1 ab	6.5 ab	5.2 ab	9.1a
SC10	15.6	12.3	13.3	10.3	14.4	9.3 a	9.1 ab	7.8 a	6.5 a	9.1 a
Lsd (0.05)						1.943	1.663	1.663	1.612	1.663

Figures with similar letter (s) of a column don't differ significantly at 5.0% probability by DMRT

3.8. Yield per Clump

The salinity tolerant *in vitro* selected somaclones also produced approximately 3 time higher yield per clump in R1 generation except in some lines (Tale 8). In Isd 28, the highest average cane yield was 18.4kg in SC4, which is 3.6 times higher over mother clone. Other somaclones also produced similar cane yield per clump. The lowest average cane yield of somaclone of Isd 28 was 14.5 kg per clump in SC1 and it was 2.86 times higher over control. Similar cane yield in Isd 35 was obtained in R1 generation. The highest average cane yield was 22.8 kg per clump in SC2 and the lowest one was 12.3 kg per clump in SC10 of Isd 35. Mother

clone (control) of Isd 36 produced 6.8 kg cane per clump in R1 generation where somaclone SC6 produced the highest 18.4 kg cane per clump and the lowest was 6.9 kg in SC8 of Isd 36. The average highest and lowest cane yield was 16.8 kg and 5.4 kg per clump in SC5 and SC4, respectively in Isd 37, where mother clone had 4.6 kg per clump. Similar types of observation were noticed in Isd 38 in R1 generation. The highest cane yield was 20.1 kg per clump in SC9 followed by 15.6 kg per clump in SC3 and the lowest average cane yield was 6.4 kg per clump in Isd 38.

In R2 generation, though the cane yield production per clump reduced distinctly compared to R1 generation average cane yield of somaclones in R2 generation was almost higher over mother clone. In Isd 28, the significantly highest cane yield was in SC2, SC3 and SC10, and the lowest yield was in mother clone. The highest average cane yield per clump was 10.4 kg in SC1 of Isd 35 and other somaclones had almost similar cane yield to that of mother clone (Table 8). All somaclones of Isd 36 produced similar cane yield to that of mother clone. Most of the somaclone of Isd 37 produced significantly higher cane yield over mother clone except SC1, SC3 and SC9. Similar trend was also observed in Isd 38, where all somaclones produced higher cane yield over mother clone except SC1, SC2 and SC4 in R2 generation (Table 8).

4. Discussion

The results obtained from the experiment explained that the changes on the morpho-physiological parameters of the somaclones produced under *in vitro* salinity stress of six sugarcane varieties in two successive years were remarkable. Present result agreed to earlier report of Begum *et al. (*2012) who stated that change in physiological characters of sugarcane under drought condition is genotype specific. Sugarcane plants are mixoploid, the source of the detected enhancement of stress tolerance can be attributed to the pre-existing variability in addition to somaclonal variation. These findings are in agreement with Taghian (2002). In the present experiment, the selected somaclones which were produced under induced salinity stress *in vitro* showed different number of millable cane, different types of LAI, and different types of stalk height and stalk diameter and yield in two different years. Salinity tolerant SC4, SC5 and SC8 of Isd 28, SC7, SC9 of Isd 35 showed higher number of millable cane, leaf area index (LAI) and yield than their mother clone in both R1 and R2 generation. Somaclones of other varieties showed insignificant difference with their mother clone. The stress tolerant somaclones showed higher stalk height than mother in R1 generation where as R2 generation showed insignificant difference. So SC4, SC5 and SC8 of Isd 28, SC7, SC9 of Isd 35 were selected as salinity tolerant.

The chlorophyll stability index (CSI) is an indication of the stress tolerance capacity of plants. In the present experiment, i*n vitro* salinity tolerant somaclones of Isd 28, Isd 35, Isd 36 and Isd 38 did not differ significantly on CSI to respective mother clone in R1 generation. The highest CSI was obtained in SC1 and SC2 of Isd 35. In Isd 37, SC3, SC9 and SC10 showed higher CSI and in Isd 38 SC3 and SC7 showed higher CSI value than their mother clone. A high CSI value means that the stress did not have much effect on chlorophyll content of plants. A higher CSI value helps plants to withstand stress through better availability of chlorophyll. This leads to increase photosynthetic rate, more dry matter production, and higher productivity. This indicates how well chlorophyll can perform under stress (Mohan *et al.,* 2000). On the basis of higher CSI, SC1, SC2 of Isd 35, SC3, SC9 and SC10 of Isd 37 and SC3, SC7 of Isd 38 was selected as salinity tolerant.

