Effect of salt stress on salt accumulation in roots and leaves of two sugarcane genotypes differing in salinity tolerance

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Abstract

Soil salinization is one of the main restrictions for sugarcane growth in tropical irrigated agricultural lands. Tolerant genotypes are being used to counteract salinization effects. Mechanisms of salt tolerance include restrictions of ion uptake, and compartmentation of ions outside photosynthetic tissues. We determined the Na to K, S, and Cl ratios using X-ray analysis of individual cells of root and leaf tissues of a salt tolerant genotype, PR692176, and a salt sensitive genotype, V-78-1, as a measure of the relative accumulation of ions resulting from salt treatments using sodium sulphate and sodium chloride. Our results showed that root xylem parenchyma is the region where most Na is retained under salinity, and that this retention is more effective in the salt tolerant genotype. In leaves, mesophyll and bundle sheath parenchymas were the sites for Na accumulation, and the strongest increases in Na/K ratios were observed in the sensitive genotype. Sodium chloride treatment increased Na/K ratios more markedly than sodium sulphate, and for all tissues addition of Ca to the nutrient solution reduced the increase in Na/K ratios induced by both salt treatments.

Key words: Crop physiology, Ca-Na interactions, Na/K ratios, Salinity tolerance, X-ray analysis.

Introduction

Soil salinity is one of the most important abiotic stresses limiting distribution and productivity of cultivated plants. Understanding the mechanisms involved in salinity tolerance in crop plants is essential to counteract the progressive salinization going on in extensive areas of agricultural lands (El-Saidi, 1997). This information is necessary to develop selection programs for improving salt tolerance in cultivated plants (Flowers and Yeo, 1995; Ashraf, 1997; Winicov, 1998).

In most crop plants salt sensitivity is related to their inability to prevent salt uptake and transportation to the shoot. Salt exclusion capacity at the root level is therefore considered one of the main mechanisms of salt tolerance (Gorham et al., 1985).

Other relevant mechanisms for salinity tolerance in higher plants are compartmentation of toxic ions in specialized tissues and/or organs (Wyn Jones and Gorham, 2002), maintenance of high selectivity of K^+ uptake and transport in the presence of Na^+ (Gorham, 1990), and osmotic adjustment capacity (Rhodes et al., 2002).

Under salt stress Ca^{2+} promotes K^+, and inhibits Na^+ and/or Cl^- uptake, thus maintaining high K^+/Na^+ ratios that counteract the reduction in plant growth caused by salinity stress (Rengel, 1992).

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Sugar cane is cultivated in tropical and subtropical regions under irrigation, often leading to soil salinization problems (Biswas, 1988). In Venezuela a large fraction of the traditional and more recently added areas for sugar cane cultivation are considered to have salinization problems already present or in progress, caused mainly by sodium chloride or sodium sulphate (Zérega et al., 1991a; Hernández et al., 2000). In those areas reductions of sugar cane production have been detected.

Among sugar cane cultivars a range of salt tolerance has been reported (Bernstein et al., 1966a; Tanimoto, 1969; Zérega et al. 1991b; Villafañe 1996; González et al., 1996; Wahid et al., 1997; Plaut et al., 2000; García and Medina, 2003). However, information on the mechanisms underlying these differences in salt tolerance is scarce.

This paper reports on the ion distribution in leaf and root tissues as measured by X-ray microanalysis in roots and leaves of two genotypes of sugar cane differing in salt tolerance. The salt tolerant 'PR692176' genotype (Villafañe, 1996), and the salt sensitive 'V78-1' genotype (Hernández et al., 2000) were submitted to salinity stress under environmentally controlled conditions. Salinity treatments consisted in addition of Na_2SO_4 or NaCl with or without addition of $CaCl_2$. Previous studies showed that NaCl is more toxic than Na_2SO_4, and that salinity reduces root/shoot ratios in both cultivars, but reductions are stronger in the sensitive cultivar (García and Medina, 2009, 2010).

