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Full Length Research Paper

Evaluation of sugarcane (*Saccharum officinarum* L.) somaclonal variants tolerance to salinity *in vitro* and *in vivo* cultures

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Tissue culture technique was used to obtain salt tolerant variants from embryogenic calli of sugarcane (*Saccharum sp.* Var. CP48-103) that were cultured on a selective medium containing different levels of NaCl (0, 33, 66, 99 and 132 mM). A total of four plants which regenerated from the tolerant calli were selected but the best in vigor were grown in *in vitro* and hydroponic systems under salinity stress (with the previous levels) as compared to source variety. With increasing supply of NaCl in both systems, root growth was more adversely affected than shoot growth. Chlorophyll contents showed a decreasing trend and dry matter yield of plants reduced but in a slow rate in tolerant somaclone than source variety. The tissues analysis showed that at high salt concentration, Cl^- and Na^+ content in shoot and root increased. With rising salt concentration from 0 to 132 mM, content of Cl^- in shoot and root of tolerant variant changed and was lower than the parent. In conclusion, this variant probably had lowest genetic ratio of shoot: root chloride due to minimum transport of Cl^- from the root to shoot. Also this variant had high content of Ca^{2+} in shoot and high K^+/Na^+ ratio at all salinity levels. Thus, it probably has genetic potential to avoid harmful ions accumulation.

Key words: Sugarcane, salinity, somaclonal variation, *in vivo*, *in vitro*.

INTRODUCTION

Soil or water salinity is considered to be the major environmental factor limiting plant growth and productivity, especially in arid and semi-arid irrigated regions including Iran. Salinity limits vegetative and reproductive growth of plants by inducing severe physiological dysfunctions and causing widespread direct and indirect harmful effects, even at low salt concentrations (Altman, 2003; Munns, 2002; Al-Maskri et al., 2010). Salt stress has been extensively investigated since soil salinity represents a major constraint for successful production and crop yield (Munns, 2002). The salt-affected lands extend to about 6% of the world surface and are becoming even more prevalent as the intensity of agriculture increases worldwide (Flowers and Yeo, 1995).

Sugarcane (*Saccharum officinarum* L.) is a glycophyte considered as moderately sensitive to salinity stress and a crop of major economical value in tropical and sub-tropical developing countries where salinity is an ever-increasing problem (Wahid et al., 1997), due to it is estimation that about 1 million ha of land under sugarcane cultivation are affected by salinity or sodicity. In Iran, sugarcane is grown under irrigated systems and is seriously prone to soil salinization. This problem may be a serious problem for the production and the yield of this agricultural crop. Sugarcane growth may be suppressed due to the accumulation of toxic ions (Wahid et al., 2009). Salinity in the root zones of sugarcane decreases the sucrose yield, through its effect on both biomass and juice quality. Although, the rate of canopy development and final size are an outcome of leaf and stem extension-growth, it has been shown that leaf injury and loss due to excess salt accumulation might be an important factor controlling the active size of the canopy (Lingle and Weigand, 1996).

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Rozeff (1995) suggested that a steep decline in growth may take place once the ECe rises above 3 dS/m, although, plants may survive up to 10 to 15 dS/m depending on the cultivar. Many elite cultivars used in commercial production in Iran have superior agronomic performance but may have susceptibility to salinity which limits their cultivation. One of such elite cultivar is CP48-103, which is agronomically superior on the clay-loam soils of the Khuzistan province but is susceptible to accumulation of abundant Cl^- in leaves (Soltani et al., 2008).

The complexity and polygenic nature of salinity tolerance has seriously limited the efforts to develop the tolerant crop variety through conventional breeding practices. Somaclonal variation in combination with *in vitro* mutagenesis and selection has been applied for the isolation of agronomically useful mutants (Jain, 2000; Zhambrano et al., 2003). Many examples related to different vegetative propagated species, show that the combination of *in vitro* culture with selection is relatively inexpensive, simple and efficient (Ahloowalia, 1998).

To achieve salt tolerance, plant cells evolve several biochemical and physiological pathways. These processes are thought to operate additively to ensure plants and cells survival and they include the exclusion of Na^+ ions and their compartmentation into vacuoles as well as the accumulation of compatible solutes such as proline, glycinebetaine and polyols (Parida and Das, 2005).

