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Analysis of toxic and osmotic effects of sodium chloride on leaf growth and economic yield of sugarcane

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Abstract. Sugarcane (*Saccharum officinarum* L.) an important sugar crop, shows high sensitivity to salinity at various growth stages. This study was conducted to determine the comparative response of a salt-tolerant (CP-4333) and a salt-sensitive (CP-71-3002) sugarcane clone to the toxic or osmotic effects of sodium chloride at two growth stages. Clones indicated significant differences in terms of reductions in dry weight and area of leaves under salinity at the grand growth stage. Leaf dry weight was more affected than leaf area, resulting in reduced specific leaf weight. The tolerant clone produced supplementary tillers in greater number that appeared to offload the ion excess. Clones indicated significant difference with regard to increase in leaf Na^+ and Cl^- and decrease in K^+, but no difference in K^+: Na^+ ratio. Na^+ and Cl^- were negatively correlated while K^+ was positively correlated with leaf growth parameters, indicating an adverse effect of Na^+ and Cl^- and importance of K^+ to salt tolerance. The tolerant clone displayed higher water content, water, and turgor potentials of leaf than sensitive clone, but the osmotic potential did not significantly change. Soluble sugars of tolerant clone indicated a ~2 fold increase over control, indicating their osmo-protective role. Free proline accumulation was more specific to the sensitive clone and was correlated with Na^+ and Cl^- showing its synthesis due to ion-toxicity. At maturity, increased salinity reduced the millable cane yield, extractable juice and juice-brix percentage, but increased juice osmolality. All these parameters were negatively correlated with EC, Na^+, and Cl^- and displayed their specific effect on the sugar levels in the internodes. In summary, ion-toxicity was the main determinant of salt tolerance at the grand growth stage while the osmotic component of NaCl mainly appeared to affect the transport of sucrose to stalks, followed by stimulated sucrolytic activity in the internodes, resulting in reduced final cane yield.

Key words: Brix; Internodes; Invertase; Ion-toxicity; K^+; Specific leaf weight; Sugars; Tillering; Turgor.

Introduction

A soil is considered saline or salt-affected when the electrical conductivity of extract from water-saturated, root-associated soil exceeds 4 dS m^{-1}. Salt affected land on earth comprises 19% out of 2.8 billion hectares of arable land (Szabolics, 1989). The main reason for this in irrigated areas is the build-up of excess ions in the upper soil profiles while in non-irrigated areas the culprit is a low precipitation/evaporation ratio. The build-up of toxic ions in the rhizosphere initially causes injury to plant roots, and then their gradual accumulation in the aerial parts causes heavy damage to plant metabolism, reducing growth and yield. Injury to leaves due to excess ion-accumulation might be an important factor controlling the active size of the canopy (Francois and Maas, 1999). Increased salinity has an inverse relationship with stomatal conductance and net photosynthetic rate (Curtis and Läuchli, 1986; Lopez et al., 2002), leading to reduced photo-assimilation and dry matter production (Rozeff, 1995; Lingle and Weigand, 1997).

Salt tolerant plants adopt many strategies that range from morpho-anatomical to physiological and biochemical in nature (Cheeseman, 1988; Zhu, 2001). The physiological ones include the exclusion of ions into physiologically less active parts (Schachtman and Munns, 1992), better selectivity of K^+ over Na^+ (Wilson et al., 2000), and synthesis of compatible osmotica for osmo-protection (Sakemoto and Murata, 2002). Plant tolerance to salinity may be more related to the Na^+:K^+ ratio in the cell than the absolute Na^+ concentration (Benzyl and Reuveni, 1994; Qian et al., 2001). The salinity resistance in maize has been mainly related to greater flux and cytoplasmic K^+ concentration (Hajibagheri et al., 1989). Reduction in water uptake by the root and hampered cell-water relations are both due to the osmotic component of salinity (Cheeseman, 1988; Wahid et al., 1999a). Tolerant plants adjust osmotically by the synthesis of highly water soluble compatible osmotica (e.g. glycinebetaine, free proline, and low molecular weight sugars) and maintain turgor. Among these, free proline ameliorates salt-induced oxidative damage to membranes (Jain et al., 2001), and glycinebetaine buffers the cellular redox potential (Hare et al., 1998), maintains Na^+ balance between the cytoplasm and the vacuole (Subbarao et al., 2001), and protects cytoplasmic membranes during salt stress (Hare et al., 1998; Rehman et al., 2002; Sakamoto and Murata, 2002). Likewise, both reducing and non-reducing sugars contribute to turgor maintenance under salt or water stress (Cheeseman, 1988; Garg et al., 2002).

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Sugarcane (*Saccharum officinarum* L.) is widely grown in tropical and subtropical areas and is mostly irrigated with subsoil or canal water. It is ranked moderately sensitive to salinity with a threshold value of 1.4 dS m^{-1} (Maas, 1986). Salinity has a greater effect on the gas exchange parameters of sugarcane (Plaut et al., 2000). A reduction in the elongation and expansion of sugarcane leaves under salinity has been attributed to a lowered efficiency of growing tissues to utilize sugars for growth (Kumar et al., 1994). A majority of plants divert normal metabolic pathways and increasingly synthesize the compatible solutes to mitigate the adverse effects of salinity (Hare et al., 1998). This is true for sugarcane too, as the synthesis of compatible solutes, including 3-dimethylsulfoniopropionate and free proline, has been reported in sugarcane leaves under osmotic stress (More et al., 1994; Paquet et al., 1994). However, more work is imperative on this aspect of sugarcane.

Sugarcane shows a steep decline in growth and productivity with a progression in root zone salinity although large genotypic differences occur (Rozeff, 1998; Wahid et al., 1997; Nasir et al., 2000; Akhtar et al., 2003). Rozeff (1995) reported a 50% reduction in the sprouting of sugarcane at 13.3 dS m^{-1} while the same reduction of yield occurred at 9.5 dS m^{-1} level of salinity. An excess of ions adversely affected the elongation and differentiation of cane stalk internodes and storage of sucrose therein (Akhtar et al., 2001). Lingle and Weigand (1997) noticed an increase in the juice osmolality and a decrease in total soluble solids and sucrose per unit increase in salinity. This may be due to a salt-induced stimulation of the sucrolytic activities of acid and neutral invertases (Balibrea et al., 2000; Tazuke and Wada, 2002). Furthermore, the older stalk sections had a higher content of juice-Na^+, little or no change in Cl^-, and lower content of K^+ than the younger ones (Lingle et al., 2000).