Selected salinity tolerant somaclones which were produced *in vitro* in present study showed different types of brix percentage both in R1 and R2 generation. It might be due to eco-physiological variation in somaclones. Reports indicated that brix % may vary due to heterozygous nature in sugarcane Isd 28 (Jabber *et al.,* 2006). Similar report was also given by Kashem *et al. (*2005) and Rahman *et al. (*2008) in variety Isd 36 and Isd 35, respectively. Among them salinity tolerant SC7 of Isd 28, SC1, SC2, SC6, SC7 and SC10 of Isd 35, SC4, SC6 of Isd 36, SC2, SC3, SC9, SC10 of Isd 37, SC7, SC8 of Isd 38 showed higher brix percentage than their mother clone in both the years. So, on the basis of brix percentage, described somaclones were selected as stress tolerant. Our results from morpho-physiological evaluation of somaclones which were produced *in vitro* salinity stress in the field in two successive year agree with the earlier report of Taghian (2002) who explained that sugarcane plants are mixoploid, the source of the detected enhancement of stresses tolerance can be attributed to the pre-existing variability in addition to somaclonal variation. He also observed the enhancement of both salt and drought tolerance was around 13% as indicated form stress tolerance indices, and suggested recurrent *in vitro* selection for further improvement of the traits may be effective. He also suggested that the selected clones to be tested for salt and drought tolerance to observe its performance under stressful environments at maturity stage instead of formative phase (60-120 days). Similar observations about superior sugarcane somaclones with thick stalk, increased stalk number and high sugar contents were also reported by Chen *et al.(*1987), Jimenez *et al.* (1991), Naritoom *et al.* (1993), El- Farash *et al.* (1996) and Taghian and Fahmy (1998).

Considering the major parameter SC4, SC7, SC8 of Isd 28, SC1, SC7, SC9 of Isd 35, SC4, SC6 of Isd 36, SC3, SC9 ,SC10 of Isd 37 and SC3, SC7, SC8 of Isd 38 were selected as salinity tolerant which were further tested under induced salinity stress in R2 generation.

References

[1] Ali, M. I.; Imam, S. A. and Ali. M. K. 1989. Ropa Akh Chas (Sugarcane cultivation by Spaced transplanting) in Bangla, Sugarcane Research and Training Institute, Ishurdi, Pabna, Bangladesh.

[2] Begum, M. K.; Alam, M. R.; Islam, M. S. and. Arefin, M. S. 2012. Effect of water stress on physiological characters and juice quality of sugarcane. Sugar Tech., 14(2):161–167.

[3] Bruno, L. 2012. Saline Agriculture in the 21st Century: Using Salt Contaminated Resources to Cope Food Requirements. Journal of Botany, 2012 (2012), Article ID 310705, 7 pages http://dx.doi.org/10.1155/2012/310705

[4] Chen, W.H.; Davey, M.R.; Power, J.B. and Cocking, E. C. 1987. Control and maintenance of plant regeneration in sugarcane callus cultures. J. Exp. Bot., 39:251-251.

[5] El-Farash, E.M.; El-Aref, H.M. and Fahmy, F.G. 1996. Exploring genotypic potential of selected sugarcane somaclones for improve sugar content and yield. Assiut J. Agric. Sci. 27: 225-238.

[6] Jabber, M. A.; Ghafur, M. A.; Begum, M. K. and Arefin, M. S. 2006. Maturity status of new promosing sugarcane genotypes and evaluation for gur quality. Bangladesh J. Sugarcane, 24-27: 41-46.

[7] Jimenez, E.; Perez, P. J.; Martin, D. and Garcia, I. 1991. Study of sugarcane (Sacchaum spp.) populations obtained by in vitro micropropagation. Centro Agricola, 18: 74-78.