Although, there are numerous reports on selection and physiological characterization of salt-tolerant clones using whole plants in diverse plant species, limited data are available on this in Iran condition, and in sugarcane there has not been concerted effort in this direction. In an earlier report, Patade et al. (2006) studied the effects of salt and drought stresses on irradiated cells of sugarcane and obtained plants tolerant to higher salt stress. Gandonou et al. (2006) studied the effects of salt stress by exposing the callus to a single level of 68 mM NaCl, and observed that physiological and biochemical indicators could play a crucial role in salt tolerance. Radiation induced mutagenesis followed by *in vitro* selection which was employed for salt tolerance in popular Indian sugarcane (Patade et al., 2008). The objective of this study was to evaluate the effects of salinity on some growth attribute and nutrient accumulation of a salt tolerant sugarcane variant at early growth stage, in comparison with the parent variety.

MATERIALS AND METHODS

This study was carried out in the Biotechnology-Tissue Culture Laboratory, Department of Sugarcane Research Center, Karun Agro-industrial Co., Iran. Healthy young leaf explants including apical meristems were obtained from the shoot of commercial sugarcane variety CP48-103. These sections were washed thoroughly under running tap water for 20 min followed by sterilization in a 1.5% NaOCl solution for 20 min, and then washed with sterile distilled water and transferred to laminar air flow cabinet. The explants were cut into thin smaller pieces of 1 to 1.5 cm and prepared for culture.

In vitro performance

Calli were established from the smaller pieces of explants on callus induction, made on Murashige and Skoog (1962) medium, supplemented with 30 g/l sucrose, 8 g/l agar and 3 mg/l 2,4-D. Constituents of all media were products of Sigma Co., USA. The medium was adjusted to pH 5.8 with NaOH (0.1 N), autoclaved at 120°C and 1 bar for 20 min. After 4 weeks, embryogenic calli were separated from the explants and transferred to MS media supplemented with different levels of NaCl (0, 33, 66, 99 and 132 mM) during serial subculture (in a step-wise manner). Cultures were grown in 100 ml glass jars containing 25 ml of culture medium closed with aluminum foil caps. Plantlets were regenerated and then rooted after 3 to 4 weeks of transfer of high healthy callus on regeneration and root medium, that is, MS medium of the same composition as earlier mentioned, but with special hormones (Barba et al., 1977) and none 2,4-D in a growth chamber under long-day conditions (16/8 h light/dark cycle) at a temperature of 25 ± 2°C and relative humidity of 60 to 70%. Light was provided by white fluorescent tubes (60 W, photon flux density 50 $\mu mol/m^2/s^1$). The best and healthy plantlets were selected as tolerant somaclonal variants for the next evaluations.

In vivo performance

Four weeks-old selected variants from tissue culture were used for salinity tolerance evaluation under *in vivo* condition including the $^1/_4$ strength modified Hoagland's solution (Hoagland and Arnon, 1950). So, healthy plant was transferred to dark plastic boxes with 50 × 30 × 20 cm^3 (length × width × depth) specification. Holes were made in the boxes lids used, with 30 x 20 cm spacing to accommodate five plants per box. Only $^2/_3$ of the boxes were filled with the solution to ensure the presence of adequate air inside the box and a special aeration mechanism system was used. Nutrient solutions were renewed every 14 days together with addition of the salinity levels. The solution was tested every week to regulate the pH and EC, and distilled water was added daily to replace transpiration losses. The growth room used for growing plants had approximately twelve and half hours of daylight with mean irradiance value of 800 W/m^2. The mean temperature and relative humidity values were 27.5 ± 3.5°C and 60 ± 5%, respectively.

Morphological and biochemical analysis

Since the morphological features of the somaclonal plants were not sufficient, biochemical analysis were also used as compared to their parental variety. Fully expanded green leaf number (as long as 75% of the leaf was still green, it was considered as a green leaf) and plant height were determined weekly. Transpiration rates of nutrient solution-grown plants were determined every two days by weighing the pots at 2.5-h intervals between 10:00 and 15:00 h, with different weight of pots which include plant with no plant pot as control. The acceptable agreement between the methods (transpiration with a photosynthesis meter, $r^2 = 0.82$), gave confidence in the method.