As a C_4 plant, sugarcane has a higher water and temperature optima for growth. According to irrigation schemes, sugarcane needs to be watered at frequent (15 d) intervals for optimum growth (Qureshi et al., 2002). With irrigation water, in marginally or moderately saline fields, an excess of soluble salts are inevitably taken up, which accumulate in the aerial parts and reduce growth and yield (Meinzer et al., 1994; Akhtar et al., 2003). Reduction in sugarcane growth might either be due to additive or individualistic effects of osmotic and toxic components of salinity. However, which of the two is more causative is not yet clear. It is surmised that both osmotic and toxic components of salinity differently affect the growth and yield of sugarcane at different growth stages. This hypothesis was tested based on changes in the growth, ions, and osmolytes in leaves at the grand growth stage, with cane yield and sugar characteristics measured at the maturity stages in this crop of commercial value.

Materials and Methods

Plant Material and Experimental Details

Sugarcane clones CP-4333 and CP-71-3002 were previously tested and declared tolerant and sensitive to NaCl salinity with average salt tolerance limits (EC_{50}) of 15.5 and 8.6 dS m^{-1}, respectively (Wahid et al., 1997). Stalk nodes (50 in number) of the clones were sown in tanks built in greenhouse measuring 2.35 m (long)×1.50 m (wide)×0.45 m (deep), lined with a double layer of polyethylene sheet and filled with soil. Experimental design was randomized complete block. Each treatment was replicated three times in each block. After sprouting, the clones were allowed to grow up to the onset of the grand growth stage (180 days after planting). A salt (NaCl) solution was gradually added to soil at this time to achieve 80, 120 and 160 mmol L^{-1} levels in four days, in addition to a control (no salt added). Physico-chemical characteristics of (a) soil were: organic matter 1.05%, total N 0.72%, cation exchange capacity 16.7 meq 100 g^{-1}, pH 7.3 and (b) soil extract were: ECe 2 dS m^{-1}, SAR 0.17, Na^+ 52 mg kg^{-1}, K^+ 430 mg kg^{-1}, Cl^- 223 mg kg^{-1}, SO_4^{2-} 49 mg kg^{-1}, Ca+Mg 935 mg kg^{-1}, NH_4^+ 7.1 mg kg^{-1}, NO_3^- 14.8 mg kg^{-1}, as determined by standard methods of soil analysis (Black, 1965). Tanks were irrigated with subsoil at 12- to 15-day intervals, having the following characteristics: ECw 0.6 dS m^{-1}, SAR 4.9, Na^+ 13 mg L^{-1}, K^+ 8 mg L^{-1}, Cl^- 271 mg L^{-1}, and SO_4^{2-}, CO_3^{2-} and HCO_3^- in traces. ECe level of the soil was measured at the end of experiment, and it had slightly increased.

Growth, Water and Ionic Relations

All measurements were taken on leaf lamina 60 d after the induction of salinity treatments. Leaf area was measured of intact plants as leaf length × leaf width × 0.68 (a factor derived from the slope of correlation between measured leaf area and the product of length × width of leaf). A leaf was considered green showing <20% symptoms of salt damage in the form of tip burning and chlorosis, as described elsewhere (Wahid et al., 1999b). Fresh weight was taken immediately after removing the green leaves, and dry weight was taken after drying them at 70°C for five days. Percent leaf water content was measured as: [(fresh weight-dry weight) × 100]/fresh weight. Specific leaf weight was computed as leaf dry weight/leaf area (Hunt, 1982). The water potential of three leaves below the top visible dewlap (TVD) leaf was measured using a pressure chamber (Scholander Bomb, Germany), and the values were averaged. For the measurement of osmotic potential, the fresh excised leaf samples were quickly frozen on dry ice, thawed, sap expressed, and collected in a microfuge tube. The sap was centrifuged at 1.2×10^4 ×g for 5 min in order to pellet the suspended materials and determined for osmotic potential using a vapor pressure osmometer (Model 5100 C, Wescor Inc., Utah). The turgor potential was determined from the difference of water and osmotic potentials. For the determination of mineral ions from leaves, the dried powdered material (1.0 g) was digested in an HNO_3:$HClO_4$ mixture (3:1 ratio) at 280°C for 2 h, cooled, and made up to known volume using deionized water (Yoshida et al., 1976). Na^+ and K^+ were determined on flame-photometer (Sherwood Model 410, Cambridge, UK). A portion of the material (1.0 g) from above was boiled

in deionized water for 2 h, filtered hot, and made up to known volume using deionized water to estimate Cl^- using chloride meter (Model VC-HI, Central Kagaku, Japan).

Free proline was determined from the fresh leaves extracted with sulphosalicylic acid and the extracts reacted with acid-ninhydrin, according to the method of Bates et al. (1973). To determine water soluble sugars from leaf blades, 0.5 g of tissue was excised, cut into small pieces, and vigorously shaken with 20 ml of 80% ethanol in a water bath at 70°C for 2 h. Extract was centrifuged at 1×10^3 ×g for 5 min, reduced to a final 5 ml volume under vacuum at 55°C, and stored at -20°C until analyzed. After thawing, an aliquot was colorimetrically determined for sugars by reaction with anthrone reagent (Yoshida et al., 1976).

