

Solute accumulation and distribution during shoot and leaf development in two sorghum genotypes under salt stress

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Abstract

Seedlings of two forage sorghum genotypes (*Sorghum bicolor* (L.) Moench) differing in salt tolerance were subjected to 0 and 100 mM NaCl and shoot development, leaf elongation, and organic and inorganic solutes contents in leaves were measured. Salt stress reduced both shoot development and leaf elongation and enhanced leaf senescence and injury. It also led to accumulation of toxic ions (Na^+ and Cl^-), organic solutes (carbohydrates, amino acids and proline), and reduction of K^+ content in leaf blades. Toxic ion accumulation was higher in the basal zone of the leaf blade and occurred during the period of intense leaf growth while organic solutes accumulation, mainly proline, was higher in the apical zone and occurred when the leaves practically had reached their final size. All these changes were more conspicuous in the sensitive than in the tolerant genotype. The latter also retained more toxic ions in leaf sheath tissue than the former. It is suggested that the reduction in shoot development and leaf elongation were related to toxic ion accumulation and depletion of K^+ ions in the leaf blades. The accumulation of organic solutes in leaves did not appear to be related to salt tolerance. Proline accumulation appears to be a reaction to salt stress damage and not a plant response associated with salt tolerance.

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1. Introduction

Soil salinity is considered one of the most important limitations for food production in irrigated soils of the arid and semi-arid areas of

the world (Munns, 2002). In Brazil, this problem occurs especially in its northern region, where approximately 25 percent of irrigated areas had become saline (Gheyi, 2000).

Salinity reduces plant shoot growth and development, and in grasses this effect is conspicuous on the leaves. NaCl inhibition of leaf expansion may be associated with reduction in leaf turgor, reduction in net CO_2 assimilation, excessive accumulation of toxic ions and/or disturbance in

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mineral nutrition (Greenway and Munns, 1980; Bernstein et al., 1993b).

In sorghum, as in other grasses, leaf elongation is confined to basal part of the leaf, which is enclosed within a whorl of encircling older leaf sheaths. There are some evidences that salt-induced leaf growth inhibition in these plants is related to toxic ion accumulation and mineral disturbance within the growing part of the leaf (Wolf et al., 1990; Läuchli et al., 1994; Bernstein et al., 1995; Hu and Schmidhalter, 1998). Sorghum plants also seem to be able to reduce Na^+ and Cl^- transport from the roots to the shoot (Lacerda et al., 2001) and/or to compartmentalize part of them in specific places in the stems, roots and leaves (Boursier and Läuchli, 1989; Shannon, 1992; Lacerda et al., 2001).

Accumulation of compatible organic solutes, so called osmolytes, in leaves is also a common response to salt stress in sorghum plants. Soluble carbohydrates, amino acids, organic acids, proline and betaines are some of the most common compatible organic solutes found in these plants (Grieve and Maas, 1984; Weimberg et al., 1984; Läuchli et al., 1994; Rosa-Ibarra and Maiti, 1995; Hasegawa et al., 2000). Additionally, increase in leaf proline content is also conspicuous in salt stressed sorghum plants (Lacerda et al., 2001). In the cells, the toxic ions usually accumulate in the vacuoles and the osmotic equilibrium between vacuoles and cytoplasm may be reached by an increase in the synthesis and accumulation of compatible organic solutes in the later (Serrano and Gaxiola, 1994; Hasegawa et al., 2000). These osmolytes may also contribute to the stabilization of protein molecules and membranes (Hare et al., 1998; Hamilton and Heckathorn, 2001) or may serve as reserve for plant metabolism (Serrano and Gaxiola, 1994).

Maintenance of lower concentration of toxic ions, and/or accumulation of compatible osmotica and reduced mineral disturbance in the growing zone of the leaf have been correlated to salt tolerance in sorghum genotypes (Läuchli et al., 1994). There is also indication that salt-induced reduction of photosynthesis in mature leaves may contribute to the growth reduction of young leaves (Munns, 2002). So, protection of mature zones of

the leaf blade by similar mechanisms can also contribute to salt tolerance in plants.

Therefore, the objective of this paper was to test the hypothesis that salt tolerance of two sorghum genotypes seedlings was related to differential toxic ions, osmolytes and mineral nutrients accumulation and/or distribution during shoot and leaf development.

2. Material and methods

2.1. Plant material, seed germination and seedling development conditions

Two forage sorghum (*Sorghum bicolor* (L.) Moench) genotypes, one salt tolerant (CSF20) and another salt sensitive (CSF18), obtained from the Empresa Pernambucana de Pesquisa (IPA) were used (Lima, 1998). The tolerance of these genotypes was determined by Barreto et al. (1995) and confirmed by a screening test, together with other fifteen sorghum genotypes, in a previous experiment (Lacerda, 2000).

Seeds were selected for size and shape, and surface sterilized with 2% sodium hypochlorite for 10 min (Gomes Filho and Prisco, 1978). After extensive rinses with running water and demineralized water, the seeds were germinated in rolls of neutral pH 'germitest' paper partially immersed in 1/5 strength of Clark's nutrient solution, pH 5.5 (Clark, 1975). At the 7th day four seedlings, selected for uniformity in size and form, were transplanted into 2.5 l pots containing aerated full strength Clark's nutrient solution having two times the P concentration, in the absence (control) and presence of 100 ml NaCl (salt stressed). The saline treatment consisted of adding NaCl to the nutrient solution at increments of 25 mM NaCl every 12 h until reaching the final concentration of 100 mM.

