Osmotic adjustment in roots and leaves of two sorghum genotypes under NaCl stress

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Seedlings of two sorghum genotypes [*Sorghum bicolor* (L.) Moench], one salt tolerant (CSF 20) and the other salt sensitive (CSF 18) were grown in nutrient solution containing 0, 50 and 100 mmol.L^{-1} NaCl for seven days and the osmotic potential (Ψ_s) and the contribution of organic and inorganic solutes to the Ψ_s were determined in the leaves and roots. Salinity reduced the Ψ_s of the cellular sap of leaves and roots in both genotypes, mainly in the salt sensitive one. The higher decrease in the Ψ_s in the salt sensitive genotype was mostly due to higher accumulation of Na^+ and Cl^- that probably exceeded the amount needed for the osmotic adjustment. Among the inorganic solutes, K^+ contributed the most to the Ψ_s in control unstressed seedlings, but its contribution decreased as salt stress increased, especially in the salt sensitive genotype. Soluble carbohydrates and amino acids were the organic solutes that contributed the most to the leaf and root Ψ_s, respectively. No statistically significant difference in these organic solute contributions to the leaf Ψ_s between genotypes was observed. Their contributions to the root Ψ_s, however, were higher in the salt tolerant genotype, especially at higher NaCl concentration. Proline contribution to leaf and root Ψ_s was quite small in both genotypes and its accumulation was not related to salt tolerance. Our results suggest that the salt tolerant genotype was able to maintain a more adequate osmotic pool in the leaves and roots under salt stress than the salt sensitive genotype.

Key words: ions, organic solutes, osmotic adjustment, salinity, *Sorghum bicolor*.

Ajustamento osmótico em raízes e folhas de dois genótipos de sorgo submetidos a estresse com NaCl: Plântulas de dois genótipos de sorgo [*Sorghum bicolor* (L.) Moench], um tolerante (CSF 20) e outro sensível (CSF 18), foram cultivadas em solução nutritiva contendo 0, 50 e 100 mmol.L^{-1} de NaCl durante sete dias. O potencial osmótico (Ψ_s) e a contribuição de solutos orgânicos e inorgânicos foram, então, determinados. O estresse salino reduziu o Ψ_s do suco celular em folhas e raízes dos dois genótipos, principalmente do sensível. A maior queda no Ψ_s no genótipo sensível, pareceu ser, pelo menos em parte, resultado dos maiores acúmulos de Na^+ e Cl^- que, provavelmente, excederam a quantidade necessária ao ajustamento osmótico. Entre os solutos inorgânicos, K^+ foi o que mais contribuiu para o Ψ_s de folhas e de raízes em plantas não-estressadas, sua contribuição, porém, decresceu com a intensificação do estresse salino, principalmente no genótipo sensível. Os carboidratos solúveis e os aminoácidos foram os solutos orgânicos que mais contribuíram para o Ψ_s nas folhas e raízes, respectivamente. Não se observaram diferenças estatísticas para a contribuição de tais solutos para o Ψ_s foliar entre os genótipos. Suas contribuições para o Ψ_s de raízes, no entanto, foram maiores no genótipo tolerante, especialmente no tratamento com maior concentração de NaCl. A contribuição da prolina para o Ψ_s foi pequena nas folhas e raízes dos dois genótipos e sua acumulação não se correlacionou com a tolerância à salinidade. Os resultados sugerem que o genótipo tolerante foi capaz de manter um "pool" osmótico mais adequado em suas raízes e folhas do que o sensível sob estresse salino.