Stalk Yield and Juice Analysis

At maturity (330 d old crop and grown under salinity for 150 days), fresh cane stalk weight per plant was determined after stripping and removing the tops. Cane stalk was passed three times through a cane-crusher to recover the maximum amount of juice, which was then filtered (through 200 µm sieve). A portion of it was immediately frozen for sugar analysis while the remainder was used for the rest of the analyses. Brix percentage (reflecting apparent sucrose in the juice) was determined by Brix Refractometer (Epic Inc. New York). To determine the osmolality, 1 ml of juice was centrifuged at 0.8×10^4 ×g to pellet the suspended materials and osmotic potential of supernatant was measured with vapor pressure osmometer (Model 5100C, Wescor Inc. Utah). EC of the non-frozen portion of juice was immediately determined using conductivity meter (Model PTI-118, UK). For the analysis of mineral ions, the juice was reduced to dryness under vacuum at 55°C on a rotary evaporator, digested in acid mixture, and determined for mineral nutrients as described above. For the determination of total sugars, 2 ml of the frozen juice was diluted to 5 ml, and rest of the procedure was the same as described above.

Results

Growth and Ionic Characteristics

Sugarcane clones indicated significant ($p<0.01$) differences for the reduction in dry weight, number, and area of leaves and tillering capacity per plant, with significant ($p<0.01$) interaction of salinity and clones. Although NaCl salinity reduced these parameters in both the clones, CP-4333 greatly excelled CP-71-3002 (Figure 1). Salinity had a more pronounced effect on dry weight than leaf area, which was evident from a significantly ($p<0.01$) more decreased specific leaf weight especially of CP-71-3002.

Both the clones indicated a significant ($p<0.01$) difference for the accumulation of leaf-Na^+ and Cl^- with increased salinity but K^+ content indicated a concomitant decrease showing a significant ($p<0.01$) interaction of the clones with salinity (Table 1). Applied salinity reduced the K^+:Na^+ ratio, but there was no significant ($p>0.05$) difference between the clones. Although Na^+ and Cl^- were detrimental to the growth of clones, the difference between the clones was evident. CP-4333 accumulated markedly less of both Na^+ and Cl^- and more of K^+ than CP-71-3002. The trend of Na^+ and Cl^- accumulation with growth parameters, however, was negative (Table 2). This carried greater physiological importance in terms of substantial difference between the clones for the excess of Na^+ and Cl^- on the dry weight, area, and specific weight of leaves. However, a positive correlation of K^+ with these attributes suggested the importance of K^+ to growth in terms of more leaves for the interception of light for photosynthesis as was apparent from the substantially higher leaf dry weight and leaf area of CP-4333 (Figure 1).

Water Relations and Osmolytes Accumulation

Both clones differed significantly ($p<0.05$) in leaf water status (Figure 2). Although applied salinity reduced water content and water potential of leaves, a significant ($p<0.01$) difference was discernible between the clones.

Table 1. Changes in mineral ions of differentially salt tolerant sugarcane clones under NaCl salinity.

Clones	NaCl (mmol L^{-1})	Leaf Na^+ (mg g^{-1} dry weight)	Leaf K^+ (mg g^{-1} dry weight)	Leaf K^+:Na^+	Leaf Cl^- (mg g^{-1} dry weight)
CP-4333	Control	16.43±0.65	240.67±46.13	14.67±2.93	36.33± 2.40
	80	42.00±6.71	224.43±34.43	5.40±0.89	68.40±10.53
	120	61.53±6.38	203.07±28.01	3.30±0.34	81.10± 9.08
	160	70.33±9.21	180.67±16.67	2.59±0.27	94.50±11.10
CP-71-3002	Control	13.40±2.80	237.57±28.37	18.46±5.27	37.20±5.50
	80	48.70±5.77	185.90±19.91	3.86±0.78	79.40±8.29
	120	76.27±5.85	159.93±16.66	2.12±0.38	98.83±7.09
	160	88.33±8.05	137.30± 8.52	1.57±0.32	117.90±7.96
Standard Error					
Clones (C)		12.79**	19.08**	2.15ns	4.90**
Salinity (S)		18.09**	271.93**	3.04**	6.93**
C×S		25.59**	38.15**	4.30ns	9.81**

Significant at **, 1% levels of probability. ns, non-significant. Mean ± standard deviation.