All experiments were conducted in a growth room at 25 ± 3 °C, relative humidity of $70 \pm 10\%$, photoperiod of 16 h, and photon flux density of $230 \mu\text{mol m}^{-2} \text{s}^{-1}$. During the experimental period the volume of nutrient solution in the pots was maintained by adding demineralized water, and the pH 5.5 was daily adjusted by

adding either HCl or NaOH. Nutrient solutions from both treatments were changed every 4 days.

2.2. Shoot development, leaf elongation and leaf senescence

Leaf elongation was measured daily by means of a ruler to access both shoot development and leaf elongation. The length of every visible leaf was measured from a fixed reference point (5 mm above the base of the stalk) to the leaf tip (Bernstein et al., 1993b). Shoot development was expressed by the plastochron index (Erickson and Michelini, 1957) determined on the basis of leaf length measurements at the time in which successive leaves reached a reference length of 7 cm (Bernstein et al., 1993a,b).

At the end of the experimental period the total length of leaf blade and of the non-viable area (chlorotic and necrotic region at the leaf tip) of the three following different fully expanded and mature leaves were measured: Leaf 1, Leaf 2 and Leaf 3 (2nd, 3rd and 4th leaf from the top, respectively). The youngest not fully expanded leaf and the leaves older than Leaf 3 were not used in the assay.

Leaf senescence was evaluated by the chlorophyll content determined according to Arnon (1949).

2.3. Leaf solute accumulation and distribution

Organic and inorganic solutes contents were also measured in the leaf 1, 2 and 3 (as described before) at the end of the experimental period.

Solute accumulation and distribution within leaves of salt stressed seedlings, along the experimental period, were also studied. The mature leaf 2 was harvested at days 0, 2, 4, 6 and 8 from the start of salt addition. Starting from day 2 the leaf blades were divided into three parts of approximately the same size (basal, median and apical) and the organic and inorganic solutes and water contents were determined. Starting from the 4th day, the organic and inorganic solutes contents of the leaf sheath (approximately 1.0 cm from the ligule) were also determined.

The amount of osmotically active solutes in different leaf zones, i.e. the 'osmotic pool', was

estimated by dividing the total amount of the analyzed inorganic and organic solutes (Na^+ , Cl^- , K^+ , Ca^{2+} , Mg^{2+} , proline, soluble carbohydrates and amino acids) by the amount of water present in the plant tissue. The contribution of each solute was expressed as a percentage of the total 'osmotic pool'.

2.4. Organic and inorganic solutes determinations

Organic and inorganic solutes were extracted from 300 mg of fresh mass, cut into small pieces, with 15 ml of demineralized water plus 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 more, and the volume completed to 50 ml with demineralized water according to a technique suggested by Lerner et al. (1978) and Weimberg et al. (1984). In this extract soluble carbohydrates (Hodge and Hofreiter, 1962), free amino acids (Moore and Stein, 1948) and proline (Bates et al., 1973) were determined. The contents of Na^+ and K^+ and of Ca^{2+} and Mg^{2+} were determined by emission and atomic absorption spectrophotometry, respectively. Cl^- was determined by visible spectrophotometry, according to Gaines et al. (1984).

2.5. Experimental design and statistical analysis

The experimental design was a completely randomized 2×2 factorial with three replicates per treatment. The data were subjected to analysis of variance, and the means were compared by Tukey's test at 5% probability. When appropriate, the data were subjected to regression analysis (Snedecor and Cochran, 1971).

3. Results

3.1. Shoot development, leaf senescence and solute accumulation in leaves of different ages

Shoot development of both genotypes, measured by the plastochron index, was inhibited by salinity (Fig. 1A). This inhibition was greater in the sensitive than in the tolerant genotype and the

