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Full Length Research Paper

Physiological and biochemical characteristics of *Sorghum bicolor* and *Sorghum sudanense* subjected to salt stress in two stages of development

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This study compared the salt tolerance of *Sorghum bicolor* and *Sorghum sudanense* plants subjected to salt stress in two distinct phases of development. We analyzed the growth, gas exchange, the relative chlorophyll content (SPAD index) and the accumulation of organic and inorganic solutes. The experimental design was a randomized 2 × 3 × 2 factorial that included two species (*S. bicolor* and *S. sudanense*), three concentrations of salts in the irrigation water (electrical conductivities of 0.0, 4.0 and 8.0 dS m^{-1}) and two periods of salt stress application to the plants [from sowing until 24 days later (Phase I) and from the 25th to the 49th day after sowing (Phase II)] with five replicates. Based on gas exchange and growth results, the plants in Phase I were more sensitive to the effects of salt stress than were those in Phase II. However, we did not observe major differences in salt tolerance between *S. bicolor* and *S. sudanense*. The Phase I sorghum plants showed a lower accumulation of organic solutes and a higher concentration of toxic ions, which confirmed that Phase I was more sensitive to salinity than Phase II.

Key words: Photosynthesis, plant growth, salinity, sorghum, salt tolerance.

INTRODUCTION

Agricultural productivity is limited by physical and chemical factors, which include soil salinity and sodicity (Rengasamy, 2002). Problems associated with salinity are more pronounced in arid and semiarid regions. In these regions, intense evaporation in combination with low rainfall contributes to the increased concentration of salts in the soil and surface water (Daker, 1988). In plants, salinity creates the following two effects: an osmotic effect, which results from high concentration of solutes in the soil solution and causes a water deficit, and an ionic effect, which arises from the accumulation of toxic ions, changes the homeostasis of the cells and causes nutritional disorders (Hasegawa et al., 2000).

Responses to salinity depend on the plant species, the cultivar and the stage of plant development, as well as the salt concentration, the duration of exposure to stress and the method of salt application (Shannon and Grieve, 1999; Munns and Tester, 2008). In glycophytes, the seedling stage is more sensitive to salts than germination stage (Hosseini et al., 2002; Marques et al., 2011). In later stages of development, all major plant processes (including photosynthesis, respiration, protein synthesis, water relations and enzymatic reactions) are affected by salinity (Munns, 2002; Parida and Das, 2005), but the degree of inhibition depends upon the level of tolerance

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to that stress.

Sorghum was identified as a key plant species for comparative genome analysis with grasses and as a source of beneficial genes for agriculture (Mullet et al., 2001). *Sorghum bicolor* and *Sorghum sudanense* are two species of great importance for agriculture because hybrids from these species have disease resistance and high yields as forage (Zhang et al., 2008). The salt tolerance in *S. bicolor* has been evaluated in plants at different stages of development. Specifically, salt tolerance has been investigated during germination (Oliveira and Gomes-Filho, 2009), in the early seedling stages (Swami et al., 2011), in plants in vegetative growth phase (Bavei et al., 2011) and in the final stages of physiological maturity (Jafari et al., 2009). These studies show that *S. bicolor* is moderately tolerant to salinity, which indicates this plant may help improve our understanding of the effects of salt stress in agronomically important crops. On the other hand, there have been few studies investigating the salinity tolerance of *S. sudanense* (Feijão et al., 2011).

This study aimed to compare the salt tolerances of *S. bicolor* and *S. sudanense* when subjected to salt stress at two distinct phases of development. We investigated the resultant growth, gas exchange, relative chlorophyll content (SPAD index) and accumulation of organic and inorganic solutes in each of these plants.

MATERIALS AND METHODS

Sowing, growth conditions and harvest of material

Seeds of *S. bicolor* (genotype CSF 20) and *S. sudanense* (variety Sudan IPA 4202) were sown in pots containing 10 L of sifted sand and washed with distilled water. Six seeds were sown in each pot. Seven days after sowing, the seedlings were thinned, based on the criterion of uniformity, leaving only two seedlings per pot. The plants were subjected to three salt concentrations in the irrigation water, which corresponded to the electrical conductivities (ECw) of 0.0, 4.0 and 8.0 dS m^{-1}. The salt was applied to the plant in the following two distinct phases of development: from sowing until 24 days after sowing (Phase I) and from the 25th to the 49th day after sowing (Phase II). The salts added to the irrigation water were NaCl, $CaCl_2.2H_2O$ and $MgCl_2.6H_2O$ in the proportion of 7:2:1 (Rhoades et al., 2000). Irrigation water was applied daily, according to the principle of lysimeter drainage (Bernardo et al., 2008), keeping the soil at field capacity and adding a leaching fraction of 15% to prevent excessive accumulation of salts. In Phase I of development, seeds or plants were irrigated daily with water having an ECw of 0.0, 4.0 or 8.0 dS m^{-1}, and every five days, the plants were irrigated with Hoagland nutrient solution (diluted 1:2). In Phase II, plants initially irrigated with distilled water (from sowing until the 24th day of the experiment) were irrigated daily from the 25th to the 49th day of the experiment with water having an ECw of 0.0, 4.0 or 8.0 dS m^{-1}. Every five days the plants were irrigated with the aforementioned nutrient solution. During the experimental period, the average daytime and nighttime temperatures and relative humidity were 36 ± 3.5°C, 30.5 ± 2.5°C and 75.0 ± 1.5%, respectively. At 25 and 50 days after sowing, the plants in Phases I and II of development, respectively, were collected and divided into leaves, stems and roots. Some of the leaves were frozen, lyophilized and converted to powder, which was used for the analysis of organic and inorganic solutes. The remaining leaves, stems and roots were used to determine the dry mass to estimate growth.