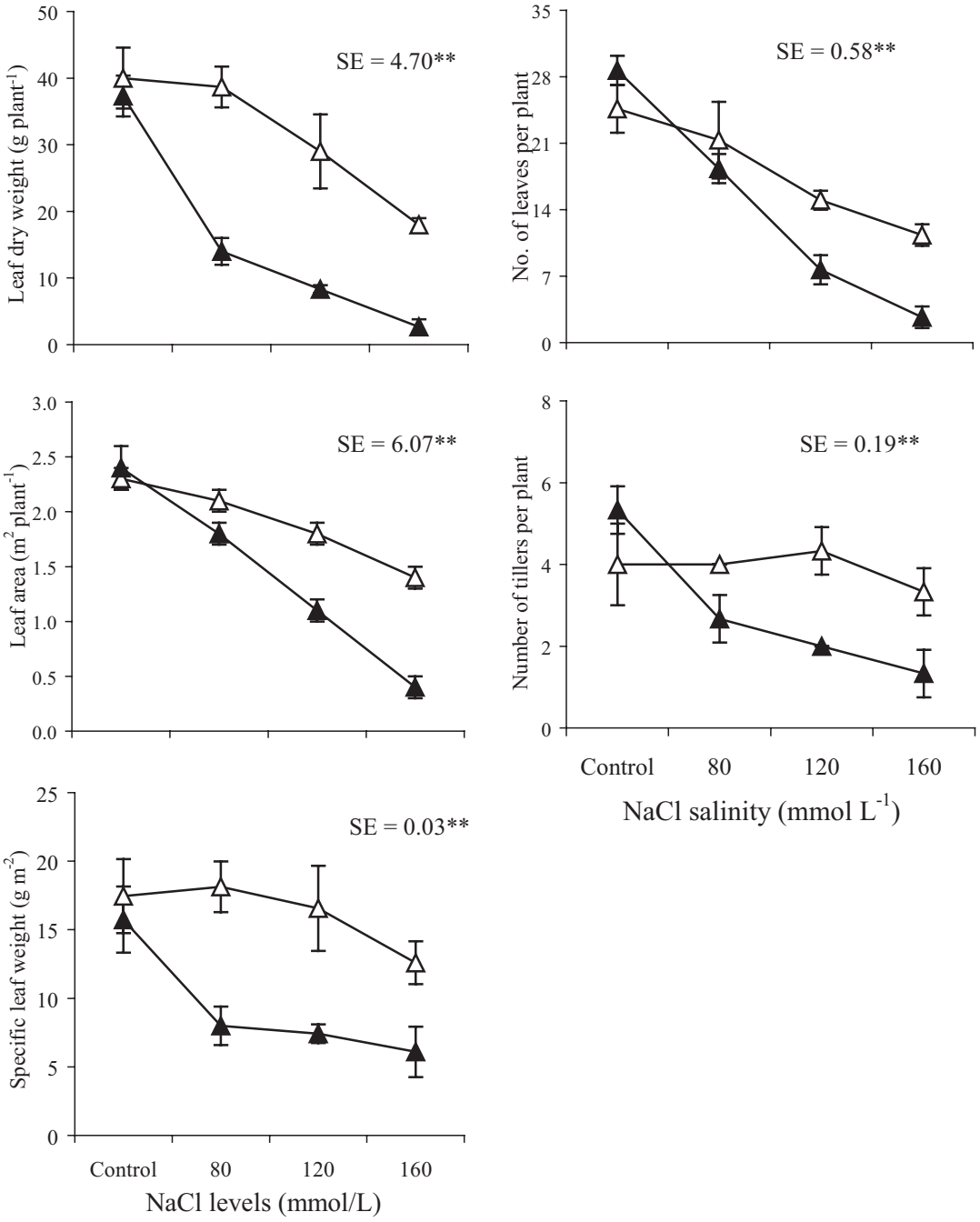

Figure 1. Changes in some growth characteristics of differentially salt tolerant sugarcane clones under NaCl salinity. In this and subsequent figures, open symbols for CP-4333 and shaded for CP-71-3002. Values are means ± standard deviation. Vertical bars denote standard error of means. SE is the standard error of clones × salinity interaction. Interaction is significant at *, $p<0.05$; **, $p<0.01$ and ns, non-significant.

Table 2. Correlation co-efficient (r) between growth characteristics and leaf-ion content leaves of sugarcane clones (n = 8).

Growth parameters	Na^+	K^+	$Na^+:K^+$	Cl^-
Leaf dry weight	-0.873**	0.977**	0.735*	-0.893**
Number of leaves per plant	-0.983**	0.971**	0.754*	-0.969**
Leaf area per plant	-0.937**	0.971**	0.704ns	-0.959**
Specific leaf weight	-0.717*	0.836**	0.573ns	-0.756*

Significant at *, $p<0.05$; **, $p<0.01$. ns, non-significant. Mean ± standard deviation.

CP-4333 maintained greater relative water content and higher water potential. Although the clones did not differ (P> 0.05) in leaf osmotic potential, CP-4333 exhibited a steady leaf turgor and better osmotic adjustment.

There was a significant ($p<0.01$) difference in the clones with regard to accumulation of free proline and soluble sugars. However, an interaction of salinity and clones was noted for free proline, but not for soluble sugars. Soluble sugars indicated a ~2 fold increase in CP-4333 at highest salt level over controls (Figure 3), confirming their greater role in the salt tolerance of CP-4333. No correlation of soluble sugars appeared with any of the ions (data not given). However, free proline accumulated in greater amounts in CP-71-3002 (sensitive clone) and had a positive correlation with Na^+ (r = -0.769; n=8) and Cl (r =-0.719; n=8), suggesting that its accumulation was due to the toxicity of these ions.

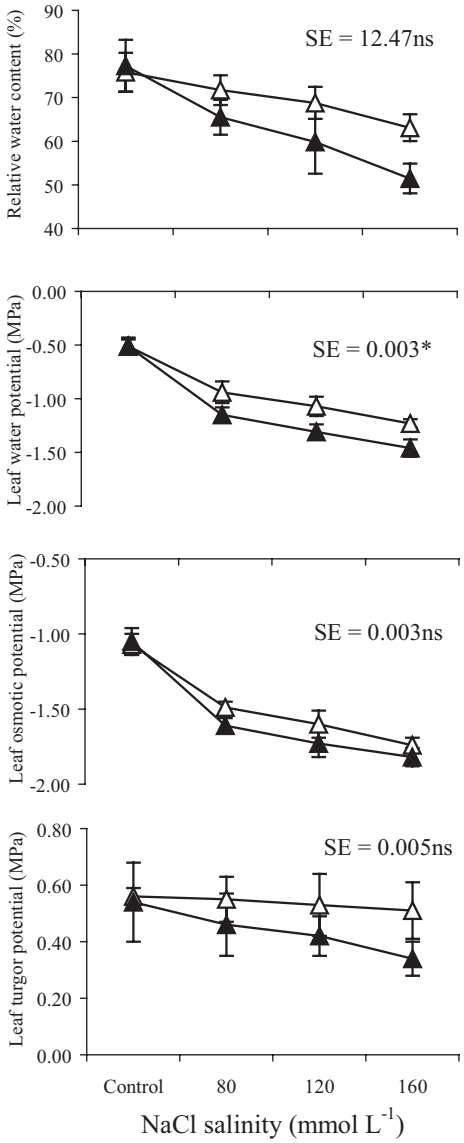

Figure 2. Changes in water relations characteristics of differentially salt tolerant sugarcane clones under NaCl salinity. For details see Figure 1.

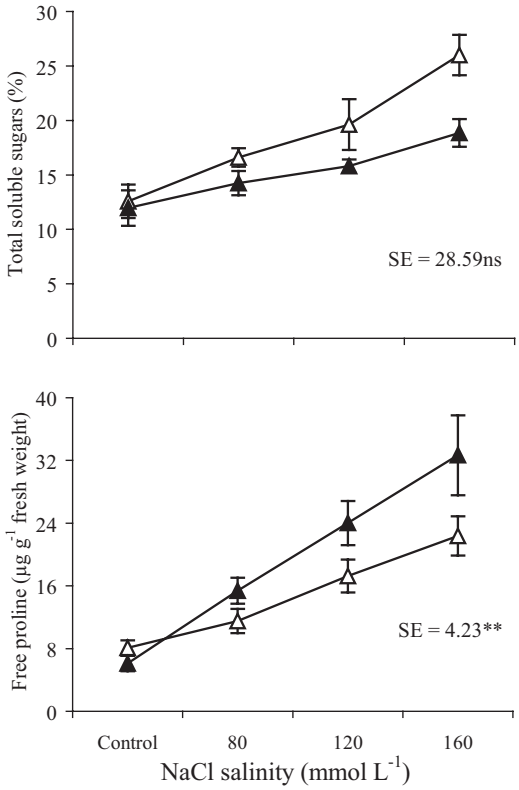

Figure 3. Changes in soluble sugars and free proline content of differentially salt tolerant sugarcane clones under NaCl salinity. For details see Figure 1.