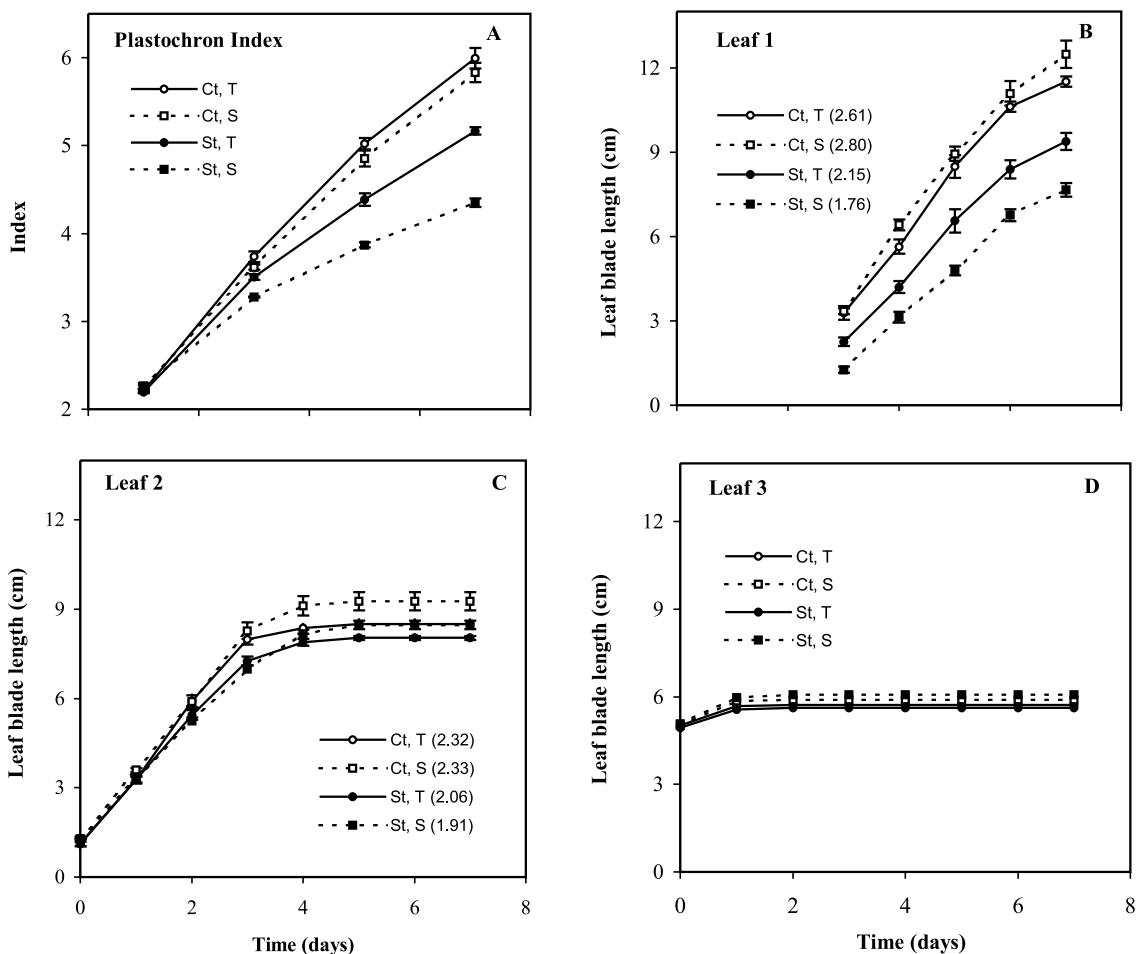


Fig. 1. Plastochron index and leaf blade length of different leaves of two forage sorghum genotypes (T = salt tolerant genotype; S = salt sensitive) as a function of time. Seedlings were grown in nutrient solution containing 0 (Ct = control) or 100 mM NaCl (St = salt stressed); values between parentheses are the angular coefficients of the linear part of the growth curves.

difference between genotypes increased with time. Salt stress led to reductions in both leaf elongation rate and final leaf length of leaves 1 and 2 (Fig. 1B, C). Salinity inhibited 11.2% of the leaf elongation rate of the leaf 2 and 17.5% of the leaf 1 of the salt tolerant genotype, while in the sensitive genotype the reductions were 18.3 and 37.0%, respectively. Leaf elongation rate and the final leaf length of the leaf 3 of both tolerant and sensitive genotypes were not affected by salt stress (Fig. 1D). This could be due to the fact that when salt stress was imposed this leaf had practically reached its final size.

The chlorophyll contents of the leaves 1, 2 and 3 were reduced by salinity, and this effect was more

conspicuous in the sensitive genotype (Table 1). The leaves 2 and 3 of salt stressed plants showed symptoms (starting from the tip of their blades) of tissue dehydration and chlorosis followed by tissue necrosis. These symptoms were conspicuous in the sensitive genotype, in which the injured zone corresponded to 32 and 52% of the length of the leaves 2 and 3, respectively. Although the leaves of the salt tolerant genotype had also been affected, the injury reached only 19 and 27% of the leaves 2 and 3, respectively (data not shown).

Leaf toxic ions content ($\text{Na}^+ + \text{Cl}^-$) in control and salt stressed seedlings of both genotypes increased from the older (leaf 3) to the younger

Table 1

Chlorophyll content of different leaves from seedlings of two forage sorghum genotypes grown in nutrient solution containing 0 (Control) or 100 mM NaCl (stressed)

NaCl (mM)	Total Chlorophyll (% of dry mass) ^a					
	Leaf 1		Leaf 2		Leaf 3	
	Tolerant	Sensitive	Tolerant	Sensitive	Tolerant	Sensitive
0	1.92 aA	2.05 aA	1.95 aA	2.02 aA	1.41 aA	1.33 aA
100	1.71 aA	1.52 bA	1.52 bA	1.18 bB	0.95 bA	0.68 bB

^a The values are means of three replicates. Leaves were numbered from the younger to the older (Section 2). Means followed by the same capital letter in the rows or small letter in the columns, for each leaf type, do not differ statistically at 5% probability, by Tukey's test.

leaf (leaf 1), and this increase was much more apparent in the leaves of salt stressed plants of the sensitive genotype (Table 2). On the other hand, younger leaves of control seedlings of both genotypes had a higher K⁺ content than the older ones (Table 2). When the seedlings were stressed the leaves 1 and 2 from both genotypes suffered a

reduction in K⁺ content. This reduction was greater in the sensitive than in the tolerant genotype, and was more conspicuous in the younger leaf (leaf 1).

Accumulation of soluble carbohydrates and proline increased in both salt sensitive and salt tolerant genotypes with salt treatment, in all

Table 2

Organic and inorganic solute contents of different leaves from seedlings of two forage sorghum genotypes grown in nutrient solution containing 0 (Control) or 100 mM NaCl (stressed)