Plants were sampled when they were 60 and 150 days old for tissue cultures and hydroponics, respectively. Each harvested plant was partitioned into stem, leaf and root for analysis. Morphological aspects, that is, leaf area, dry matter accumulation, dry matter partitioning and total chlorophyll rates were analyzed in these components. Leaf area was measured using a leaf area meter (LI-3050A, LI-COR, USA) in square centimeter. Dry matter accumulation was quantified by obtaining dry weights of plants at 70°C for 48 h in a dry oven. Dry matter partitioning to shoots and roots was

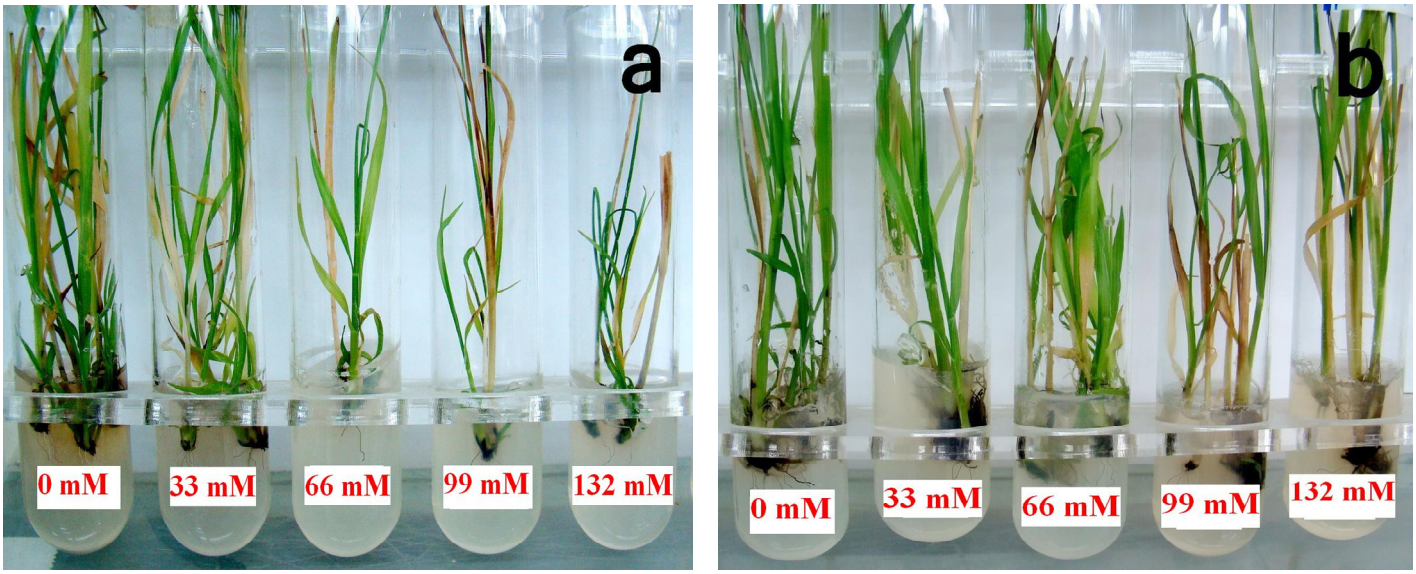

Figure 1. (a) Source variety and (b) its best variant growth response to different salinity levels (mM, NaCl) in, *in vitro* system.

estimated by calculating shoot/root and leaf/stem ratios in dry weight basis. Total chlorophyll rates were measured using the chlorophyll meter (SPAD 502, Minolta, Japan). Tissue chloride content was determined by coulometric–amperometric titration (Soltani et al., 2008) on water extracts of samples taken from dried and ground plant material. The potassium and sodium contents were estimated by flame photometer (Jenway PFP 7, ELE Instrument Co. Ud.) method (Yoshida et al., 1976). Calcium was estimated by Versene titration method as described by Jackson (1973). All measurements were conducted on three replicate plants per each treatment.