Cane Yield and Juice Characteristics

Although CP-71-3002 had a greater stripped cane yield than CP-4333 under non-saline condition, it suffered a substantial loss due to salinity (Table 3). The most significant ($p<0.01$) effect of NaCl was on the quantity of extractable juice. The lowest recovery and most viscous juice occurred at 160 mmol L^{-1} in CP-71-3002, indicating a more reduced water uptake by this clone. This was further evident from the increased juice osmolality having a direct correlation with EC, Na^+, Cl^- but an inverse correlation with juice-K^+ (Table 4). EC of juice increased with a significant ($p<0.05$) difference between the clones. Juice-Na^+ was considerably ($p<0.01$) greater in CP-71-3002 than CP-4333, but juice-Cl^- was similar ($p>0.05$). The K^+ and K^+:Na^+ ratio was slightly higher ($p<0.05$) in the juice of CP-4333 than CP-71-3002. Both clones exhibited differential capability to synthesize total sugars ($p<0.01$) although salinity had no effect on their accumulation. Contrarily, brix percentage was significantly ($p<0.01$) higher in the juice of CP-4333 than CP-71-3002 (Table 3). Correlations between sugar relations and juice ions revealed that increased EC, Na^+ and Cl^- content of juice had a negative relationship with the extractable juice and brix percentage (Table 4). Juice K^+, on the other hand, indicated a strong positive correlation and apparently a profound effect on the extractable juice and brix percentage of the clones.

Table 3. Stripped cane yield, extractable juice, and juice characteristics of differentially salt tolerant sugarcane clones under NaCl salinity.

Clones	NaCl (dS/m)	Cane stalk yield (kg/plant)	Extractable juice (l/plant)	EC (dS/m)	Osmolality (mOsmol/kg)	Na (mmol/L)	K (mmol/L)	K/Na ratio	Cl (mmol/L)	Total sugars (%)	Brix (%)
CP-4333	Control	4.80±1.54	1.52±0.09	4.93±0.35	554.3±21.1	10.13±0.64	134.0±10.2	13.22±1.54	37.43±1.75	35.0±1.64	13.4±1.46
	80	4.95±0.32	1.52±0.06	5.80±0.56	634.0±29.5	20.37±1.86	117.9± 6.9	5.81±0.43	48.93±3.66	34.0±2.26	11.8±0.47
	120	4.71±0.65	1.21±0.12	7.33±0.40	676.7±35.1	26.50±2.29	99.7±10.0	3.77±0.34	66.80±3.90	36.9±2.05	10.6±1.42
	160	2.96±0.28	0.90±0.10	8.73±0.93	744.3±35.9	29.23±2.53	93.5±5.8	3.15±0.37	76.27±5.20	37.0±1.35	9.96±0.45
CP-71-3002	Control	5.79±0.64	1.70±0.15	5.33±0.25	511.3±20.7	9.77±1.66	139.4±10.1	14.57±1.68	34.77±2.80	37.2±2.18	13.9±1.34
	80	2.67±0.54	1.12±0.10	6.60±0.36	648.7±43.5	26.90±1.44	93.3±5.9	3.47±0.36	56.40±2.62	40.7±1.91	9.9±0.70
	120	1.43±0.22	0.62±0.06	8.43±0.82	760.7±36.4	33.00±1.59	82.6±2.6	2.51±0.25	76.47±6.81	41.5±2.30	8.5±0.66
	160	0.69±0.15	0.27±0.09	9.80±0.7	845.7±17.0	40.57±2.42	79.3±5.4	2.03±0.18	89.47±5.12	40.7±3.33	7.2±0.45
Standard Error											
Clones (C)		0.10**	0.70**	0.09**	250.52**	2.66**	5.31**	0.29*	3.67**	0.49**	0.26**
Salinity (S)		0.15**	0.99**	0.13**	354.29**	3.77**	7.51**	0.40**	5.20**	0.70ns	0.36**
C×S		0.21**	1.40**	0.18**	501.04**	5.33*	10.62**	0.57*	7.35*	0.99ns	0.51**

Significant at *, 5% and **, 1% levels of probability. ns, non-significant. Mean ± standard deviation.

Discussion

This study revealed a significant difference between the clones in terms of reduction in growth and yield characteristics, and these observations are consistent with our previous finding (Wahid et al., 1997). Better growth and yield of CP-4333 lied in the higher number and area of leaves together with effective regulation of ions in the leaves (Figure 1). Specific leaf weight, a determinant of dry matter accumulation (Hunt, 1982), was affected more in the sensitive clone due to its high sensitivity to Na^+ and Cl^- for dry weight rather than leaf expansion (Plaut et al., 2000). Greater reductions in these parameters together with increased signs of salt damage have been regarded as salt sensitivity criteria for many crops (Lutts et al., 1996; Wahid et al., 1999b; Plaut et al., 2000). The symptoms of salt injury were noted to a lesser extent, particularly in the younger leaves of CP-4333 (data not shown). Enhanced production of supplementary tillers was greatly advantageous to salt tolerant clone in displaying greater photosynthetic area and dry matter yield (Wahid et al., 1997; Grattan et al., 2002).