NaCl (mM)	Solute (mol kg ⁻¹ dry mass) ^a					
	Leaf 1		Leaf 2		Leaf 3	
	Tolerant	Sensitive	Tolerant	Sensitive	Tolerant	Sensitive
Na⁺						
0	0.015 bA	0.019 bA	0.035 bA	0.025 bA	0.023 bA	0.029 bA
100	0.555 aB	0.657 aA	0.358 aB	0.593 aA	0.315 aB	0.369 aA
Cl⁻						
0	0.240 bA	0.201 bA	0.210 bA	0.143 bA	0.122 bA	0.101 bA
100	0.639 aB	0.746 aA	0.502 aA	0.527 aA	0.312 aA	0.307 aA
K⁺						
0	0.720 aA	0.676 aA	0.601 aA	0.511 aB	Nd	Nd
100	0.490 bA	0.390 bB	0.510 bA	0.405 bB	Nd	Nd
Proline						
0	0.004 aA	0.006 bA	0.006 bA	0.008 bA	0.007 bA	0.011 bA
100	0.006 aB	0.010 aA	0.019 aB	0.049 aA	0.033 aB	0.061 aA
Soluble carbohydrates						
0	0.494 bA	0.451 bA	0.577 bA	0.567 bA	0.805 bA	0.796 bA
100	0.632 aA	0.669 aA	0.869 aB	0.950 aA	0.986 aB	1.260 aA

^a The values are means of three replicates. Leaves were numbered from the younger to the older (see Section 2). Means followed by the same capital letter in the rows or small letter in the columns, for each leaf type and solute, do not differ statistically at 5% probability, by Tukey's test.

analyzed leaves (Table 2). Under salt treatment all leaves of the sensitive genotype showed higher proline accumulation than the tolerant one. The same tendency was observed for soluble carbohydrates, except that in leaf 1 no statistical difference between the genotypes was detected. These organic solutes accumulated preferentially in the older and more injured leaves of both genotypes and it was much more apparent with respect to proline content in salt stressed seedlings. The solute content in leaf 3 was approximately 2 and 1.3 times higher than in leaf 2, in the salt sensitive and salt tolerant genotypes, respectively (Table 2).

3.2. Leaf growth and solute accumulation as a function of stress time

The relationship between solute accumulation and salt tolerance during leaf growth in salt stressed seedlings was studied. The dry matter yield of leaf 2 increased with time, but the high initial growth rates observed during the first days, after 4 days of NaCl treatment, continuously decreased in both genotypes, especially in the sensitive one (Fig. 2A). Accumulation of Na^+ (Fig. 2B) and Cl^- (Fig. 2C) in this leaf coincided with the reduction in growth rates and occurred with higher intensity in the salt sensitive genotype. The Na^+ content in the leaf sheath was higher than in the leaf blade in both genotypes, and salt tolerant genotype maintained a significantly higher Na^+ sheath/blade ratio during all the stress periods (Table 3).

The Na^+/K^+ ratio in leaf 2 blade increased with the time of salt treatment up to the 4th day and then kept essentially constant in both genotypes (Table 4). The average K^+ content in the salt tolerant and sensitive genotypes during the period of 4–8 days of stress were 580 and 300 mmol kg^{-1} , respectively. The former value corresponded to the K^+ leaf content of the salt tolerant genotype but the later only to about 50% of the K^+ leaf content of the salt sensitive genotype at the beginning of stress treatment. On the other hand, the leaf blade of the salt sensitive genotype accumulated much more Na^+ than the one from the salt tolerant, and at the end of the experimental period the Na^+ content in leaf blades of this

genotype was about 1.9 times higher than in the salt tolerant. Consequently, the salt sensitive kept a Na^+/K^+ ratio about 2.5 times higher than the salt sensitive genotype after 2 days of salt stress (Table 4).

The accumulation of soluble organic solutes as a function of time was quite different from the toxic Na^+ and Cl^- ions accumulation. Carbohydrates (Fig. 2D) and proline (Fig. 2E) content rapidly increased after 4 days of salt treatment, when leaf 2 had practically reached its final size, in both genotypes (Fig. 2A). The accumulation of these solutes, mainly proline, was always greater in the sensitive genotype. Amino acids concentration, on the contrary, (Fig. 2F), reduced with time, reaching a minimum between 4 and 6 days of stress, in both genotypes.

3.3. Leaf blade water content and solute distribution as a function of stress time

The water content decreased with time of stress in the basal, median and apical zones of leaf 2, in both sorghum genotypes (Table 5). The greatest reduction in water content occurred in the apical and the lowest in the basal zone of leaf 2 blade. This difference was more conspicuous in the salt sensitive than in the salt tolerant genotype, i.e. there was a progressive loss of leaf water starting from the tip toward the base of the leaf blade, and this was more severe in the apical zone of the salt sensitive genotype. The accumulation of Na^+ and Cl^- followed an opposite pattern, i.e. these ions contents decreased from the base to the tip of the leaf blade. Additionally the sensitive genotype showed the highest toxic ion content in the basal zone and the lowest water content in the apical zone of the leaf blade.

Soluble carbohydrates and proline contents increased with the time of seedling exposure to salt stress at different zones of leaf 2 blade and this was conspicuous in the apical zone, in both genotypes (Fig. 3). This pattern of solute accumulation was the opposite of the one observed for toxic ions (Fig. 4). The accumulation of carbohydrates was slightly higher in the salt sensitive genotype, especially in the apical zone. The accumulation of proline occurred in all leaf blade