Gas exchange, chlorophyll and growth plant

A day before each harvest, the gas exchange of the plants was measured using a portable infrared gas analyzer (IRGA, mod. LCi, ADC, Hoddesdon, UK), which included an artificial light source (1,200 mmol m^{-2} s^{-1} intensity) (ADC, Hoddesdon, UK). The measurements were performed in the first two fully expanded leaves from the apex of each plant and at room temperature and humidity. The relative chlorophyll content was estimated in these leaves using a portable chlorophyll meter (SPAD-502 Minolta, Osaka, Japan). We determined the leaf area (LA), using an area meter (LI-3100, Li-Cor, Inc. Lincoln, NE, USA). The dry mass of different plant parts were obtained after drying at 60°C for 48 h. The dry mass of the shoot (DMS) was the sum of the dry mass of the leaves (including the mass of the lyophilized material) and the stem of each plant. From the DMS and the dry mass of the roots (DMR), we estimated the DMR/DMS ratio.

Preparation of extracts

To determine the content of organic and inorganic solutes, extracts were prepared as described by Cataldo et al. (1975), with minor modifications. In each test tube, 10 ml of deionized water was added to 100 mg of freeze-dried powdered leaves. The samples were shaken and incubated for 1 h in a water bath at 45°C; the tubes were agitated every 15 min. The samples were then centrifuged for 15 min at 3000 × g, and the supernatant (extract) collected, filtered through filter paper and stored at 4°C.

Determination of organic and inorganic solutes

The content of soluble carbohydrates was determined according to the method of Dubois et al. (1956). We also determined the free amino acid content (Yemm et al., 1955) and the proline content (Bates et al. 1973). The Na^+ and K^+ contents were determined by flame photometry, while the Cl^- content was determined by the method described by Gaines et al. (1984).

Experimental design and statistical analysis

The experimental design was completely randomized in a 2 × 3 × 2 factorial. The design included two species (*S. bicolor* and *S. sudanense*), three concentrations of salts in the irrigation water (ECw of 0.0, 4.0 or 8.0 dS m^{-1}) and two periods of salt stress application to the plants (Phase I and Phase II), with five replicates. The results were subjected to analysis of variance, and the means at each stage of development were compared by Tukey test at 5% probability, using the program Assistat.

RESULTS

Gas exchange

The photosynthetic rate (*A*) of the plants of *S. bicolor* and *S. sudanense* was not affected by salinity in Phase I of development (Figure 1A). In this same period, stomatal conductance (g_s) was significantly reduced with increasing salinity in both species, which did not differ significantly