Statistical analysis

The experiment was a factorial experiment of two factors, with three replications and arranged in a randomized completely block design. The first factor was one sugarcane variety, CP48-103, and 4 derivation salinity tolerant variants. The second factor was five salinity levels (0, 33, 66, 99 and 132 mM NaCl). The data were subjected to analysis of variance (ANOVA), and comparisons between the mean values of treatments were made by the least significant difference (LSD) test calculated at a confidence level of $P \leq 0.05$ using the statistical software SAS (v. 6).

RESULTS AND DISCUSSION

Numerous works comparing general responses of some plant species with different salinity levels, reported growth reduction under salt stress conditions (Altman, 2003; Barba et al., 1977; Jain, 2000). Under this experiment conditions, contrary to the main variety (CP48-103), root growth of tolerant variant was found not to be reduced significantly by an increase in supply of NaCl than that of shoots. Both root length and mean number of rooted shoots (Figure 1a) decreased with increase in salt concentration in main variety but not in the tolerant variant. These results are in agreement with results obtained previously, which also indicated that roots were among the first plant organs affected by salt stress and the most sensitive ones (Bhatnagar-Mathur et al., 2008).

According to Neumann (1997) report, salinity can rapidly inhibit root growth and hence the capacity for uptake of water and essential mineral nutrients from the soil. In culture conditions, tolerant variant kept normal growth and elevated NaCl concentrations, and showed no inhibitory effect on shoot growth (Figure 1b).

With increasing salt concentrations, total dry weight decreased sharply in main variety than new tolerant variant. In the highest salinity level maximum of total dry weight was 1.9 g/plant in tolerant variant and 1.1 g/plant in source variety in hydroponic system (Table 1). The increase in value of the shoot/root dry weight ratio at high NaCl concentrations indicates that roots were affected positively by salinity than shoots, especially in main variety. Under salinity stress, results showed that total dry matter production highly correlated with K^+/Na^+ ratio ($r = 0.90$ for root and $r = 0.92$ for shoot). It was indicated that higher amounts of Na^+ in plant tissues significantly reduced dry matter production (Figure 2).

All the morphological aspects expect leaf number (Table 1) were significantly higher ($P<0.05$) in the best variant than control under both culture systems at high salinity, 132 mM NaCl. Carbon partitioning depends on the strength of both source and sink. As the leaf provides the platform for photosynthesis, leaf area indicates the strength of the source of a crop. Photosynthesis and dry matter production of a plant is proportional to the amount of leaf area on the plant (Padmathilake et al., 2007). Reductions of chlorophyll content under elevated salinity

Table 1. Means of some determined morphological aspects of *in vitro* and *in vivo* source of sugarcane variety and its best variant grown in response to salinity stress.