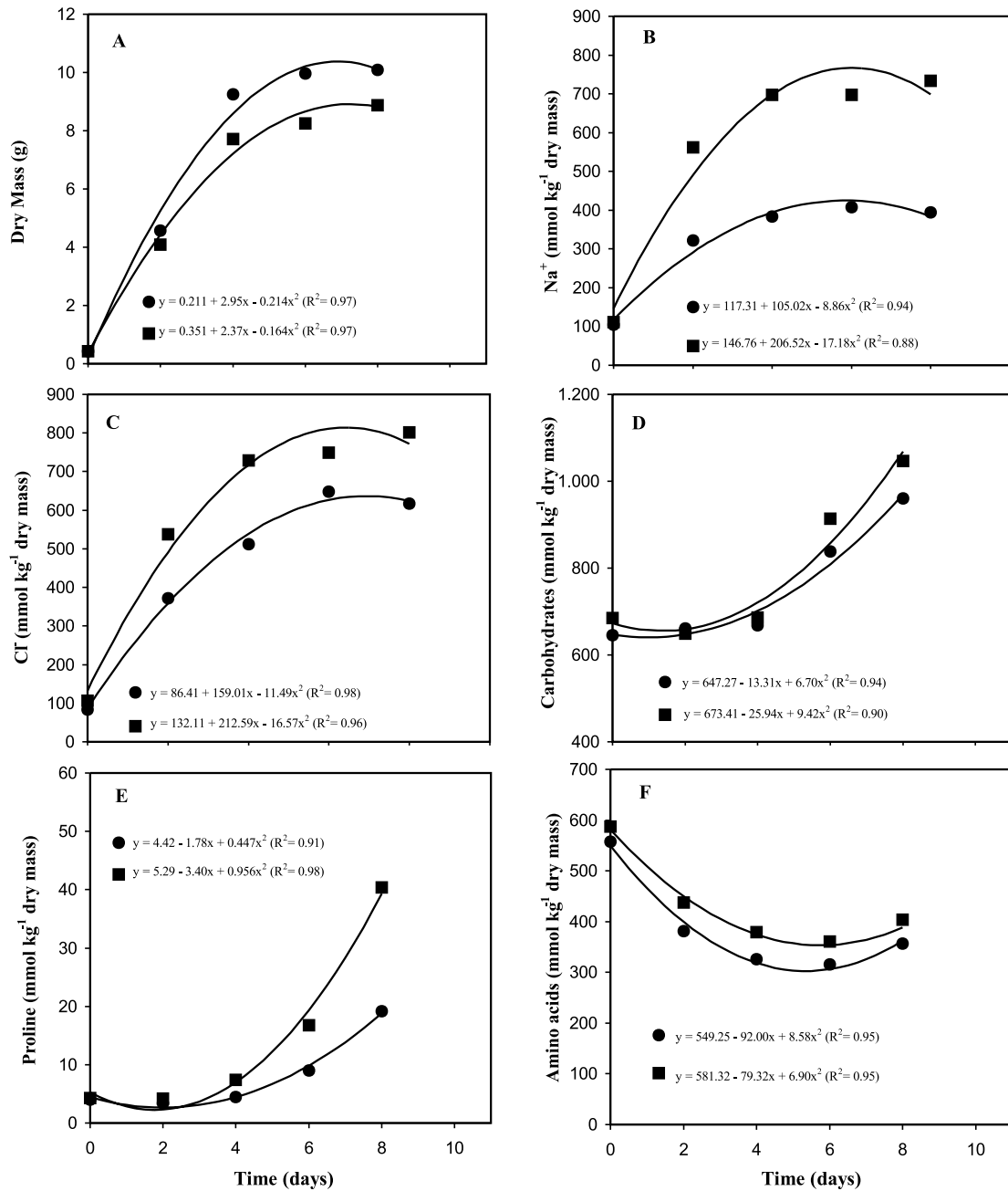


Fig. 2. Dry mass (A), Na⁺ (B), Cl⁻ (C), soluble carbohydrates (D), proline (E), and soluble amino acids (F) in leaf 2 from seedlings of two forage sorghum genotypes grown in nutrient solution containing 100 mM NaCl, as a function of the time; salt tolerant (●) and salt sensitive (■).

zones, except in the basal zone of leaf blade of the salt tolerant genotype (Fig. 3). The apical zone

showed a rapid and intense increase in proline accumulation after the 4th day from the start of

Table 3

Na⁺ content in different parts of the mature leaf 2 from seedlings of two forage sorghum genotypes, at different time of exposure to 100 mM NaCl

Time (days)	Na ⁺ (mol kg ⁻¹ dry mass) ^a					
	Sheath ^b		Blade		Sheath/blade	
	Tolerant	Sensitive	Tolerant	Sensitive	Tolerant	Sensitive
4	1.27 B	1.53 A	0.38 B	0.70 A	3.3 A	2.2 B
6	1.39 B	1.67 A	0.41 B	0.70 A	3.4 A	2.4 B
8	1.50 A	1.62 A	0.39 B	0.73 A	3.8 A	2.2 B

^a The values are means of three replicates. Leaves were numbered from the younger to the older (see Section 2). Means followed by the same capital letter in the rows, for each analyzed part, do not differ statistically at 5% probability, by Tukey's test.

^b The sheath tissue was analyzed after 4 days, when the ligule become apparent.

the stress, and this increase was much more conspicuous in the salt sensitive than in the salt tolerant genotype (Fig. 3).

As expected from the data shown in Figs. 3 and 4, the estimated values for different components of the 'osmotic pool' indicated that the relative contribution of toxic ions decreased from the basal to the apical zone of leaf 2 (data not shown) of stressed seedlings from both salt tolerant and sensitive genotypes. However, the relative contribution of organic solutes followed an opposite trend and K⁺ stayed more or less constant throughout the leaf blade length.