The objective was to determine how the salt treatments affect the distribution of Na, K, Cl and S, in roots and leaves of these genotypes plants cultivated under salt stress. The concept of ion ratios was used to interpret the relative accumulation of ions within plant tissues induced by salt treatments. Following hypotheses were tested in this experiment: a) sensitivity to salt is revealed by larger increases in Na/K ratios under salt treatments; b) NaCl treatment increases Na/K ratios more than Na_2SO_4 treatment c) increases in Na/K ratios are counteracted by Ca addition.

Materials and Methods

Plant cultivation

The assay was conducted in a growth chamber at the Southeastern Plant Environment, North Carolina State University, Raleigh, NC. *In vitro* plants of the tolerant (PR692176) and sensitive (V78-1) genotypes were obtained from the Biotechnology Laboratory (Central Azucarero El Palmar) replicated from genetic material provided by the Sugar Cane Germplasm Bank of the INIA Yaritagua Station (Yaracuy, Venezuela).

In vitro plants carefully cleaned and disinfected, were planted in germinators with a 1:1 mixture of vermiculite and sand. The containers were acclimatized for one month in a growth chamber (white fluorescent lamps, 12 h photoperiods at 600 $\mu mol\ m^{-2}\ s^{-1}$, and thermoperiod of 26º/22ºC; Thomas and Downs, 1991). Air humidity was maintained by an automatic nebulizer spraying the chamber for ten seconds every 15 minutes.

Plants were irrigated once a day with a modified Hoagland solution (Kumar et al., 1994) diluted to ¼ during the first 2 weeks and to ½ during the following 2 weeks. At the end of the acclimatization period plants were pruned again to improve stalk thickening.

Plants as uniform as possible were selected and transplanted to dark plastic containers (8 L, 16 cm wide x 42 cm length; one plant per container), perforated below to facilitate drainage and filled with sand previously washed and disinfected. Plants were watered daily with enough solution to displace completely the remaining of the previous irrigation. During the first week after transplantation irrigation solution was maintained at ¾ of the original concentration. Salinity treatments began at the

second week after transplanting. At this time 100 W infrared lamps were added to the fluorescent lamps bench, increasing PAR to 639 µmol m^{-2} s^{-1} (Thomas and Downs, 1991). Thermoperiod was changed to day/night values of 30º/26º, and relative humidity was maintained at 70% in average.

Salinity treatments

To the modified Hoagland solution of Kumar et al. (1994) sodium chloride or sodium sulphate were added with or without supplement of calcium chloride to constitute four salinity treatments: NaCl (100 mol m^{-3}), Na_2SO_4 (50 mol m^{-3}), NaCl + $CaCl_2$ (10 mol m^{-3}), and Na_2SO_4 + $CaCl_2$ (10 mol m^{-3}). Ca addition in the two last treatments reduced the Na/Ca ratio from 40 to 8, and the Na/Cl ratio from 1 to 0.8. All salt solutions and the control were prepared using demineralized water and pH was adjusted to 7.0.

Treatments began four weeks after transplantation and were maintained for two months. To avoid an osmotic shock at the beginning of the treatments salinity of irrigation was increased at a rate of 20 mol m^{-3} day^{-1} for those containing NaCl, and 10 mol m^{-3} day^{-1} for those containing Na_2SO_4. In treatments receiving additional $CaCl_2$ its concentration was increased at a rate of 2 mol m^{-3} day^{-1}. Plants were irrigated once a day during the first month and twice a day during the second month. The experimental design was a fully randomized factorial arrangement 2 x 5: 2 genotypes, 4 salinity treatments and a control, with 6 replications each.