Plant type	Salinity {NaCl (mM)}	plantlet height (cm)		Chlorophyll content (mg g^{-1})		Leaf area (cm^2 $plant^{-1}$)		Leaf number (per plant)		Mean leaf dry weight (g $plant^{-1}$)		Total dry weight (g $plant^{-1}$)		Shoot : root ratio		Leaf : stem ratio	Transpiration (l d^{-1} $plant^{-1}$)
		In vitro	*In vivo*	*In vitro*	*In vivo*	*In vitro*	*In Vivo*	*In vitro*	*In vivo*	*In vitro*	*In vivo*	*in vitro*	*in vivo*	*in vitro*	*in vivo*	*in vivo*	*in vivo*
Best Somaclon	0	12.2^{b}	24.4^{ab}	2.3^{a}	2.6^{a}	25.1^{ab}	257.1^{b}	3.8^{a}	7.8^{ab}	0.6^{a}	2.4^{a}	0.9^{a}	3.6^{a}	2.3^{f}	1.2^{d}	3.8^{ef}	0.202^{a}
	33	12.0^{b}	22.4^{b}	2.3^{a}	2.5^{a}	25.0^{b}	246.7^{c}	3.5^{b}	7.3^{c}	0.5^{ab}	2.2^{b}	0.8^{ab}	3.2^{b}	2.3^{f}	1.2^{d}	4.2^{d}	0.186^{a}
	66	11.3^{bc}	20.1^{c}	2.2^{a}	2.4^{ab}	24.5^{b}	236.4^{de}	3.1^{c}	6.7^{e}	0.4^{bc}	1.8^{c}	0.7^{bc}	2.7^{c}	2.4^{f}	1.2^{d}	4.8^{c}	0.140^{b}
	99	9.5^{de}	18.3^{c}	2.0^{b}	2.2^{bc}	24.1^{bc}	229.9^{e}	2.8^{d}	6.2^{f}	0.4^{bc}	1.4^{d}	0.6^{cd}	2.1^{d}	2.5^{f}	1.3^{d}	5.7^{b}	0.113^{c}
	132	8.6^{e}	15.8^{d}	1.7^{c}	2.0^{c}	23.3^{c}	218.5^{f}	2.1^{e}	5.4^{g}	0.3^{cd}	1.1^{e}	0.5^{de}	1.9^{de}	2.5^{f}	1.3^{d}	6.2^{a}	0.058^{e}
Source variety	0	13.5^{a}	24.9^{a}	1.9^{b}	2.2^{bc}	26.5^{a}	266.3^{a}	3.8^{a}	7.9^{a}	0.6^{a}	2.5^{a}	0.8^{ab}	3.5^{ab}	3.1^{e}	1.4^{d}	3.0^{g}	0.201^{a}
	33	10.6^{cd}	18.1^{c}	1.5^{c}	2.0^{c}	23.3^{c}	241.3^{cd}	3.6^{ab}	7.6^{b}	0.4^{bc}	2.1^{b}	0.7^{bc}	2.8^{c}	3.6^{d}	1.7^{c}	3.6^{f}	0.149^{b}
	66	5.3^{f}	10.9^{e}	1.0^{d}	1.3^{d}	19.8^{d}	200.3^{g}	3.5^{b}	7.2^{c}	0.3^{cd}	1.5^{d}	0.5^{de}	2.1^{d}	4.1^{c}	2.0^{b}	4.0^{ed}	0.110^{c}
	99	3.6^{g}	7.7^{f}	0.8^{de}	1.1^{d}	17.1^{e}	186.4^{h}	2.9^{cd}	7.0^{cd}	0.2^{de}	1.0^{e}	0.4^{e}	1.6^{e}	4.8^{b}	2.3^{a}	4.8^{c}	0.076^{d}
	132	2.2^{h}	4.7^{g}	0.7^{e}	0.9^{e}	15.0^{f}	174.5^{i}	2.8^{d}	6.8^{de}	0.1^{e}	0.5^{f}	0.2^{f}	1.1^{f}	5.3^{a}	2.5^{a}	5.4^{b}	0.045^{e}

Means sharing same letter are not significantly ($p > 0.05$) different.

conditions were observed in some salt-sensitive plant species (Munns, 2002). In contrast, chlorophyll content in salt tolerant plants either do not decline or rise with increasing salinity (Patade et al., 2006). Chlorophyll concentration can be used as a sensitive indicator of the cellular metabolic state; thus, its decrease signifies toxicity in tissues due to accumulation of ions (Don et al., 2010).

In our experiment, chlorophyll contents decreased with a slow slope (20%) and rapid slope (59%) with increasing NaCl supply of up to 132 mM in tolerant variant and source variety, respectively.

The rate of salt accumulation in shoots of salt tolerant plants can be determined by the rate of transpiration. Transpiration rate generally tend to decline with increasing rhizospheric salinity in both sensitive and tolerant plants (Michael et al., 1997). It might be due to salt accumulation in the mesophyll which reduced stomatal aperture (Flowers et al., 1995). Our results showed that the salt tolerant variant have been able to transport lesser harmful salt ions (Na^{+} and Cl^{-}) to shoot tissues (Table 2), and then had a higher transpiration than source variety (Table 1).