The contribution of toxic ions to the 'osmotic pool' of the median zone of leaf 2 blade was higher in the salt sensitive than in the salt tolerant genotype while K⁺ contribution was greater in the salt tolerant genotype, at all salt treatment time tested (Table 6). Although the salt sensitive genotype had slightly higher soluble carbohydrate contents during leaf development (Fig. 3), the

contribution of this solute to the 'osmotic pool' of the salt tolerant genotype was higher than that of the salt sensitive (Table 6), especially during the period of most intensive leaf growth (Fig. 2A). During this period the contribution of proline to the 'osmotic pool' was about the same for both genotypes, however, at the last days of the experimental period it became higher in the salt sensitive than in the salt tolerant genotype.

4. Discussion

Sorghum when exposed to high levels of NaCl exhibits reduced leaf growth rate, leaf emergence rate, and overall shoot development (Bernstein et al., 1993b). These results were confirmed here using two sorghum genotypes differing in their tolerance to salinity. Under salt stress these sorghum genotypes not only reduced both leaf elongation rate and the final length of leaves 1

Table 4

Na⁺/K⁺ ratio in the mature leaf 2 from seedlings of two forage sorghum genotypes, at different time of exposure to 100 mM NaCl

Genotypes	Na ⁺ /K ⁺ ratio				
	Time (days)				
	0	2	4	6	8
Tolerant	0.18 A	0.39 B	0.66 B	0.77 B	0.71 B
Sensitive	0.20 A	0.83 A	2.13 A	1.90 A	2.32 A

Means within the column followed by the same letter do not differ statistically at 5% probability, by Tukey's test. The values are means of three replicates.

Table 5

Water content in different parts of the mature leaf 2 from seedlings of two forage sorghum genotypes, at different time of exposure to 100 mM NaCl

Time (days)*	Water Content (%)*					
	Basal		Median		Apical	
	Tolerant	Sensitive	Tolerant	Sensitive	Tolerant	Sensitive
2	87.3	88.0	86.6	86.5	84.4	84.0
4	86.0	86.6	84.5	84.5	81.5	80.5
6	85.7	85.6	83.9	83.7	79.8	76.1
8	85.1	84.1	83.9	82.0	77.8	74.6

*, The values are means of three replicates.

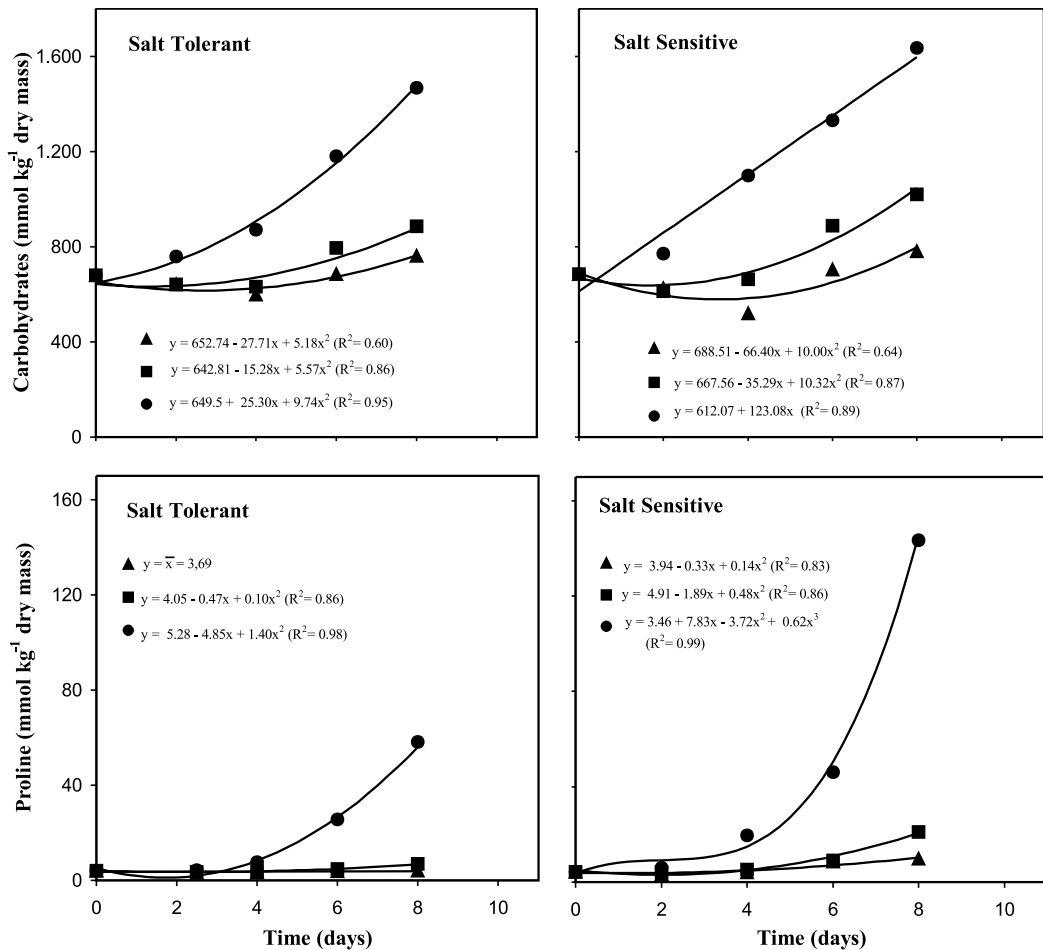


Fig. 3. Soluble carbohydrates and proline at different leaf blade zones of leaf 2 from seedlings of two forage sorghum genotypes grown in nutrient solution containing 100 mM NaCl, as a function of the time; basal (▲), median (■) and apical (●) zones of the leaf blade.