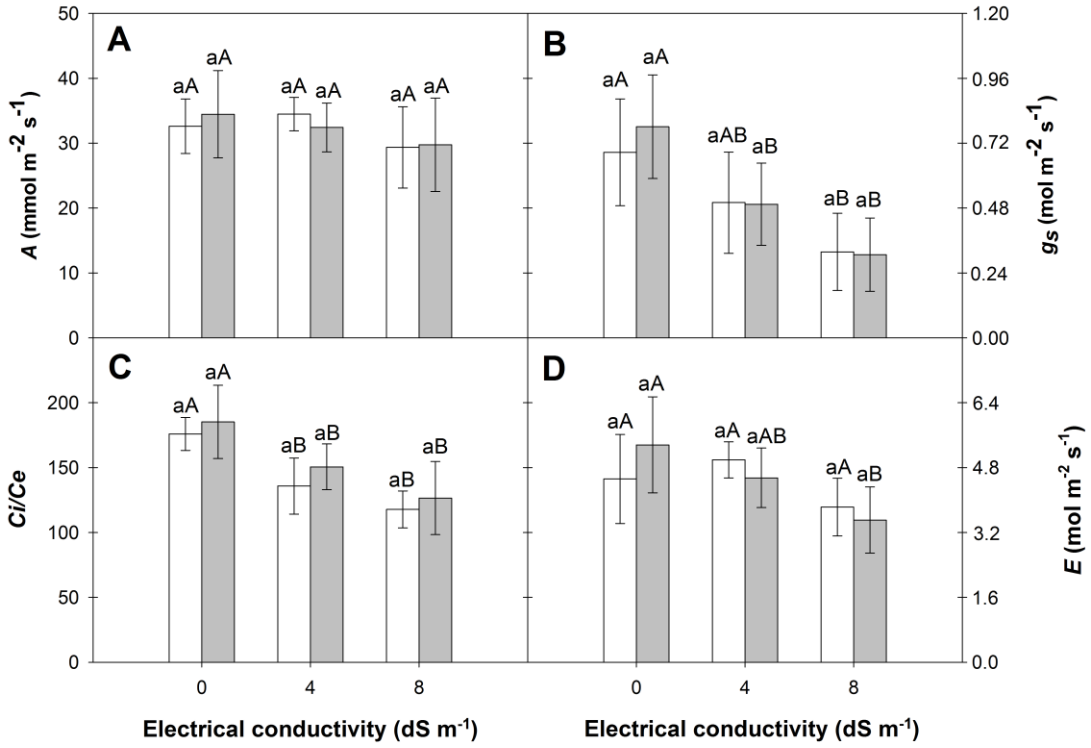

Figure 1. Gas exchanges in plants of two species of sorghum, *Sorghum bicolor* (□) and *Sorghum sudanense* (■), subjected to three saline treatments (0.0, 4.0 and 8.0 dS m^{-1}) applied from sowing to 24 days later (Phase I). A – Photosynthesis (*A*), B – Stomatal conductance (g_s), C – Ratio between the external and internal concentrations of CO_2 (C_i/C_e), D – Transpiration (*E*). Means followed by same lowercase letter for species and capital letter to saline treatments are not significantly different ($P \leq 0.05$). Error bars in each column represent the standard error.

from each other (Figure 1B). At the highest level of salinity (8.0 dS m^{-1}), the g_s was reduced by 53% in *S. bicolor* and by 60% in *S. sudanense*, compared with respective controls (Figure 1B). The relationship between the internal and external concentration of CO_2 (C_i/C_e) in Phase I of development was significantly reduced with increasing salinity; we observed 33 and 32% reductions, respectively, in *S. bicolor* and *S. sudanense* (Figure 1C). In addition, the C_i/C_e at this stage of plant development also did not differ significantly between the species studied. Also in Phase I, transpiration (*E*) was not affected by salinity in *S. bicolor*, but it decreased by 34% in *S. sudanense,* compared with controls (Figure 1D). Like the other gas exchange parameters of the plants in Phase I, there was no significant difference in transpiration between *S. bicolor* and *S. sudanense*.

Salinity reduced the photosynthetic rate of *S. bicolor* plants in Phase II of development; however, this reduction was similar in both saline treatments (Figure 2A). In *S. sudanense*, the photosynthetic rate remained unchanged in plants at 8.0 dS m^{-1} salinity compared with control (Figure 2A). In both species, the g_s in Phase II plants treated with 8.0 dS m^{-1} salinity did not differ significantly from the control (Figure 2B). In plants treated with 0.0 and 8.0 dS m^{-1} salinity in Phase II, the C_i/C_e, was higher in *S. bicolor* than in *S. sudanense* (Figure 2C). Salinity did not affect the C_i/C_e of the two species of sorghum in Phase II; however, in saline treatments, this parameter was higher in *S. sudanense* than in *S. bicolor* (Figure 2D).

Chlorophyll

The relative chlorophyll content (SPAD index) of *S. bicolor* plants was not altered by the salinity in either phase of development (Figure 3). In Phase I *S. sudanense* plants, SPAD index increased as a direct function of salinity (Figure 3A). At a salinity equivalent to ECw of 8.0 dS m^{-1}, the SPAD index increased 15% over the control levels. In Phase II plants, the SPAD index of *S. sudanense* was higher than that of *S. bicolor* (Figure 3B).

Plant growth

Salinity strongly reduced the leaf area (LA) of Phase I plants in both species. The effect was especially dramatic