Sodium and chloride concentration in shoots and roots of sugarcane differently increased with salinity genotypically (Patade et al., 2006). In this study, shoot Na^{+} concentration increased to 0.345% in tolerant variant and to 0.580% of dry weight in source variety with the application of 132 mM NaCl as compared to without salt, cases respectively (Table 2). Similarly, increase was found in root Na^{+} concentration but in high amounts than shoot Na^{+} (Table 3), and increased to 0.655% in tolerant variant and to 1.105% of dry weight in source variety, respectively. It is interesting to note that tolerant variant had lower ratio of shoot to root Na^{+}, absorbed and also transported lower rate of Na^{+} from root to shoot tissues, an important characteristic of salt tolerant genotypes, as compared to parent variety. While the salt levels increased, root and shoot Cl^{-} content also increased in both experimental plants, but the trend was slow and had a low rate in tolerant variant than the its parent. Although, the root/shoot ratio of Cl^{-} content in the source variety was higher than the variant (2.10 against 1.25), the Cl^{-} content in the shoot and root of tolerant variant was lower than parent variety (Tables 2 and 3).

In the absence of stress, K^{+} concentration showed a low significant difference among the two experimental plant types. Although it had low concentration in tolerant variant, but with increased in salinity, it changed adversely and

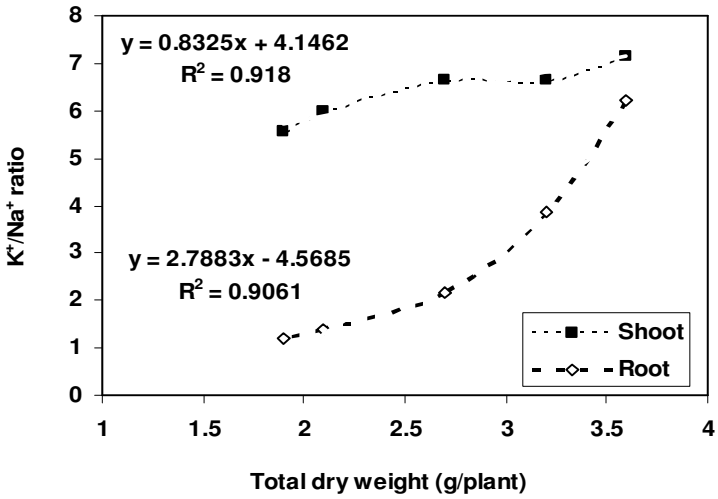

Figure 2. Trend of total dry weight changes with change in K^+/Na^+ content in the shoots and roots of sugarcane salt tolerant variant under increased salinity.

Table 2. Nutrient composition (% of dry weight) of shoot of the source sugarcane variety and its best variant under increased salinity in two culture systems.

Plant type	Salinity (mM, NaCl)	Na^+		Cl^-		K^+		Ca^{2+}	
		In vitro	*In vivo*	*In vitro*	*In vivo*	*In vitro*	*In vivo*	*In vitro*	*In vivo*
Best variant	0	0.24^{c}	0.20^{a}	0.19^{a}	0.18^{a}	1.47^{b}	1.43^{ab}	0.11^{a}	0.12^{c}
	33	0.28^{b}	0.22^{a}	0.20^{a}	0.19^{a}	1.52^{a}	1.46^{a}	0.12^{a}	0.14^{ab}
	66	0.31^{b}	0.24^{b}	0.31^{b}	0.26^{b}	1.53^{a}	1.48^{a}	0.15^{b}	0.15^{a}
	99	0.35^{a}	0.27^{c}	0.43^{c}	0.41^{c}	1.53^{a}	1.47^{a}	0.16^{bc}	0.15^{a}
	132	0.38^{a}	0.31^{c}	0.49^{c}	0.45^{c}	1.53^{a}	1.47^{a}	0.17^{c}	0.14^{ab}
Source variety	0	0.27^{c}	0.24^{a}	0.24^{a}	0.20^{a}	1.52^{a}	1.48^{a}	0.07^{a}	0.09^{c}
	33	0.35^{c}	0.31^{b}	0.29^{a}	0.26^{a}	1.50^{a}	1.46^{a}	0.07^{a}	0.10^{bc}
	66	0.44^{b}	0.39^{c}	0.37^{b}	0.35^{b}	1.41^{ab}	1.37^{b}	0.09^{b}	0.11^{b}
	99	0.56^{a}	0.49^{c}	0.47^{c}	0.40^{b}	1.32^{c}	1.28^{c}	0.10^{b}	0.11^{b}
	132	0.61^{a}	0.55^{d}	0.53^{d}	0.48^{c}	1.27^{d}	1.23^{c}	0.15^{c}	0.13^{a}

Means sharing same letter are not significantly ($p > 0.05$) different.