X-rays microanalysis

Sixty days after treatment initiation, three plants per treatment were collected for the analysis of ion accumulation in absorbing root tissues located 10 and 100 mm from the root tip, and leaf tissues located at the medium third of the blade, about 10 mm from central vein of the most recently expanded leaf (Top Visible Dewlap, TVD leaf). The elements Na, K, Cl, and S were analyzed in the Analytical Instrumentation Facility of North Carolina State University (Raleigh, NC) using Energy Dispersive X-ray analysis (EDX) (Yeo et al., 1977). Ten mm long segments of roots and 10 x 5 mm sections of TVD leaf were cut with a razor blade and frozen in vials with liquid nitrogen. This material was freeze-dried and maintained in a desiccator until analysis. The root tissues analyzed were cortical parenchyma (third layer of the central cortex), xylem parenchyma, and metaxylem vessels. In the leaves the tissues studied were upper and lower epidermises, chlorophyllous parenchyma of the mesophyll and the bundle sheath, and metaxylem vessels. The freeze-dried material was mounted on carbon sample supporters using a double adhesive carbon tape. The X-ray detector (Link Pentafet Oxford) was mounted on a Hitachi S-3200 scanning electron microscope. The X-ray spectrum was collected over individual cells, within a square scanning area, covering the whole cell. For all samples the conditions were 12 kV voltage acceleration, 100s of exposure time, 15 mm between the detector and the plant material, and a 90º incidence angle of the electron beam.

The Na, K, Cl and S spectra of each tissue sample were submitted to a semiquantitative analysis using the Link Isis program (Oxford Instruments). The relative content of each element in % of the whole x-ray spectra allowed the calculation of Na/Cl ratios (NaCl treatments), Na/S ratios (Na_2SO_4 treatments) and Na/K ratios for all treatments. For each tissue the measurements obtained from the three replicates were averaged.

Results and Discussion

X-ray spectra obtained from root and leaf tissues of both genotypes showed that K was the dominant ion in control plants as reported for maize (Yeo et al. 1977) and *Phaseolus coccineus* roots (Kramer et al. 1977) (data not shown).

Root tissues

The sodium sulphate treatment increased strongly the Na/K ratios in all root tissues examined both at 10 and 100 mm from tip (Table 1). At 10 mm the increase was consistently higher in the salt sensitive cultivar, and Ca addition increased the Na/K ratios in parenchymatic tissue of both cultivars. At the 100 mm level the xylem parenchyma showed the strongest increases in Na/K ratios, which were higher in the tolerant cultivar. Ca addition caused a reduction in Na/K ratios only in the salt sensitive cultivar.

The Na/S ratios increased in all tissues and root locations of both cultivars indicating a stronger Na uptake compared to sulphate (Table 1). Ca additions only reduced Na/S ratios in the salt sensitive cultivar at the 100 mm level.

The sodium chloride treatment also increased the Na/K ratios of all root tissues of both genotypes but the relative increase was much more pronounced at the 10 mm location in the salt sensitive genotype (Table 2). Ca supplement partially reduced this increase. Na/K ratios were always higher in all tissues at the 100 mm level, but the increases were more pronounced in the xylem parenchyma. The Na/Cl ratios showed a similar pattern, and again the Ca supplement was effective in counteracting these effects.

Previous studies in sugar cane indicate that salt transport to the leaves may be regulated by salt retention in the lower stems sections (Bernstein et al., 1966b; Lingle et al., 2000), or in the roots (Segovia, 1982) or both (Plaut et al., 2000). Our results confirm that in sugar cane, as in maize and barley, Na is sequestered in the xylem parenchyma

Table 1. X-ray spectra Na/K and Na/S ratios, measured on individual cells located 10 or 100 mm from the root tip, in plants belonging to salt tolerant (PR692176) and salt sensitive (V78-1) sugarcane genotypes stressed for 60 days with 100 mM Na_2SO_4 with or without Ca supplement (10 mM).

Genotype Treatment	Tissue	Distance from root tip (mm)			
		10		100	
		Na/K	Na/S	Na/K	Na/S
PR692176					
Control	Cortex parenchyma	0.02	0.51	0.10	0.51
	Xylem parenchyma	0.00	0.10	0.13	0.65
	Metaxylem vessel	0.01	0.45	0.06	0.39
Na_2SO_4	Cortex parenchyma	0.83	1.08	2.08	1.39
	Xylem parenchyma	0.32	0.45	3.16	2.74
	Metaxylem vessel	0.46	0.77	0.65	0.73
Na_2SO_4 + Ca	Cortex parenchyma	1.26	1.30	2.85	2.73
	Xylem parenchyma	0.73	0.85	3.01	2.81
	Metaxylem vessel	0.62	0.74	0.89	0.96
V78-1					
Control	Cortex parenchyma	0.00	0.30	0.00	0.20
	Xylem parenchyma	0.00	0.01	0.00	0.05
	Metaxylem vessel	0.00	0.02	0.01	0.02
Na_2SO_4	Cortex parenchyma	1.21	1.16	1.59	1.40
	Xylem parenchyma	0.91	0.95	2.01	1.78
	Metaxylem vessel	0.75	0.87	0.98	0.93
Na_2SO_4 + Ca	Cortex parenchyma	1.53	3.69	1.33	1.03
	Xylem parenchyma	1.39	2.05	1.65	1.12
	Metaxylem vessel	0.74	0.93	0.88	0.76