Changes in ionic status of green leaves are crucial to adjudge the sustenance of the active photosynthetic canopy in giving higher dry matter yield (Francois and Maas, 1999). The increased content of Na^+ has been reported to suppress the leaf gas exchange and PSII photochemical activity (Dionisio-Sese and Tobita, 2000). In this study, the accumulation of Na^+ and Cl^- and reduction in K^+ took place, but with a substantial difference between the clones (Table 1). These findings strongly suggest that lowered content of Na^+ and Cl^- in the leaves of CP-4333 is crucial for better growth and tillering, which was hardly displayed by CP-71-3002.

In contrast to some earlier reports (Wilson et al., 2000; Qian et al., 2001), sugarcane clones did not show difference in terms of the K^+:Na^+ ratio (Table 1), thus making it hard to declare it a salt tolerance criterion for this crop. Lingle et al. (2000) reported such a trend in the cane stalk stressed with saline irrigation water. This view was further supported by a strong relationship of K^+, but not of the K^+:Na^+ ratio, with leaf growth parameters (Table 2). Therefore, absolute rather than relative content of K^+ seems to be a plausible strategy of evaluating salt tolerance in sugarcane as it has crucial roles in osmotic and stomatal regulation and enzymatic reactions (Shrivastava et al., 1997; Gratten and Grieve, 1999; Dionisio-Sese and Tobita, 2000; Lopez et al., 2002).

Osmotic adjustment is the improvement in cell water balance due to the accumulation of inorganic and organic osmolytes. Enhanced production and retention of non-toxic compatible osmolytes is a strategy of tolerant plants in countering the damaging effects of salinity. They play numerous roles together with improved cell water balance (cf. Introduction section). Both the sugarcane clones indicated remarkable differences for relative leaf water content, leaf water, and turgor potentials, which were substantially greater in CP-4333 (Figure 2). However, there was no dif-

Table 4. Correlation co-efficient (r) between juice-EC, juice mineral ions and quantity of extractable juice total sugars and brix percentage (n = 8).

Juice characteristics	Juice-EC	Na^+	K^+	Cl^-
Quantity of extractable juice	-0.950**	-0.928**	0.916**	-0.984**
Juice osmolality	0.967**	0.951**	-0.935**	0.986**
Total sugars	-0.699ns	0.684ns	0.715*	-0.661ns
Brix percentage	-0.889**	-0.984**	0.979**	-0.914**

Significant at *, $p<0.05$; **, $p<0.01$. ns, non-significant. Mean ± standard deviation.

ference in the leaf osmotic potential between the clones, and the reasons for this are different. The tolerant clone accumulated considerably higher K^+ (Table 1) and had a ~2 fold greater accumulation of soluble sugars (compared to control), but relatively low free proline content at the highest salinity level (Figure 3). All these osmolytes improved cell water balance and enabled this clone to adjust osmotically (Figure 2, 3). The sensitive clone, on the other hand, had much reduced water content and very low turgor potential although it also had a higher free proline content. As is revealed from the data, this clone accumulated soluble sugars and K^+ in amounts inappropriate to generate turgor and thus showed reduction in osmotic potential. Free proline accumulation held significant correlations with Na^+ and Cl^- (cf. Results section), and its production was more specific to the sensitive clone (Figure 3). This suggested that its production was because of salt damage to the cell cytoplasm. This corroborates with what has been reported for soybean (Moftah and Michel, 1987), rice (Lin and Kao, 1996), sorghum (Wahid et al., 1998), and Kentucky bluegrass cultivars (Qian et al., 2001). A hampered leaf water status of the CP-71-3002 was further attributed to a reduced water absorption by a weak root system as reported earlier for this clone (Wahid et al., 1997), followed by its reduced transport to the aerial parts due to the osmotic effect of salinity.

High net leaf carbon assimilation rate followed by rapid translocation of synthesized sucrose to the internodes determines the final yield in sugarcane (Lingle, 1999). Applied salinity has a well-pronounced effect on the biosynthesis of sucrose in the leaf and its translocation to stalk for storage (Lingle and Weigand, 1997; Akhtar et al., 2001). Salinity modulates the activities of sugar metabolizing enzymes in a number of crops. It reduced activities of sucrose synthase and starch phosphorylase and enhanced those of acid and neutral invertases (Krishnamurthy and Bhagwat, 1995; Dubey and Singh, 1999; Tazuke and Wada, 2002). Since phloem loading from source tissue and translocation of photo-assimilates to sink tissue (in addition to other factors) is affected by the sub-optimal availability of water in the medium, the osmotic effect of salinity becomes crucial to this process. This has been evidenced by an increased extracted-juice osmolality (Table 3). Subsequent to translocation of sugars in the internodes, the excess of ions may stimulate the activity of invertases, which tends to reduce sucrose yield by sucrolytic activity (Balibrea et al., 2000; Tazuke and Wada, 2002). As noted here, juice EC, Na^+ and Cl^- content increased, which had a strong negative trend with extractable juice and brix percentage, as a measure of sucrose concentration (Table 4). It is believed that excess Na^+ and Cl^- in the internodes stimulated the sucrolytic action of invertase, leading to a decreased brix percentage. Although K^+ had a positive correlation with extractable juice and brix percentage, its effect appeared to be masked by ion-specific action of Na^+ in the internodes.

To conclude, the above-ground growth of sugarcane was largely affected by NaCl-toxicity, and the effect of Cl^- appeared to be more than that of Na^+. Soluble sugars and K^+ appeared to lessen the adversaries of ions on tolerant clone by improving the cell water balance to a great extent, while free proline accumulated exclusively as a result of ion-toxicity. At maturity mainly osmotic and, to a lesser extent, toxic effects were evident, the former during the phloem translocation of sucrose and the latter during the sucrolytic action of excess ions on invertases in the internodes. This differential effect i.e. the toxic effect of salinity on leaf growth and osmotic effect on sugar accumulation in stalk may be specific to sugarcane, a fact which should be established in other crops for their better management in saline areas.

Literature Cited

Akhtar, S., A. Wahid, M. Akram, and E. Rasul. 2001. Effect of NaCl salinity on yield parameters of some sugarcane genotypes. Int. J. Agri. Biol. **3:** 507-509.

Akhtar, S., A. Wahid, and E. Rasul. 2003. Emergence, growth and nutrient composition of sugarcane sprouts under NaCl salinity. Biol. Plant. **46:** 113-117.