Table 3. Nutrient composition (% of dry weight) of root of the source sugarcane variety and its best variant under increased salinity in two culture systems.

Plant type	Salinity (mM, NaCl)	Na^+		Cl^-		K^+		Ca^{2+}	
		In vitro	*In vivo*	*In vitro*	*In vivo*	*In vitro*	*In vivo*	*In vitro*	*In vivo*
Best variant	0	0.28^{a}	0.24^{a}	0.16^{a}	0.12^{a}	1.58^{a}	1.49^{a}	0.10^{a}	0.08^{a}
	33	0.34^{a}	0.31^{a}	0.19^{a}	0.16^{a}	1.54^{a}	1.35^{b}	0.10^{a}	0.09^{b}
	66	0.45^{b}	0.41^{b}	0.33^{b}	0.29^{b}	1.02^{b}	1.25^{c}	0.11^{b}	0.11^{c}
	99	0.60^{c}	0.55^{c}	0.53^{c}	0.48^{c}	0.85^{bc}	1.20^{cd}	0.12^{c}	0.11^{c}
	132	0.69^{c}	0.62^{c}	0.62^{c}	0.56^{c}	0.77^{c}	1.20^{cd}	0.11^{b}	0.11^{c}
Source variety	0	0.35^{a}	0.32^{a}	0.20^{a}	0.15^{a}	1.48^{a}	1.41^{a}	0.03^{a}	0.04^{a}
	33	0.67^{b}	0.62^{b}	0.28^{a}	0.24^{a}	1.42^{b}	1.35^{b}	0.04^{a}	0.04^{a}
	66	0.98^{c}	0.95^{c}	0.54^{b}	0.49^{b}	1.36^{c}	1.30^{bc}	0.04^{a}	0.05^{ab}
	99	1.03^{c}	0.95^{c}	0.93^{c}	0.87^{c}	1.30^{d}	1.28^{c}	0.06^{b}	0.06^{bc}
	132	1.15^{d}	1.06^{c}	1.11^{c}	1.04^{c}	1.25^{d}	1.21^{d}	0.09^{c}	0.10^{c}

Means sharing same letter are not significantly ($p > 0.05$) different.