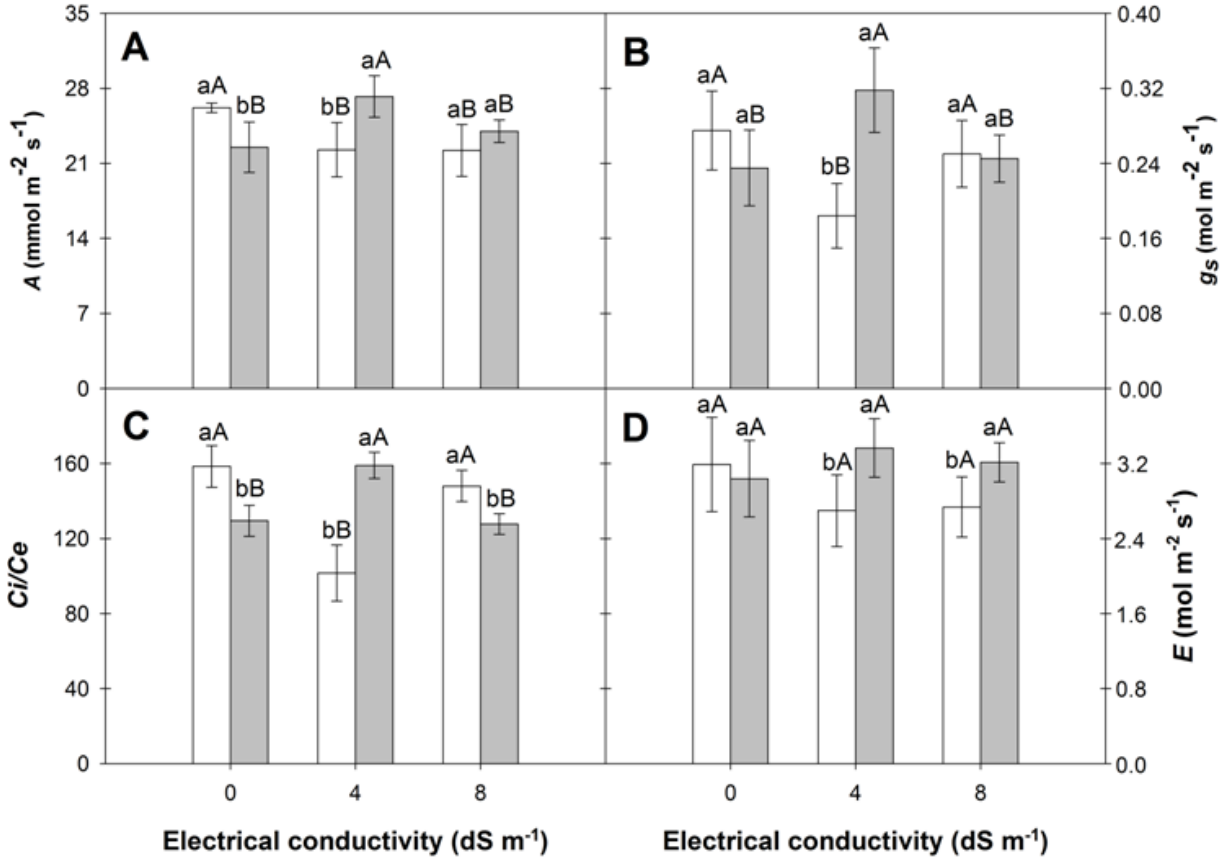

Figure 2. Gas exchanges in plants of two species of sorghum, *S. bicolor* (□) and *S. sudanense* (■), subjected to three saline treatments (0.0, 4.0 and 8.0 dS m^{-1}) applied from the 25th to the 49th day after sowing (Phase II). A, Photosynthesis (*A*); B, Stomatal conductance (g_s); C, Ratio between the external and internal concentrations of CO_2 (C_i/C_e), D, Transpiration (*E*). Means followed by same lowercase letter for species and capital letter to saline treatments are not significantly different ($P \leq 0.05$). Error bars in each column represent the standard error.

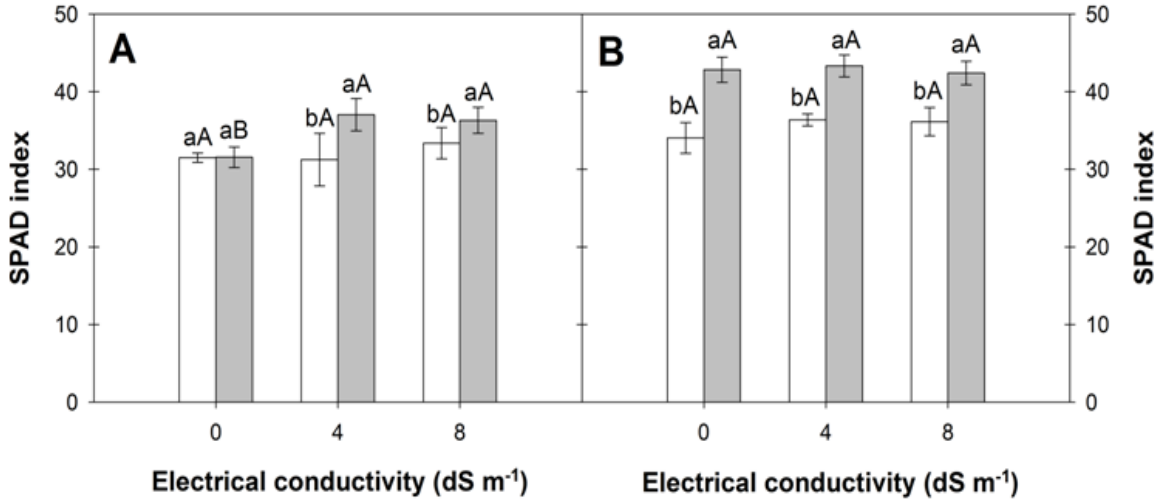

Figure 3. Relative chlorophyll content (SPAD index) of plants of two species of sorghum, *S. bicolor* (□) and *S. sudanense* (■), subjected to three saline treatments (0.0, 4.0 and 8.0 dS m^{-1}), applied from sowing to 24 days later (A, Phase I) or from the 25th to the 49th day after sowing (B, Phase II). Means followed by same lowercase letter for species and capital letter to saline treatments are not significantly different ($P \leq 0.05$). Error bars in each column represent the standard error.