Palavras-chave: ajustamento osmótico, íons, salinidade, solutos orgânicos, *Sorghum bicolor.*

INTRODUCTION

In most saline soils Na^+ and Cl^- are the dominant ions, and usually they exceed by far the plant demand/necessity. The excess of soluble salts in the root environment causes osmotic stress, which may result in disturbance of the plant water relation, in the uptake and utilization of essential nutrients, and also in toxic ion accumulation. As a result of these changes, the activities of various enzymes and the plant

metabolism are affected (Läuchli et al., 1994; Bernstein et al., 1995; Munns, 2002; Lacerda et al., 2003). Some plants are able to tolerate drought and saline stresses by reducing the cellular osmotic potential as a consequence of a net increase in solute accumulation, in a process called osmotic adjustment (Hazegawa et al., 2000; Munns, 2002; Serraj and Sinclair, 2002). It is accepted that during osmotic adjustment the cells tend to compartmentalize most of the absorbed ions in vacuoles at the same time that they synthesize and accumulate compatible organic solutes in the cytoplasm in order to maintain the osmotic equilibrium between these two compartments (Serrano and Gaxiola, 1994; Hare et al., 1998; Hasegawa et al., 2000). The osmotic regulation, that occurs in both roots and leaves, contributes to maintain water uptake and cell turgor, which are essential to sustain physiological processes such as cell expansion, stomatal opening, photosynthesis, and many others plant processes (Zhang et al., 1999).

During osmotic adjustment, however, plants spend a significant amount of metabolic energy for uptake and intracellular compartmentalization of ions and for biosynthesis of compatible organic solutes. Although this appears to be essential for plant survival under salt and water stress conditions, some authors believe that organic solute accumulation is a consequence of lower photoassimilate utilization and/or lower relative growth rather than an adaptive plant response to cope with osmotic stress (Munns, 1988; Kramer and Boyer, 1995; Serraj and Sinclair, 2002).

In spite of these controversies, osmotic adjustment is receiving increasing recognition as a major plant acclimatization mechanism to water and salt stress (Zhang et al., 1999). Several ions, amino acids, quaternary amines, organic acids, sugars and polyols were found among the solutes that accumulate during osmotic adjustment of salt stressed cells and tissues (Rodríguez et al., 1997; Zhang et al., 1999). However, their presence, amount, and distribution within the cells vary widely among plant species and cultivars (Hare et al., 1998; Zhang et al., 1999).

Therefore, the objective of this paper was to test the hypothesis that differential salt tolerance in sorghum seedlings was related to quantitative and qualitative aspects of the leaf and root osmotic adjustment.

MATERIAL AND METHODS

Seeds of two forage sorghum [*Sorghum bicolor* (L.) Moench] genotypes, one salt tolerant (CSF20) and the other salt sensitive (CSF18), obtained from the Empresa Pernambucana de Pesquisa Agropecuária, were selected for size and shape and surface sterilized with 2 % sodium hypochlorite for 10 min. After extensive rinses with running tap water and demineralized water, the seeds were germinated in rolls of neutral pH "germtest" paper partially immersed in 1/5 strength Clark's nutrient solution, pH 5.5 (Clark, 1975).

Four seven-day old seedlings, selected for uniformity, were transferred to 2.6 L polyethylene pots containing Clark's nutrient solution (with double P concentration), pH 5.5. Salt (NaCl) was added to the nutrient solution in increments of 25 $mmol.L^{-1}$ every 12 h to reach NaCl concentrations of 0, 50 and 100 $mmol.L^{-1}$. The nutrient solutions were continuously aerated and the pH adjusted daily to 5.5 with HCl or NaOH. The experiment was carried out in a growth chamber programmed to a temperature of 25 ± 3°C, 230 $\mu mol.m^{-2}.s^{-1}$ of photosynthetically active radiation and 16 h photoperiod (Lacerda et al., 2003).

Seven days after the beginning of salt additions the plants were harvested and washed with demineralized water. Organic and inorganic solutes were extracted from 300 mg of fresh mass of roots and mature leaf blades with 15 mL of demineralized water containing 0.03 mL of toluene in a water-bath at 30°C for 1 h and filtered into a volumetric flask. This extraction was repeated twice and the volume completed to 50 mL with demineralized water, according to the technique described by Lerner et al. (1978) and Weimberg et al. (1984). In this extract soluble carbohydrates (Horwitz, 1975), total free amino acids (Moore and Stein, 1948) and proline (Bates et al., 1973) were determined. The contents of Na^+ and K^+, and Ca^{2+} and Mg^{2+} were determined by emission and atomic absorption spectrophotometry, respectively, and the content of Cl^- by visible spectrophotometry (Gaines et al., 1984).