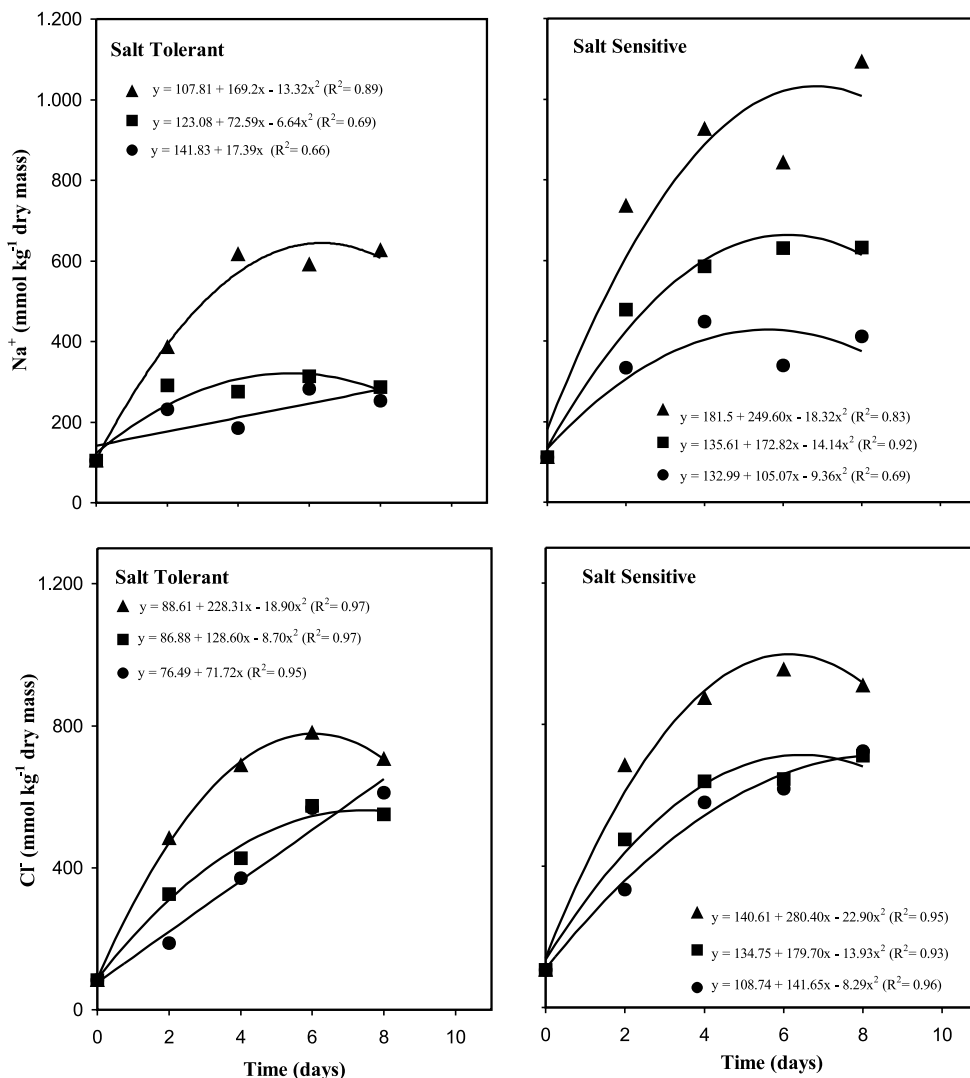


Fig. 4. Na⁺ and Cl⁻ contents at different leaf blade zones of leaf 2 from seedlings of two forage sorghum genotypes grown in nutrient solution containing 100 mM NaCl, as a function of the time; basal (▲), median (■) and apical (●) zones of the leaf blade.

and 2 but also reduced the plastochron index (Fig. 1). So, stressed plants also had less and smaller leaves than control, especially those of the salt sensitive genotype.

Additionally, based on the reduction of chlorophyll content and on the increasing intensity of leaf injury symptoms at leaves 1, 2 and 3 an enhancement of leaf senescence was obvious. Salinity-induced enhancement of leaf senescence, greater in the salt sensitive genotype, and probably related to hormonal imbalance (Prisco and

O'Leary, 1972; Dangl et al., 2001), seems to have a good correlation with genotype salt sensitivity, as it was suggested for rice (Lutts et al., 1996b).

Under saline conditions the salt sensitive genotype not only accumulated more toxic ions, i.e. Na⁺ and Cl⁻, in the leaves than the salt tolerant (Table 2), especially in the leaves 1 and 2, but also showed larger reduction in leaf elongation (Fig. 1). In this experiment, the accumulation of toxic ions coincided with a period of very intense leaf growth (Fig. 2B and C), suggesting that salt tolerance

Table 6

Contribution of different solutes to the total 'osmotic pool' at the median zone of the mature leaf 2 of two forage sorghum genotypes, after different times of exposure to 100 mM NaCl

Days	Contribution to 'Osmotic Pool' (%) ^a									
	Na ⁺ + Cl ⁻		K ⁺		Carbohydrates		Proline		Other solutes ^b	
	ST ^c	SS	ST	SS	ST	SS	ST	SS	ST	SS
0	9.1	11.3	25.9	24.4	38.0	35.2	0.2	0.2	26.8	28.9
2	25.1	37.2	33.4	23.1	26.6	22.2	0.1	0.1	14.8	17.4
4	31.1	45.8	24.9	11.4	28.1	24.7	0.2	0.2	15.7	17.9
6	33.2	41.2	20.4	10.9	29.8	29.3	0.2	0.3	16.4	18.1
8	30.5	40.8	18.6	9.5	32.3	31.0	0.2	0.6	18.4	18.1

^a Total 'osmotic pool' was estimated by dividing the amount of all analyzed solutes (Na⁺, Cl⁻, K⁺, Ca²⁺, Mg²⁺, proline, soluble carbohydrates and amino acids) by the amount of water in the leaf tissue: the contribution of each solute was expressed as a percentage of the total 'osmotic pool'. The values are means of three replicates.