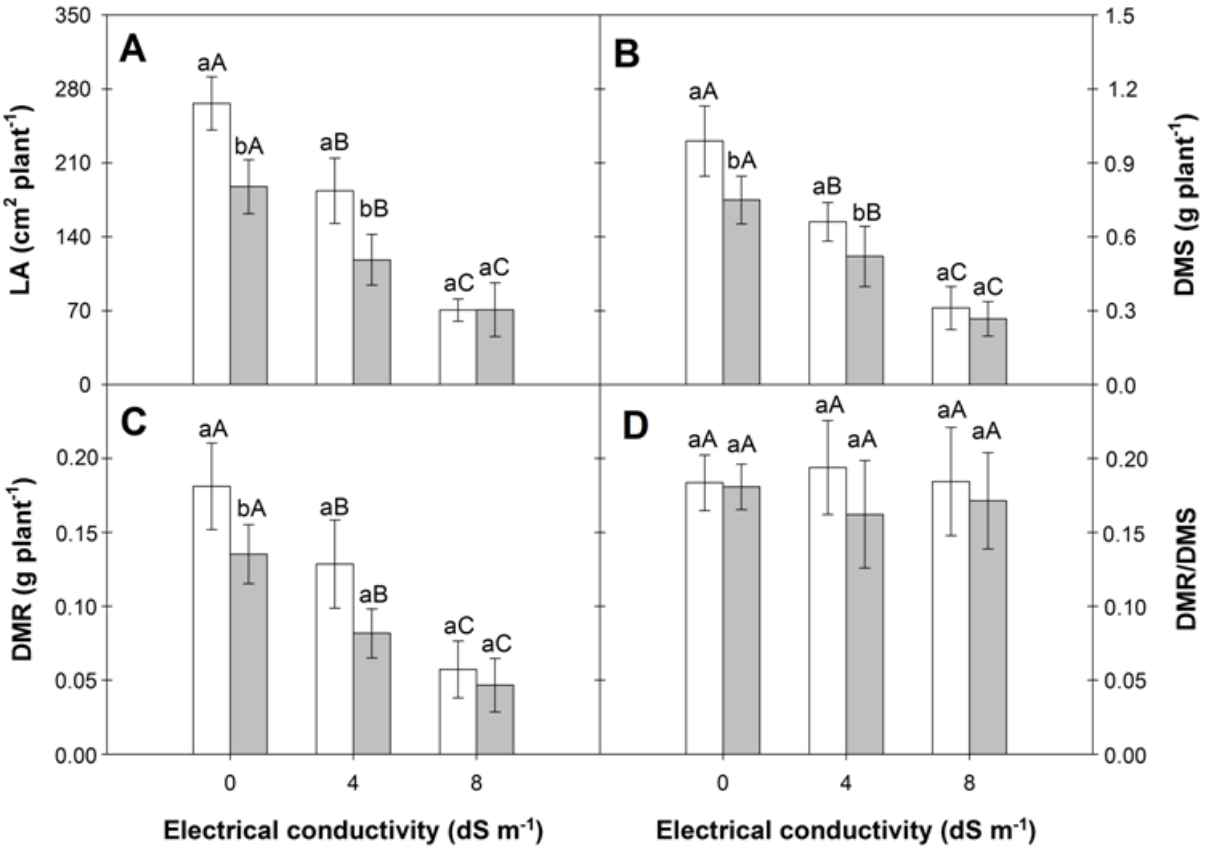

Figure 4. Plant growth of two species of sorghum, *S. bicolor* (□) and *S. sudanense* (▩), subjected to three saline treatments (0.0, 4.0 and 8.0 dS m^{-1}) applied from sowing to 24 days later (Phase I). A, Leaf area (LA), B, Dry mass of shoot (DMS), C, Dry mass of root (DMR); D, Ratio between dry mass of root and dry mass of shoot (DMR/DMS). Means followed by same lowercase letter for species and capital letter to saline treatments are not significantly different ($P \leq 0.05$). Error bars in each column represent the standard error.

at a salinity of 8.0 dS m^{-1}; we observed a 73% decrease in LA in *S. bicolor* and a 62% decrease in *S. sudanense* (Figure 4A). Under control conditions or a 4.0 dS m^{-1} saline treatment, the mean values of LA in *S. bicolor* were significantly higher than those in *S. sudanense* (Figure 4A). In Phase I, the dry mass of the shoot (DMS) and of the root (DMR) decreased with increasing salinity in both species studied (Figure 4B and C). In this phase, the DMS at 8.0 dS m^{-1} salinity was reduced by 70 and 65% in *S. bicolor* and *S. sudanense*, respectively, compared with controls. In control conditions and at the lower dose of NaCl, the DMS of *S. bicolor* was greater than that of *S. sudanense* (Figure 4B). The DMR of *S. bicolor* and *S. sudanense* plants at 8.0 dS m^{-1} salinity in Phase I was reduced by 72 and 70%, respectively, compared with controls. The two species differed significantly from one another only in the control group (Figure 4C). In Phase I of development in both species, the DMR/DMS ratio was not altered by increasing salinity (Figure 4D). Increasing salinity during Phase II of development did not affect the LA of either species; however, the DMS and DMR were reduced progressively as salinity increased (Figure 5). At this stage of development, treatment with 8.0 dS m^{-1} salinity reduced the DMS of *S. bicolor* and *S. sudanense* by 33 and 42%, respectively, compared with controls (Figure 5B). The DMR was reduced by 24 and 39% relative to controls, in *S. bicolor* and *S. sudanense*, respectively (Figure 5C). In Phase II, the DMR/DMS ratio was reduced with increasing salinity only in *S. bicolor*, which showed a reduction of 20% in plants at 8.0 dS m^{-1} salinity compared with controls. There were no significant differences in the mean values of DMR/DMS ratio between the two species of sorghum (Figure 5D).