Samples of roots and mature leaf blades stored in plastic bags at -20°C were thawed, pressed in a hydraulic press, centrifuged at 2,500 g_n for 20 min and the supernatant used as "cellular sap". The osmotic potential (Ψ_s) in combined supernatants of three replicates and in the nutrient solution were determined by a cryoscopic method using a microosmometer (Osmette 2007, Precision System, Inc, USA), according to Slavik (1974).

The Ψ_s of each solute was estimated by van't Hoff equation, and summed up to obtain the calculated Ψ_s or expressed as percentage of the total measured Ψ_s. The measured and calculated Ψ_s were corrected for maximum turgor (Slavik, 1974; Huang and Redmann, 1995)

The experimental design was a completely randomized 2 x 3 factorial design with three replicates. The data were subjected to analysis of variance and the means were compared by the Tukey test at 5% probability.

RESULTS AND DISCUSSION

The calculated and measured osmotic potentials (Ψ_s) decreased in both the leaves and roots of the two sorghum genotypes with the increase in NaCl concentration in the nutrient solution (table 1). The average of measured Ψ_s in the leaves was about 2.1 times lower than in the roots. The calculated osmotic potentials were about 24 % lower than the measured ones, probably because the contribution of other solutes to the osmotic pool, such as nitrate, sulfate, phosphate, organic acids, ammonium quaternary compounds, and others were not determined in this experiment. However, the correlation between measured and calculated Ψ_s for both leaves and roots was statistically significant within each one of the genotypes studied (data not shown).

The intensity of reduction in the Ψ_s was dependent upon plant part and genotype (table 1). The increase in NaCl concentration from 0 to 100 mmol.L^{-1} corresponded to a decrease in nutrient solution Ψ_s of 0.467 MPa. The same increase in NaCl salinity was responsible for decreases in measured leaf and root Ψ_s of 0.491 and 0.106 MPa for the salt tolerant genotype, and of 0.846 and 0.216 MPa for the salt sensitive genotype, respectively. Therefore, the measured solute accumulation was always higher in the leaves than in the roots, and the salt sensitive genotype also accumulated more solutes than the salt tolerant one. These results suggest that the leaves of the salt sensitive genotype may over-accumulate solutes during osmotic adjustment.

The contribution of the different organic and inorganic solutes to the Ψ_s depended on plant part and genotype (tables 2 and 3). The contribution of Na^+ and Cl^- in the leaves increased with NaCl concentration in the nutrient solution, especially in the salt sensitive genotype (table 2). The amounts of Na^+ and Cl^- accumulated in the leaves of plants treated with 100 mmol.L^{-1} NaCl contributed to about 30 and 33 % of the measured Ψ_s in the salt tolerant and salt sensitive genotype, respectively. These potentially toxic ions may be accumulated in the apoplast, accelerating tissue dehydration, and/or in the cytoplasm causing injury to metabolic systems (Munns et al., 1995; Munns, 2002), mainly in salt sensitive genotypes.

Potassium ion and Ca^{2+} contributions to the leaf Ψ_s decreased, while that of Mg^{2+} increased, in both genotypes, increasing NaCl concentration in the nutrient medium (table 2). Calcium ion and Mg^{2+} contributions, however, were much smaller than that of K^+. Potassium ions were the major inorganic solute in the control treatment, but when plants were exposed to 100 mmol.L^{-1} NaCl its contribution to leaf Ψ_s decreased by about 43 and 60 % in the salt tolerant and salt sensitive genotypes, respectively.

Table 1. Leaf and root osmotic potential (Ψ_s) in two sorghum genotypes grown in nutrient solution containing different NaCl concentrations.