Table 2. X-ray spectra Na/K and Na/Cl ratios, measured on individual cells located 10 or 100 mm from the root tip, in plants belonging to salt tolerant (PR692176) and salt sensitive (V78-1) sugarcane genotypes stressed for 60 days with 100 mM NaCl with or without Ca supplement (10 mM).

Genotype Treatment	Tissue	Distance from root tip (mm)			
		10		100	
		Na/K	Na/Cl	Na/K	Na/Cl
PR692176					
Control	Cortex parenchyma	0.02	0.31	0.10	0.78
	Xylem parenchyma	0.00	0.06	0.13	0.46
	Metaxylem vessel	0.01	0.10	0.06	0.10
NaCl	Cortex parenchyma	1.38	0.68	2.25	0.89
	Xylem parenchyma	0.82	0.32	3.79	1.26
	Metaxylem vessel	0.56	0.29	0.73	0.35
NaCl + Ca	Cortex parenchyma	0.79	0.34	1.23	0.60
	Xylem parenchyma	0.40	0.27	3.75	0.82
	Metaxylem vessel	0.37	0.23	0.81	0.30
V78-1					
Control	Cortex parenchyma	0.00	0.46	0.00	0.30
	Xylem parenchyma	0.00	0.01	0.00	0.23
	Metaxylem vessel	0.00	0.04	0.01	0.22
NaCl	Cortex parenchyma	2.71	1.14	2.16	0.76
	Xylem parenchyma	1.49	0.77	3.36	0.93
	Metaxylem vessel	1.01	0.43	1.31	0.68
NaCl + Ca	Cortex parenchyma	2.15	0.40	2.06	0.43
	Xylem parenchyma	1.27	0.33	2.97	0.71
	Metaxylem vessel	0.84	0.29	1.01	0.32

of mature roots, regulating the transport of this ion to the shoot. 'PR692176' showed a higher capacity for Na sequestration (larger Na/K ratios), a fact that might be related to the higher salinity tolerance of this genotype. However, this should be verified by analyses of Na/K ratios in older regions of the roots (>250 mm) and basal shoot sections. In *Phaseolus coccineus* a Na/K ratio of 32 was measured in the xylem parenchyma located at 300 mm from the root tip in plants stressed with 100 mM NaCl (Kramer et al., 1977), whereas in maize cultivated under similar conditions, Yeo et al. (1977) measured ratios between 10 and 20 in the same tissue at 250 mm from the root tip.

Leaf tissues

The salt treatments modified strongly the ion ratios of all leaf tissues measured, but as the most consistent results were obtained by the mesophyll parenchyma, bundle sheath parenchyma, and the

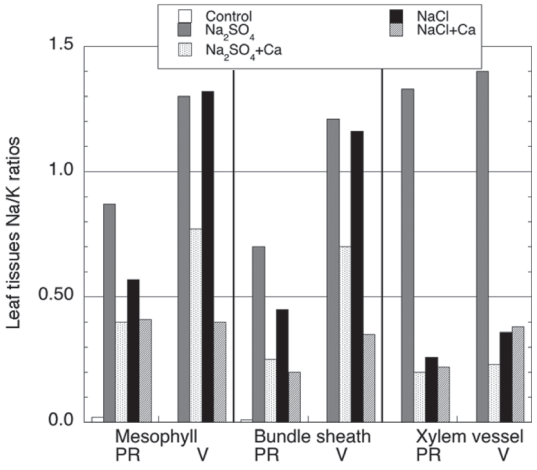

Figure 1. Na/K ratios in leaf tissues of sugar cane cultivars tolerant (PR) and sensitive (V) to salt.

xylem vessels, the following considerations will be restricted to those tissues.