Organic solutes

The content of organic solutes varied greatly according to the stage of plant development, the species and the level of salinity (Figure 6). In plants in Phase I of development, the carbohydrate content of *S. bicolor* plants increased 118% at 8.0 dS m^{-1} salinity, compared with control; in *S. sudanense* plants, no change in carbohydrate content

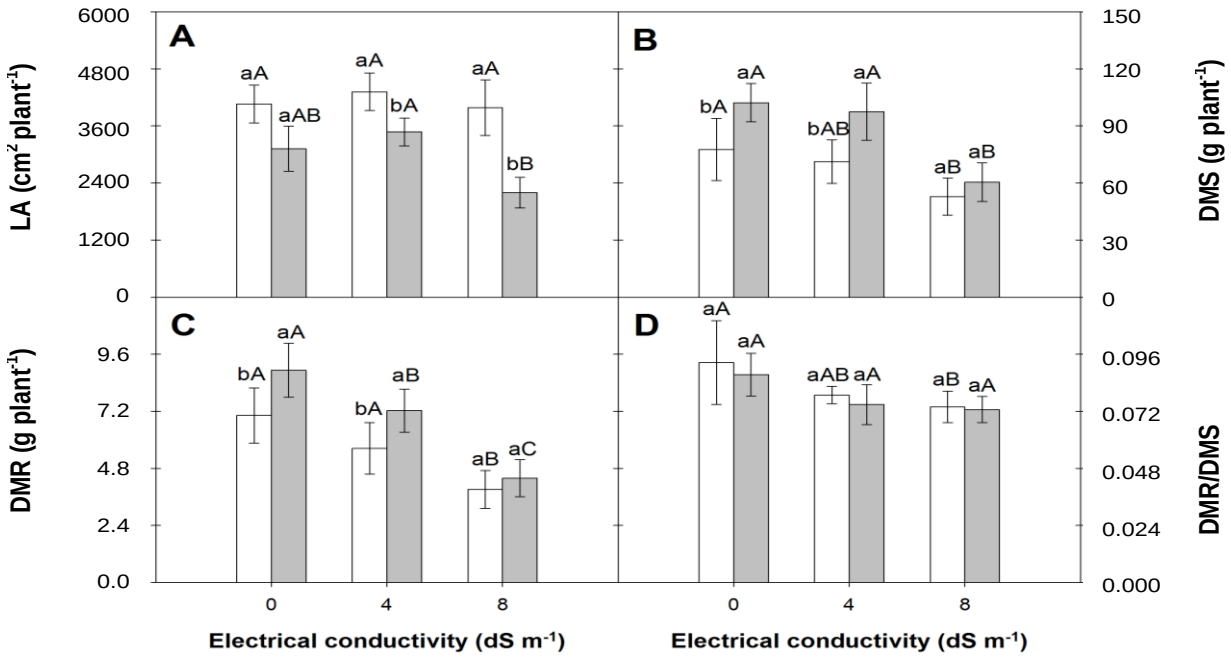

Figure 5. Plant growth of two species of sorghum, *S. bicolor* (□) and *S. sudanense* (▩), subjected to three saline treatments (0.0, 4.0 and 8.0 dS m^{-1}) applied from the 25th to the 49th day after sowing (Phase II). A, Leaf area (LA), B, Dry mass of shoot (DMS), C, Dry mass of root (DMR), D, Ratio between dry mass of root and dry mass of shoot (DMR/DMS). Means followed by same lowercase letter for species and capital letter to saline treatments are not significantly different ($P \leq 0.05$). Error bars in each column represent the standard error.

was observed (Figure 6A). At the highest level of salinity, the proline content increased 34% in *S. bicolor* but was reduced by 82% in *S. sudanense* compared with controls (Figure 6C). Salinity did not affect the content of free amino acid in *S. bicolor.* However, at the highest salinity level, free amino acid content was reduced 33% in *S. sudanense,* as compared with control (Figure 6E).

In plants in Phase II of development, the carbohydrate content did not change with salinity in *S. bicolor.* In *S. sudanense,* however, the carbohydrate content was reduced 30% on average, compared with the control group in plants exposed to the 4.0 or 8.0 dS m^{-1} salinity treatment (Figure 6B). Also in this same stage of development, the proline content of *S. bicolor* plants exposed to the 4.0 or 8.0 dS m^{-1} salinity treatment was reduced an average of 18%, compared with controls. In *S. sudanense*, the highest level of salinity (8.0 dS m^{-1}) promoted an increase in the proline content (approximately 60%) (Figure 6D). The content of free amino acid in *S. bicolor* did not change with salinity. However, in *S. sudanense* plants exposed to either saline treatment, the free amino acid content increased by approximately 50%, compared with control (Figure 6F).

Inorganic solutes

In Phase I plants subjected to 8.0 dS m^{-1} salinity, the content of Na^+ increased by 80 and 57% relative to control plants of *S. bicolor* and *S. sudanense*, respectively (Figure 7A). Following exposure to 8.0 dS m^{-1} salinity, the K^+ content was reduced by 45 and 37% in *S. bicolor* and *S. sudanense*, respectively (Figure 7B). The content of Cl^- was most dramatically increased by salinity. In *S. bicolor*, this increase was 212%, and in *S. sudanense* it was 101%, when we compare the highest dose of salts with the respective controls (Figure 7C). There was an increase of 212% in the Na^+/K^+ content in plants of *S. bicolor* exposed to 8.0 dS m^{-1} salinity compared with control. At this same level of salinity, there was a 168% increase in Na^+/K^+ content in *S. sudanense* (Figure 7D). Distinct from our findings in Phase I plants, the content of Na^+ in plants in Phase II of development was reduced by 19% in *S. bicolor* compared with control following the application of salinity at 8.0 dS m^{-1} (Figure 8A). In *S. sudanense*, the Na^+ content of plants subjected to 8.0 dS m^{-1} did not differ from that of control plants. The K^+ content was also reduced in a manner similar to that observed in plants in Phase I. However, the reductions in K^+ content were smaller in Phase II of development (Figure 8B). The K^+ content in plants subjected to 8.0 dS m^{-1} salinity was reduced by 29% and by approximately 15% in *S. bicolor* and *S. sudanense,* respectively, compared with controls (Figure 8B). The content of Cl^- in plants in Phase II was strongly increased in both species; at 8.0 dS m^{-1} salinity, we observed increases of 650 and