Balibrea, M.E., J. Dell'Amico, M.C. Bolarin, and F. Perez-Alfocea. 2000. Carbon partitioning and sucrose metabolism in tomato plants growing under salinity. Physiol. Plant. **110:** 503-511.

Bates, L.S., R.P. Waldren, and I.D. Teare. 1973. Rapid determination of free proline for water stress studies. Plant Soil **39:** 205-207.

Benzyl, M.L., and M. Reuveni. 1994. Cellular mechanisms of salt tolerance in plant cells. Hort. Rev. **16:** 33-69.

Black, C.A. 1965. Methods of Soil Analysis. American Society of Agronomy, Madison, Wisconsin.

Bray, S., and D.M. Reid. 2002. The effect of salinity and CO_2 enrichment on the growth and anatomy of the second trifoliate leaf of *Pheseolus vulgaris*. Can. J. Bot. **80:** 349-359.

Cheeseman, J.M. 1988. Mechanism of salinity tolerance in plants. Plant Physiol. **87:** 547-550.

Curtis, P.S. and A. Läuchli. 1986. The role of leaf area development and photosynthetic capacity in determining growth of Kenaf under moderate salt stress. Aust. J. Plant Physiol. **13:** 553-565.

Dionisio-Sese, M.L. and S. Tobita. 2000. Effect of salinity on sodium content and photosynthetic responses of rice seedlings differing in salt tolerance. J. Plant Physiol. **157:** 54-58.

Dubey, R.S. and K.A. Singh. 1999. Salinity induced accumulation of soluble sugars and alters the activity of sugar metabolizing enzymes in rice plants. Biol. Plant. **42:** 233-239.

Francois, L.E. and E.V. Maas. 1999. Crop response and management on salt-affected soils. *In* M. Pessarakli (ed.), Handbook of Plant and Crop Stress. Marcel Dekker Press Inc., New York, pp. 169-201.

Garg, A. K., J-K. Kim, T. G. Owens, A. P. Ranwala, Y. D. Choi, L. V. Kochian, and R. J. Wu. 2002. Trehalose accumulation in rice plants confers high tolerance levels to different abiotic stresses. Proc. Natl. Acad. Sci. USA **99:** 15898-15903.

Grattan, S.R. and C.M. Grieve. 1999. Mineral nutrient acquisition and response of plants grown in saline environments. *In* M. Pessarakli (ed.), Handbook of Plant and Crop Stress. Marcel Dekker Press Inc., New York, pp. 203-229.

Grattan, S.R., L. Zeng, M.C. Shannon, and S.R. Roberts. 2002. Rice is more sensitive to salinity than previously thought. Calif. Agri. **56:** 189-195.

Hajibagheri, M.A., A.R. Yeo, T.J. Flowers, and J.C. Collins. 1989. Salinity resistance in *Zea mays*: Fluxes of potassium, sodium and chloride, cytoplasmic concentrations and microsomal membrane lipids. Plant Cell Environ. **12:** 753-757.

Hare, P.D., W.A. Cress, and J.V. Staden. 1998. Dissecting the roles of osmolytes accumulation during stress. Plant Cell Environ. **21:** 535-553.

Hunt, R. 1982. Plant Growth Curves: an Introduction to Functional Approach to Plant Growth Analysis. Edward Arnold, London.

Jain, M., G. Mathur, S. Koul, and N.B. Sarin. 2001. Ameliorative effect of proline on salt stress-induced lipid peroxidation in cell lines of groundnut (*Arachis hypogea* L.). Plant Cell Rep. **20:** 463-468.

Krishnamurthy, R. and K.A. Bhagwat. 1995. Effect of NaCl on enzymes in salt tolerant and salt sensitive rice cultivars. Acta Agron. Hung. **43:** 51-57.

Kumar, S., K.M. Naidu, and H.J. Sehtia. 1994. Causes of growth reduction in elongating and expanding leaf tissue of sugarcane under saline conditions. Aust. J. Plant Physiol. **21:** 79-83.

Lin, C.C. and C.H. Kao. 1996. Proline accumulation is associated with inhibition of rice seedlings root growth caused by NaCl. Plant Sci. **114:** 121-128.

Lingle, S.E. 1999. Sugar metabolism during growth and development in sugarcane internodes. Crop Sci. **39:** 480-486.

Lingle, S.E. and C.L. Weigand. 1997. Soil salinity and sugarcane juice quality. Field Crop. Res. **54:** 259-268.

Lingle, S.E., R.P. Weidenfeld, and J.E. Irvine. 2000. Sugarcane response to saline irrigation water. J. Plant Nutr. **23:** 469-486.

Lopez, C.M.L., H. Takahashi, and S. Yamazaki. 2002. Plant-water relations of Kidney bean plants treated with NaCl and foliarly applied glycinebetaine. J. Agron. Crop Sci. **188:** 73-80.

Lutts, S., J.M. Kinet, and J. Bauhormont. 1996. NaCl induced senescence in leaves of rice (*Oryza sativa* L.) cultivars differing in salinity resistance. Ann. Bot. **78:** 389-398.

Maas, E.V. 1986. Salt tolerance of plants. Appl. Agric. Res. **1:** 12-26.

Meinzer, F.C., Z. Plaut, and N.Z. Saliendra. 1994. Carbon isotope discrimination, gas exchange, and growth of sugarcane cultivars under salinity. Plant Physiol. **104:** 521-526.

Moftah, A.E., and B.E. Michel. 1987. The effect of sodium chloride on solute potential and proline accumulation in soybean leaves. Plant Physiol. **83:** 238-240.

More, P.V., B.B. Desai, and U.D. Chavan. 1994. Effect of salt stress on biochemical parameters of sugarcane. J. Maharashatra Agric. Univ. **19:** 431-433.

Nasir, M.N., R.H. Qureshi, M. Aslam, and J. Akhtar. 2000. Screening of sugarcane lines selected through hydroponic studies in naturally salt affected field. Pak. Sugar J. **15:** 2-10.