The sodium sulphate, treatment elevated the Na/K ratios of leaf tissues of the tolerant genotype above 0.7, the increase being more pronounced in the xylem vessel with a ratio above 1.3, the increase was markedly reduced in all tissues by Ca supplement (Fig. 1). The increase observed in the sensitive cultivar was more pronounced, all values being above 1.2 (Fig. 1). Again Ca supplement counteracted this increase especially in the xylem vessels.

The sodium chloride treatment brought about a moderate increase in Na/K ratios in the tolerant genotype (0.25-0.55), and a strong increase (1.15-1.3) in the sensitive genotype in the mesophyll and bundle sheath parenchymas but not in the xylem vessel (Fig. 1). Ca supplement was effective in reducing the ratios below 0.4 in the tissues of both genotypes.

The Na/S ratios by the sodium sulfate treatment were consistently higher in the sensitive genotype in all tissues measured, and the Ca supplement reduced this effect but ratios in the sodium sulphate, plus Ca treatment were always higher in the sensitive genotype (Fig. 2 upper panel). The increase in these ratios indicates a faster uptake of Na than sulphate, into the parenchymas of the sensitive genotype, whereas uptake of Na in the tolerant genotype is not enough to elevate Na/S ratios above 1.

The sodium chloride treatment had little effect on the Na/Cl ratios in the chlorophyllous parenchyma of the tolerant genotype, indicating that absorption of both cations occurred at similar rates, whereas in the sensitive genotype this ratio increased strongly revealing preferential absorption of Na compared to Cl. Again, the Ca supplement was effective in reducing those levels to values similar to those of the tolerant genotype (Fig. 2 lower panel).

In both treatments with single salts 'PR692176'

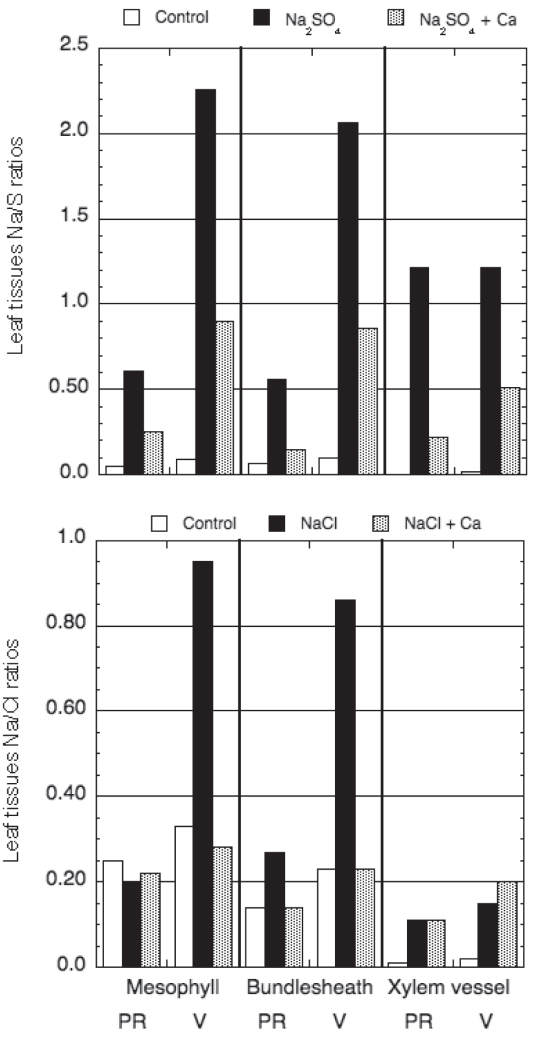

Figure 2. Na/S (upper panel) and Na/Cl (lower panel) ratios of leaf tissues from plants tolerant (PR) and sensitive (V) to salt treated with sodium sulfate and sodium chloride.