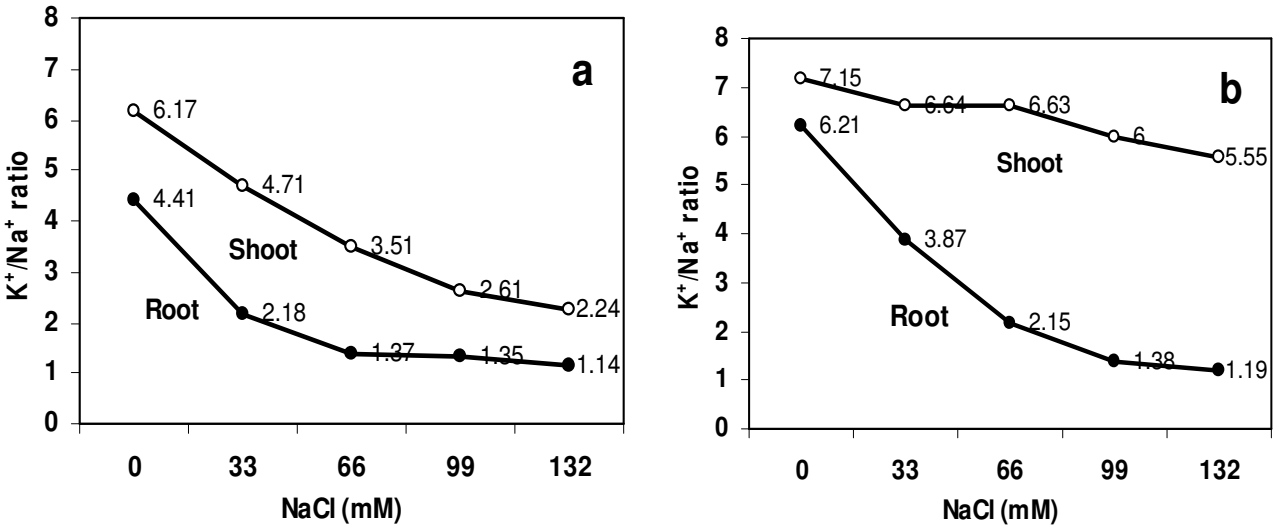

Figure 3. Changes in K^+/Na^+ content in the shoots and roots of (a) source variety and (b) its best sugarcane variant under increased salinity.

sharply in tolerant variant, resulting in a difference in chan-ged K^+/Na^+ ratio (Figure 3), though, this ratio was lower in source variety than tolerant variant, especially in the shoot than in the root tissues. Results showed high correlation between dry weight and K^+/Na^+ ratio of shoot and root at all salinity levels, r = 0.90 for root and r = 0.92 for shoot, respectively (Figure 2). These findings are in contrast with some previous results (Patade et al., 2006; Wahid et al., 1997, 2006) and is in agreement with others (Soltani et al., 2008). The Ca^{2+} analysis (Table 2 and 3) of different plants and organs showed small variation in its contents than other elements. Higher accumulation (0.17%) accumulation of Ca^{2+} in the shoot of the tolerant variant was observed compared to source variety indicating the important role of that accumulation during salt tolerance. The role of Ca^{2+} on signal transduction and in polar growth in plant systems has been well demonstrated (Arzani; 2008).

Salinity still remains the major abiotic stresses that limit and pose a threat to agricultural production in many parts of the world (Altman, 2003; Don et al., 2010). While a number of mechanisms relating to improved stress adaptation in crops have been suggested, the fact remains that their association with genetic gains for yield and their relative importance in different salinity-prone environments are still only partially defined. Therefore, a well-focused approach combining the molecular, physiological and metabolic aspect of abiotic stress tolerance is required (Bhatnagar-Mathur et al., 2008). Subclonal variations play an important role in sugarcane varietal improvement. It is proven that some tissue culture variants are superior than the donor clones in terms of higher biomass, sugar yield and disease resistance (Rajeswari et al., 2009). Plant tissue culture is recognized as an important tool to generate useful genetic variability for crop improvement. Large differences in the salt tolerance of germplasm of a number of crops have been reported (Arzani, 2008; Wahid et al., 2009) but new genetic variability induced by *in vitro* culture was first reported in sugarcane (Rajeswari et al., 2009). In this study, the somaclonal showed significant differences for various characters with its parent. The statistical analysis of the data from this study showed that the changes are genetic. Thus, tissue culture system can be applied in sugarcane breeding programs as a complimentary system for the development of subclones for commercial purpose, parental lines and energy cane. Similarly, the results of the tissue culture with hydroponic techniques showed that hydroponic should be useful for initial screening of the many commercial and new sugarcane variants before final field testing and release of new variety.

This study highlights the importance of the effects of both ionic and physiological component of the salt stress on sugarcane. The results made us to suggest that the physiological mechanisms that mediate the response to salt stress are different. We also provided evidence that the growth inhibition is mainly due to the build up of Na^+ and Cl^- ions in the activated tissues under salt stress as mentioned by El Yacoubi et al., (2010). Moreover, we demonstrated that the ion status is closely related to the nature of the stress factor applied in the medium. We revealed that stress resistance in sugarcane somaclonal variants is closely related to the retention of a high amount of K^+ and Ca^{2+} and a low level of Na^+ and Cl^-.

Conclusion

This study demonstrates that *in vitro* selection techniques

can be used to generate salt-tolerant plant lines in sugarcane and also to study physiological and biochemical indicators of salinity tolerance in this plant. Salt tolerance seems to be related to the efficiency of a tissue to absorb, deposit and transport the levels of inorganic solutes in response to salt stress. The results indicated that some mineral solutes, that is, K^+ and Ca^{2+} have a positive role to play in the tolerance of salinity by the generated plant.

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