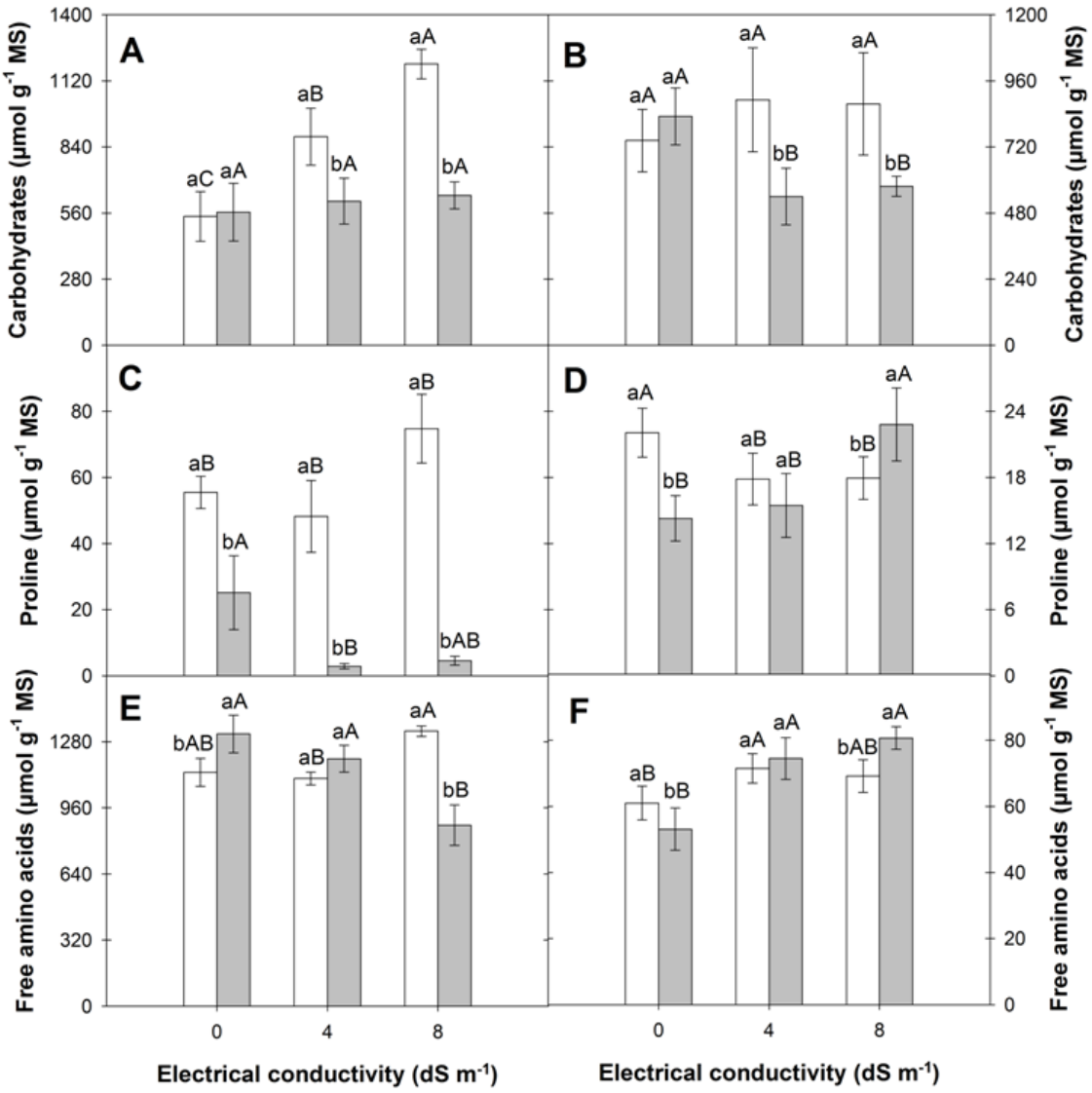

Figure 6. Organic solute content in leaves of plants of two species of sorghum, *S. bicolor* (□) and *S. sudanense* (■), subjected to three saline treatments (0.0, 4.0 and 8.0 dS m^{-1}) applied from sowing to 24 days later (Phase I - A, C and E) or from the 25th to the 49th day after sowing (Phase II – B, D and F). A, B, Carbohydrate; C, D, Proline, E, F, Free amino acid. Means followed by same lowercase letter for species and capital letter to saline treatments are not significantly different ($P \leq 0.05$). Error bars in each column represent the standard error.

630% in *S. bicolor* and *S. sudanense*, respectively, compared with controls (Figure 8C). In both species, at 8.0 dS m^{-1} salinity, the Na^+/K^+ content was not altered when compared to control plants (Figure 8D).