maintained a Na/K <1, in the mesophyll and the bundle sheath parenchymas, whereas this ratio was >1 in the 'V78-1'. This indicates that the higher salt tolerance of 'PR692176' may be associated with its ability to maintain K selectivity in presence of Na resulting in lower Na/K ratios in photosynthetically active tissues of the leaf blade as shown in other cultivated plants (Shannon, 1997; Gorham et al., 1985). Supplementing salt treatments with Ca reduced the Na/K ratio in leaf tissues, particularly in the sensitive genotype, confirming the role of Ca

in maintaining K selectivity in the presence of Na in leaf tissues (Rengel, 1992; Shannon, 1997). In both genotypes, and for all salt treatments, the Na/K ratio was lower in the bundle sheath parenchyma, compared with the mesophyll parenchyma, suggesting a higher discrimination capacity in the former tissue.

In the leaf tissues the Na/Cl ratios of plants stressed with NaCl was much lower than the Na/S ratios in plants stressed with Na_2SO_4, revealing the high permeability of leaf tissues towards Cl. The distribution of Na/Cl ratios indicated a high permeability towards Cl in the photosynthetic tissues of the tolerant genotype.

Finally, in 'PR692176' plants stressed with NaCl, the Na/K ratio in both mesophyll parenchyma and bundle sheath parenchyma, was lower than that of plants treated with Na_2SO_4, suggesting a higher selectivity towards K in presence of Na in those tissues. However, growth was much more affected by the NaCl treatment than with the Na_2SO_4 treatment (García and Medina, 2009, 2010) indicating that low Na/K ratios were not enough to counteract the toxic effect of Cl in the photosynthetic tissue. Contrary to the observations of the present study, in sorghum plants (Boursier and Läuchli, 1990) and barley (Leigh and Storey, 1993) stressed with NaCl, it was shown that Cl was excluded from the mesophyll, whereas Na was maintained at relatively high concentrations suggesting that its presence was not as toxic as that of Cl (Leigh and Storey, 1993). In the case of sorghum, a highly sensitive species towards Cl, it has been shown that salt tolerance is directly related to its ability to exclude Cl from the mesophyll and accumulate it in the epidermis and the leaf sheath (Huang and Van Steveninck, 1988).

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References

Ashraf, M. 1997. Improvement of salt tolerance in some native pulse crops. In: Jaiwal, P., Singh, R., and Gulati, A. (eds) Strategies for improving salt tolerance in higher plants. Science Publishers Inc. USA, pp. 413-434.

Bernstein, L., Francois E. and Clark, R. 1966a. Salt tolerance of N.Co. varieties of sugar cane. I. Sprouting, growth and yield. Agronomy J., 58: 489-493.

Bernstein, L., E. Francois Y R. Clark. 1966b. Salt tolerance of N.Co. varieties of sugarcane. II. Effects of soil salinity and sprinkling on chemical composition. Agron. J., 58: 503-507.

Biswas, B. 1988. Agroclimatology of the sugarcane crop. World Meteorological Organization, Technical Note No. 193, 90 p.

Boursier, P. and Läuchli, A. 1990. Growth responses and mineral nutrient relations of salt-stressed sorghum. Crop Sci., 30: 1126-1233.

El-Saidi, M. 1997. Salinity and its effect on growth, yield and some physiological proceses of crop plants. In: Jaiwal, P., Singh, R. and Gulati, A. (eds) Strategies for improving salt tolerance in higher plants. Science Publishers inc. USA, pp. 111-127.

Flowers, T. and Yeo, A. 1995. Breeding for salinity resistance in crop plants: where next? Aust. J. Plant Physiol., 22: 875-884.

García, M. and Medina, E. 2003. Crecimiento y acumulación de prolina en dos genotipos de caña de azúcar sometidos a salinización con cloruro de sodio. Rev. Fac. Agron. (LUZ), 20: 168-179.

García, M. and Medina, E. 2009. Acumulación de iones y solutos orgánicos en dos genotipos de caña de azúcar estresados con sales simples o suplementadas con calcio. Bioagro, 21(1): 3-14.

García, M. and Medina, E. 2010. Crecimiento y morfología radical en dos genotipos de caña de azúcar sometidos a salinización con sales simples o suplementadas con calcio. Rev. Fac. Agron. (Luz), 27 (1): 17-38.