NaCl (mmol.L^{-1})	Nutrient Solution Ψ_s (- Mpa)	Cellular Sap Ψ_s (- MPa) [a,b]			
		Leaves		Roots	
		Measured	Calculated	Measured	Calculated
			Salt tolerant		
0	0.035	1.039	0.752 cA	0.529	0.404 bA
50	0.265	1.217	0.889 bA	0.548	0.411 bB
100	0.502	1.530	1.204 aB	0.635	0.525 aB
			Salt sensitive		
0	0.035	1.032	0.752 cA	0.587	0.424 cA
50	0.265	1.266	0.897 bA	0.613	0.487 bA
100	0.502	1.878	1.457 aA	0.803	0.621 aA

[a] Measured and calculated Ψ_s in the cellular sap (See 'Material and Methods')

[b] Means, followed by the same capital letters (between genotypes, in each NaCl treatment) and by the same small letters (between NaCl treatment, in each genotype), do not differ statistically by the Tukey test at 5 %. The statistical analysis was made with positive osmotic potential values.

Among the organic solutes, soluble carbohydrates contributed the most to the leaf Ψ_s (table 2), and they also seemed to be important in the leaf osmotic adjustment under salt stress conditions, as suggested by Greenway and Munns (1980) and Ashraf (1994). Their contribution to the Ψ_s, however, did not change with the increase in NaCl concentration, probably because the increase in leaf soluble carbohydrate content was proportional to the increase in leaf osmolality. Although, there were no genotypic differences in soluble carbohydrate contribution to the Ψ_s, the salt sensitive genotype showed higher leaf content (data not shown).

Proline contents, of all the organic solutes analyzed, showed the highest relative increase in response to salt stress (table 2). Its contribution to the leaf Ψ_s, when plants were subjected to 100 mmol.L^{-1} NaCl, increased about 2.5 times in the salt tolerant genotype and 3.8 times in the salt sensitive one. The absolute contribution of proline, however, was the smallest, suggesting that this organic solute may not play a role in sorghum osmotic adjustment, at least under the experimental conditions used here. Probably, proline is associated to other functions in plants under salt stress (Hare et al., 1998). Similar results have been reported for other cultivated species (Huang and Redmann, 1995; Lutts et al., 1996; Meloni et al., 2003).

The amino acid contribution to the leaf Ψ_s was about 11 % in control plants but it decreased to about 8 % in plants treated with 100 mmol.L^{-1} NaCl. No difference in leaf amino acid contribution between genotypes was observed, but the salt sensitive genotype showed higher leaf amino acid content (data not shown).

The increase in the Na^+ plus Cl^- contribution to the root Ψ_s in salt stressed plants (table 3) was higher than that to the leaf Ψ_s (table 2). This was expected since a significant amount of Na^+ and Cl^- may be retained in stems and leaf sheaths, as shown by Shannon (1992) and Lacerda et al (2001, 2003). Similar to the observed in leaves, the contribution of these two ions to the osmotic pool was higher in the salt sensitive genotype, probably contributing to the higher reduction in growth observed in this genotype (Lacerda et al., 2001).

The contributions of Ca^{2+}, K^+ and Mg^{2+} to the root Ψ_s decreased with the increase of NaCl concentration in the nutrient solution (table 3). Potassium ion was the most important solute contributing to both root and leaf Ψ_s in control plants. However, its contribution under salt stress conditions strongly decreased, especially in the salt sensitive genotype.

Amino acids were the main organic solute contributing to the root Ψ_s, followed by soluble carbohydrates and proline (table 3). None of them, however, changed with the application of salt treatment. Proline contribution to the root Ψ_s, similarly to that observed in leaves, was also not significant, at least under the conditions of the experiment. Amino acid contributions to the Ψ_s were always higher in the salt tolerant genotype, regardless of salt treatment.

Table 2. Solute contribution to the leaf osmotic potential (Ψ_s) in two sorghum genotypes grown in nutrient solution containing different NaCl concentrations.