^b Other solutes: Ca²⁺, Mg²⁺ and amino acids.

^c ST = salt tolerant genotype; SS = salt sensitive genotype.

could be associated with the synchronization between the rate of ion transport to the shoot and the plant capacity to compartmentalize them in different tissues or cells (Boursier and Läuchli, 1989; Lacerda et al., 2001). Besides, a control mechanism of absorption and transport of these toxic ions to the leaves may be involved, at least in sorghum (Greenway and Munns, 1980; Salim and Pitman, 1983; Moya et al., 1999). Furthermore, the leaves that suffered the stronger elongation inhibition as a result of salt stress (Fig. 1) were the ones that showed the greatest reduction in K⁺ content, especially in the salt sensitive genotype (Table 2). The Na⁺/K⁺ ratio, which was very small in the control plants, increased substantially in the two genotypes after the plants were exposed to high levels of NaCl, particularly in the sensitive one (Table 4). Leaf elongation in this genotype, therefore, was reduced not only by accumulation of toxic ions but also by an inadequate concentration of K⁺, which is essential to cell enlargement, especially in young leaves (Taleisnik and Grunberg, 1994).

A direct relationship between leaf injury and toxic ions content and/or between leaf injury and inhibition of leaf elongation has not been observed. The injured zone increased with leaf age, while the inhibition of leaf elongation (Fig. 1) and toxic ions content decreased (Table 2). The in-

tensity of leaf injury was probably related to the duration of salt treatment. On the other hand, since the growth of younger leaves is dependent on photosynthates produced by mature leaves (Munns, 2002) and these leaves were more severely injured in salt sensitive genotype, probably the degree of injury of mature leaves could be used as a criterion for discrimination of sorghum genotypes with differential tolerance to salt.

Salt-induced organic solutes accumulation was higher in the older and more injured leaves (Table 2) and occurred especially at later stages of leaf growth, especially in the salt sensitive genotype (Fig. 2D–F). So, organic solutes accumulation, especially proline accumulation, does not seem to be related to salt tolerance or to salt stress acclimation, but probably is just an expression of the seedling reaction to the stress damage. Similar conclusions have been reached by Moftah and Michel (1987) and Lutts et al. (1996a) studying organic solute accumulation in soybean and rice, respectively. Both sorghum genotypes accumulated large amounts of carbohydrates during salt stress (Fig. 2D and Table 2) that could be used for cell osmotic adjustment (Weimberg et al., 1984; Lacerda et al., 2001). Excessively high concentration of these solutes, however, by a feedback mechanism may inhibit photosynthesis (Munns,

1993) causing a reduction of leaf growth, as it was observed in the salt sensitive genotype.

Growth in grass leaf is restricted to the small intercalary meristem at the basal part of the leaf near the point of attachment to the node (Bernstein et al., 1993a,b), i.e. there is a gradient of tissue age from the basal (youngest) to the apical (oldest) zone of the leaf blade. There are evidences that salt tolerance could be associated to low concentration of toxic ions and/or accumulation of compatible osmotica in both growing and mature zones of the leaf blade (Bernstein et al., 1995; Munns, 2002). Our data has shown large differences in organic and inorganic solutes concentrations as well as in water content at different zones of the leaf blade. Toxic ion accumulation was higher in the basal zone (Fig. 4) while dehydration (Table 5) and organic solutes accumulation (Fig. 3) were conspicuous at the apical zone. The decreases in water content and in toxic ion accumulation from the base toward the tip of the leaf blade were also found by Bernstein et al. (1995). This dehydration process starting at the leaf tip may be related to a loss in transpirational control or a reduction in water entry into this zone relative to other areas of the leaf (Bernstein et al., 1995) or to both.

The increase in proline content (Fig. 3) occurred when the water content of the tissue was low (Table 5). Proline contribution to the 'osmotic pool', however, was quite low throughout salt stress, and the salt sensitive genotype accumulated this organic solute at higher proportion than did the salt tolerant genotype (Table 6). So, probably proline accumulation is a result of tissue reaction to stress damage (Hanson et al., 1977; Ferreira et al., 1979) rather than a tissue response to salinity acclimation or adaptation. The contribution of soluble carbohydrates to the 'osmotic pool' was high in both genotypes, especially in the salt tolerant (Table 6) and during the period of most intensive leaf growth (Fig. 2A). Furthermore, the 'osmotic pool' in the leaves of the salt tolerant genotype was richer in K^+ and poorer in toxic ions than in the salt sensitive genotype (Table 6). Therefore, the microenvironment existing within the cells of the salt tolerant genotype seems to favor a more efficient metabolism with a conse-

quent better growth under stress conditions than in the salt sensitive genotype.

Our data, therefore, supported the hypothesis that the salt-induced reductions in shoot development and leaf elongation were due to differential toxic ions accumulation and/or distribution and K^+ depletion in the leaves during shoot and leaf development. Salinity enhancement of mature leaf senescence, lower in salt tolerant genotype, seems to be a consequence of better control of ions absorption and its transport to the leaves. On the other hand, osmolytes accumulation, especially proline, did not seem to be related to salt tolerance in sorghum, but appeared to be a consequence of tissue dehydration.

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