González, V., Castroni, S. and Fuchs, M. 1996. Evaluación de la reacción de genotipos de caña de

azúcar a diferentes concentraciones de NaCl. Agron. Trop., 46: 219-232.

Gorham, J. 1990. Salt tolerance in the triticeae: K/Na discrimination in *Aegilops* species. J. Exp. Bot., 41: 615-621.

Gorham, J., Wyn Jones, R. and McDonell, E. 1985. Some mechanisms of salt tolerance in crop plants. Plant and Soil, 89: 15-40.

Hernández, J., Zérega, L. and Ordosgoitti, A. 2000. Causa del necrosado del borde de las hojas y retraso en el crecimiento de la caña de azúcar (*Saccharum* spp híbrido) en el bajo Yaracuy. Rev. Fac. Agron. (LUZ), 17: 236-238.

Huang, Ch. and van Steveninck, R. 1988. Effect of moderate salinity on patterns of potassium, sodium and chloride accumulation in cells near the root tip of barley: role of differentiating metaxylem vessels. Physiol. Plant., 73: 525-533.

Kumar, S., Naidu, K. and Sehtiya, H. 1994. Causes of growth reduction in elongating and expanding leaf tissue of sugar cane under saline conditions. Aust. J. Plant Physiol., 21:71-83.

Leigh, R. and Storey, R. 1993. Intercellular compartmentation of ions in barley leaves in relation to potassium nutrition and salinity. J. Exp. Bot., 44:755-762.

Lingle, S., Wiedenfeld R. and Irvine, J. 2000. Sugarcane response to saline irrigation water. J. Plant Nut., 23: 469-486.

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

Rengel, Z. 1992. The role of calcium in salt toxicity. Plant Cell Environ., 15: 625-632.

Rhodes, D., Nadolska-Orczyk, A. and Rich, P. 2002. Salinity, osmolytes and compatible solutes. In: Läuchli, A. and Lüttge, U. (eds.) Salinity - Environment - Plants - Molecules. Kluwer Academic Publishers. Dordrecht, The Netherlands. pp. 181-204.

Segovia, A. 1982. Efectos del pH, P y frecuencia de riego sobre la producción y composición mineral de la caña de azúcar bajo condiciones de salinidad. Trabajo de Ascenso. Facultad Agronomía, Universidad Central de Venezuela. Maracay. 139 p.

Shannon, M. 1997. Genetics of salt tolerance in higher plants. En: Strategies for improving salt tolerance in higher plants. P. Jaiwal, R. Singh y A. Gulati (eds). Science Publishers, Inc. USA. p: 265-289.

Tanimoto, T. 1969. Differential physiological response of sugar cane varieties to osmotic pressures of saline media. Crop Sci., 9:683-691.

Thomas, J. and Downs, R. 1991. Phytotron procedural manual for controlled environment research at the southeastern plant environment laboratory. NC. Agric. Res. Tech. Bull., 244.

Villafañe, R. 1996. Tolerancia a la salinidad y al sodio de seis variedades de caña de azúcar en Venezuela. Agron. Trop., 46: 85-99.

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

Winicov, I. 1998. New molecular approaches to improving salt tolerance in crop plants. Ann. Bot., 82: 703-710.

Wyn Jones, G. and Gorham, J. 2002. Intra-and inter-cellular compartmentation of ions. In: Läuchli, A. and Lüttge, U. (eds.) Salinity - Environment - Plants - Molecules. Kluwer Academic Publishers. Dordrecht, The Netherlands. pp. 159-180.

Yeo, A., Kramer, D. and Läuchli, A. 1977. Ion distribution in salt-stressed mature *Zea mays* roots in relation to ultrastructure and retention of sodium. J. Exp. Bot., 28: 17-29.

Zérega, L., Hernández, T. and Valladares, J. 1991a. Caracterización de suelos y aguas afectadas por sales en zonas cañameleras de azucarera río Turbio. Caña de Azúcar, 9: 5-52.

Zérega, L., Hernández, T. and Valladares, J. 1991b. Evaluación de 14 variedades de caña de azúcar en dos suelos afectados por sales, bajo condiciones de umbráculo. Caña de Azúcar, 9: 81- 98.