NaCl (mmol.L^{-1})	Solute Contribution to the Ψ_s (%)[a,b,c]						
	$Na^+ + Cl^-$	K^+	Ca^{2+}	Mg^{2+}	CHO	Pro	AA
				Salt tolerant			
0	11.23 cA	28.32 aA	3.30 aB	0.85 cB	19.58 aA	0.22 cA	10.14 aA
50	18.69 bB	20.16 bA	2.14 bA	1.25 bB	20.40 aA	0.34 bB	10.15 aA
100	30.14 aB	16.06 cA	1.79 cA	1.64 aB	19.48 aA	0.55 aB	8.57 aA
				Salt sensitive			
0	9.00 cB	27.42 aA	4.39 aA	1.46 bA	18.22 aA	0.30 cA	12.31 aA
50	21.14 bA	15.83 bB	2.39 bA	2.23 aA	20.21 aA	0.49 bA	10.73 aA
100	32.62 aA	11.09 cB	1.67 cA	2.25 aA	20.18 aA	1.14 aA	8.79 bA

[a] CHO = soluble carbohydrates; Pro = proline; AA = amino acids.
[b] The contribution of other solutes (estimated by difference) was about 25,4 %
[c] Means, followed by the same capital letters (between genotypes, in each NaCl treatment) and by the same small letters (between NaCl treatment, in each genotype), for each solute, do not differ statistically by the Tukey test at 5 %.

Table 3. Solute contribution to the root osmotic potential (Ψ_s) in two sorghum genotypes grown in nutrient solution containing different NaCl concentrations.

NaCl ($mmol.L^{-1}$)	Solute Contribution to the Ψ_s (%)[a,b,c]						
	$Na^+ + Cl^-$	K^+	Ca^{2+}	Mg^{2+}	CHO	Pro	AA
				Salt tolerant			
0	12.10 cA	35.41 aA	1.95 aA	4.09 aB	7.16 aA	0.19 aA	15.52 aA
50	26.84 bB	22.61 bA	1.41 bB	1.82 bB	6.82 aA	0.18 aA	14.98 aA
100	35.18 aB	19.26 bA	1.05 cA	1.55 bA	8.37 aA	0.21 aA	16.95 aA
				Salt sensitive			
0	10.89 cA	34.63 aA	1.97 aA	5.78 aA	6.02 aA	0.18 aA	12.54 aB
50	32.37 bA	22.73 bA	1.71 bA	3.22 bA	6.18 aA	0.19 aA	11.61 aB
100	42.5 aA	15.22 cB	1.02 cA	2.04 cA	6.45 aB	0.17 aA	10.01 aB

[a] CHO = soluble carbohydrates; Pro = proline; AA = amino acids.
[b] The contribution of other solutes (estimated by difference) was about 22,8 %
[c] Means, followed by the same capital letters (between genotypes, in each NaCl treatment) and by the same small letters (between NaCl treatment, in each genotype), for each solute, t do not statistically differ by the Tukey test at 5 %.

Leaf and root Ψ_s were always lower in the salt sensitive genotype, suggesting that the osmotic adjustment evaluated by the decrease in tissue Ψ_s may not be related to salt tolerance in these two sorghum genotypes. According to our data, the higher decrease in Ψ_s in the salt sensitive genotype was due, at least in part, to a higher Na^+ and Cl^- accumulation, that possibly exceeded the amount needed for the osmotic adjustment and, consequently, affected plant development and growth negatively (Munns and Termaat, 1986; Munns et al., 1995; Munns, 2002). The higher decrease in leaf Ψ_s in the sensitive genotype was also related to a higher soluble organic solute accumulation, especially carbohydrates. However, we believe that this soluble carbohydrate accumulation may be the result of a reduced utilization rather than an increase in their biosynthesis to compensate changes in Ψ_s during salt stress (Munns, 1988; Lacerda, 2000; Serraj and Sinclair, 2002). It appeared that the osmotic adjustment of the salt tolerant genotype was attained by maintaining a better distribution of Na^+ and Cl^- between the vacuole and cytoplasm that resulted in a more adequate concentration of these ions in the latter. In addition, the osmotic pool of the root and leaf tissues of the salt tolerant genotype showed a more adequate K^+ and compatible organic solute concentration than the salt sensitive one. Therefore, it is suggested that the salt tolerant genotype was able to maintain an osmotic pool in the cytoplasm more adequate for cellular metabolism and, consequently, to plant growth under salt stress conditions (Prisco, 1980; Lacerda et al., 2003).

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