[8] Kashem, N.; Ahmed, T.; Jabber, M. A. and Abdullah, M. 2005. Performance of some selected sugarcane varieties in farmers' field condition under Tista meander floodplain soils. Bangladesh J. Sugarcane, 24-27: 98-101.

[9] Killen, T.C. and Andrew, R.H. 1969. Measurement of drought resistance in Corn. Agron. J., 61: 669-672.

[10] Kader, M. A. 2006. Salt Stress in Rice: Adaptive Mechanisms for Cytosolic Sodium Homeostasis. Ph.D Thesis. Dep. Plant Biol. Forest Genet. Swedish University of Agricultural Sciences .Uppsala. Sweden.

[11] Lingle, S.E. and Wiegand, C. L. 1996. Growth and yield responses of sugarcane to saline soil:II. Sucrose biochemistry in individual internodes. Proc. Inter. American Sugarcane Seminars, 93-102.

[12] Maralappanavar, M.S.; Kuruvinashetti, M.S.; and Harti, C.C. 2000. Regeneration, establishment and evaluation of somaclones in Sorghum bicolor (L.) Moench. Euphytica, 115: 173-180.

[13] Mahadevan, A. and Sridhar, R. 1982. Methods in physiological plant pathology, 2nd ed. Sivakami Pub. 316. Indira Nagar. India.

[14] Martinez-Beltran J. and Manzur C.L. 2005. Overview of salinity problems in the world and FAO strategies to address the problem. Proc. Int. Salinity Forum, Riverside, California, April 2005, p. 311–313.

[15] Mohan, M.M.; Naraynan, S.L. and Ibrahim, S.M. 2000. Chlorophyll stability index (CSI): its impact on salt tolerance in rice. Dep. Agric. Bot. Agric.College and Res. Inst., Tamil Nadu Agric. Univ., Madurai, India.

[16] Naritoom, K.; Sooksathan, K.; Korpradittskul, V.; Suksathan, I; Visessuwan, R.; Burikam, S.; Klingkong, S. and Suriyachaiyakorn, M. 1993. Plant regeneration from shoot tip culture of sugarcane. Kasetsart J. Natural Sci., 27: 286-291.

[17] Rahman, M. M.; Podder, B. P.; Kabiraj, R. C.; Alam, K. S.; and Rahman, M. A. 2008. Performance of some released sugarcane varieties under Thakurgaon Sugar Mills Zones. Bangladesh J. Sugarcane, 30: 127-131.

[18] Rosas, H. G., Garcia, S. S., Reyes, G.R., Ontiveros, J. L.R. and Villasenor, A.C.R. (2003). Preliminary results on in vitro selection for tolerance to chloride excess in avocado. Revista Chapingo Serie Hort., 9(1): 39-43.

[19] Samad, M.A., Begum, S., Majid, M.A. (2001). Somaclonal variation and irradiation in sugarcane calli for selection against red rot, water logged conditions and delayed or non-floweringcharacters. IAEA-TECDOC-1227: 45-50.

[20] Spancer, G.L. and Meade, G.P. 1963. Cane sugar hand book. 9th ed. G.P. Meade, John Wiley and Sons, Inc. New York. p. 17.

[21] Subbarao, M. and Shaw, M.A.E. 1985. A review of research on sugarcane soils of Jamaica. Proc. Meeting West Indies Sugar Technol., 2: 343- 355.

[22] Taghian, A. S. 2002. Expression of mutual tolerance to drought and salt stresses of the in vitro selected clones of sugarcane. Proc. 3rd Scient. Conf. Agric. Sci. Fac. of Agric., Assiut. Univ., Assiut, Egypt. 22 October. 2002. pp. 262-441.

[23] Taghian, A.S., and Fahmy, F.G. 1998. Genetical studies on sugarcane plants derived from tissue culture. Assiut J. Agric. Sci., 29, 1: 113-131.

[24] Watson, D.J. 1952. The physiological basis of variation in yield. Adv. Agron., 4: 101-145.

[25] Xing, W. and Rajashekhar, C.B. 2001. Glycine betaine involvement in freezing tolerance and water stress in Arabidopsis thaliana. Environ. Expt. Bot., 46: 21-28.