Paquet, L., B. Rathinasabapathi, H. Saini, L. Zamir, D.A. Gage, Z.-H. Huang, and A.D. Hanson. 1994. Accumulation of the compatible solute 3-dimethylsulfoniopropionate in sugarcane and its relatives, but not in other gramineous crops. Aust. J. Plant Physiol. **21:** 37-48.

Plaut, Z., F.C. Meinzer, and E. Federman. 2000. Leaf development, transpiration and ion uptake and distribution in sugarcane cultivars grown under salinity. Plant Soil **218:** 59-69.

Qian, Y.L., S.J. Wilhelm, and K.B. Marcum. 2001. Comparative response of two Kentucky bluegrass cultivars to salinity stress. Crop Sci. **41:** 1895-1900.

Qureshi, S.A., C.A. Madramootoo, and G.T. Dodds. 2002. Evaluation of irrigation schemes for sugarcane in Sindh, Pakistan, using SWAP93. Agric. Water Manag. **54:** 37-48.

Rehman, M.S., H. Miyake, and Y. Takeoka. 2002. Effect of exogenous glycinebetaine on growth and ultrastructure of salt stressed rice seedlings (*Oryza sativa* L). Plant Prod. Sci. **5:** 33-44.

Rozeff, N. 1995. Sugarcane and Salinity-a Review Paper. Sugarcane No. 5, pp. 8-19.

Rozeff, N. 1998. Irrigation Water Salinity and Macro Yields of Sugarcane in South Texas (USA). Sugarcane No. 2, pp. 3-6.

Sakamoto, A. and N. Murata. 2002. The role of glycine betaine in the protection of plants from stress: clues from transgenic plants. Plant, Cell Environ. **25:** 163-171.

Schachtman, D.P. and R. Munns. 1992. Sodium accumulation in leaves of *Triticum* species that differ in salt tolerance. Aust. J. Plant Physiol. **19:** 331-340.

Shrivastava, A.K., G.B. Singh, R. Darash, S.P. Shukla, and A.K. Ghosh. 1997. Influence of NaCl induced salt stress on uptake and partitioning of potassium in sugarcane. Osmotic and specific effects. J. Nucl. Agric. Biol. **26:** 64-69.

Subbarao, G.V., R.M. Wheeler, L.H. Levine, and G.W. Stutte. 2001. Glycine betaine accumulation, ionic and water relations of red beet at contrasting levels of sodium supply. J. Plant Physiol. **158:** 767-776.

Tazuke, A. and T. Wada. 2002. Activities of sucrose catalyzing enzymes of cucumber fruit as affected by NaCl addition to nutrient solution. Environ. Control Biol. **40:** 321-325.

Szabolics, I. 1989. Salt-Affected Soils. The Chemical Rubber Company Press, Inc. Boca Raton, Florida.

Wahid, A., A-ur-R. Rao, and E. Rasul. 1997. Identification of salt tolerance traits in sugarcane lines. Field Crop. Res. **54:** 9-17.

Wahid, A., E. Rasul, and A-ur-R. Rao. 1999a. Germination of seeds and propagules under salt stress. *In* M. Pessarakli (ed.), Handbook of Plant and Crop stress 2nd Edition. Marcel dekker Press Inc., New York, pp. 153-168.

Wahid, A., I. Masood, I-ul-H. Javed, and E. Rasul. 1999b. Phenotypic flexibility as marker of sodium chloride tolerance in sunflower genotypes. Environ. Exp. Bot. **42:** 85-94.

Wahid, A., I-ul-H. Javed, I. Ali, A. Baig, and E. Rasul. 1998. Short term incubation of sorghum caryopsis in sodium chloride levels: changes in pre- and post-germination physiological parameters. Plant Sci. **139:** 223-232.

Wilson, C., S.M. Lesch, and C.M. Grieve. 2000. Growth stage modulates salinity tolerance of New Zealand spinach (*Tetragonia tetragonioides*, Pall.) and Red Orach (*Atriplex hortensis* L.). Ann. Bot. **85:** 501-509.

Yoshida, S., D.A. Forno, J.L. Cock, and K.A. Gomez. 1976. Laboratory Manual for Physiological Studies of Rice. IRRI, Los Banos, Philippines.

Zhu, J.-K. 2001. Plant salt tolerance. Trends in Plant Sci. **6:** 66-71.

氯化鈉對甘蔗葉之生長及經濟產量的毒性及滲透壓之影響

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甘蔗（*Saccharum officinarum* L.）係一重要之糖作物，在生長之各階段對土壤鹽度具高敏感性。本研究旨在比較兩生長階段時，耐鹽品系（CP-4333）及不耐鹽品系（CP-71-3002）甘蔗對氯化鈉毒性或滲透壓力的反應。當遭受鹽壓力時，不耐鹽品系不論是乾重或葉面積均顯著地減少 （grand growth stage）。耐鹽品系產生為數較多之分支以對抗過量之鹽離子。不耐鹽品系其葉子的 Na^+ 及 Cl^- 顯著增加，而 K^+ 顯著減少。Na^+ 及 Cl^- 和葉子之生長參數有負相關；而 K^+ 則有正相關：顯示Na^+ 及 Cl^- 之不良影響，同時顯示 K^+ 對甘蔗耐鹽之貢獻。耐鹽品系具較高之水含量，水壓及膨壓；但滲透壓則兩品系沒有差別。耐壓品系當受鹽害時比控制組（不受鹽害者）其可溶性糖增加 2 倍。自由 proline 之累積在不耐鹽品系有較多量，顯示自由 praline 之生合成乃受離子一毒害之影響所致。在成熟階段，增加土壤鹽度會減少可採取之蔗作，可抽取蔗汁及汁一甜份之比率；但增加蔗汁之滲透壓。所有這些參數都和 Na^+ 及 Cl^- 有負相關，並且特別表現在節間的糖含量上。綜合言之，離子一毒性在 grand growth stage 乃耐鹽性之主要決定因素；而 NaCl 之滲透壓成份主要影響蔗糖從葉子到梗之運送，及隨後之增加的節間之蔗糖分解活性，導致最後蔗糖產量之下降。

關鍵詞：甜度；節間；蔗糖分解酶；離子一毒性；鉀離子；比葉重；糖；分支；膨壓。