DISCUSSION

Plant growth is a result of the integration of several physiological processes. Photosynthesis is one of the most important (Parida and Das, 2005). In some species, such as jatropha (Silva et al., 2011), citrus (Lopéz-Climent et al., 2008) and cowpea (Assis Junior et al., 2007), the net photosynthetic rate, stomatal conductance and transpiration are severely reduced by salt stress. This response, however, is not general. Amorim et al. (2010) found that photosynthetic parameters of adult dwarf cashew plants were not affected by increasing salinity. In this study, in Phase I of development, the photosynthetic rate in both species of sorghum was not altered by salinity, even with reductions in g_s and *Ci/Ce* (Figure 1). In addition, the sorghum species in Phase I did not differ in any of the gas exchange parameters evaluated. However, in Phase II of development, when stress was applied to these older plants, *S. sudanense* was less affected by salinity than was *S. bicolor*. These

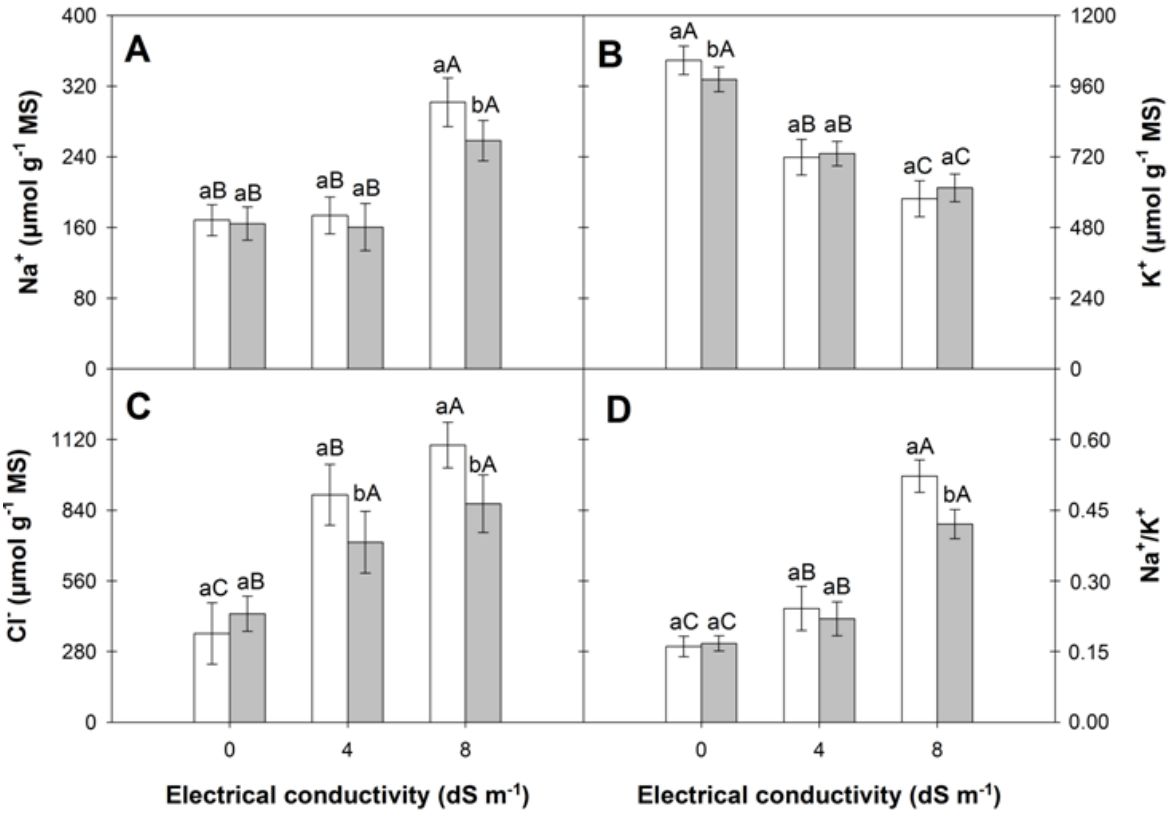

Figure 7. Inorganic solute content in leaves of plants of two species of sorghum, *S. bicolor* (□) and *S. sudanense* (■), subjected to three saline treatments (0.0, 4.0 and 8.0 dS m^{-1}) applied from sowing to 24 days later (Phase I). A, Na^+ content, B, K^+ content, C, Cl^- content, D, Na^+/K^+ ratio. Means followed by same lowercase letter for species and capital letter to saline treatments are not significantly different ($P \leq 0.05$). Error bars in each column represent the standard error.

results indicate that the effect of salinity on gas exchange in *S. bicolor* and *S. sudanense* depends on both the stage of development in which the stress is applied and the salt concentration.

The relative chlorophyll content varies among species or cultivars and according to environmental conditions (Campbell et al., 1990; Castelli et al., 1996). The salinity may increase the chlorophyll content at low levels of stress. Chlorophyll content may even decrease at high levels of salinity stress (Ashraf and Harris, 2004). In plants in Phase I of development, *S. sudanense* experienced an increase in the value of the SPAD index, which is proportional to the chlorophyll content (Yamamoto et al., 2002). A similar result was not found in *S. bicolor* (Figure 3). Paulus et al. (2010) also observed increases in chlorophyll content of lettuce at the highest levels of salinity. In Phase II of development, there were no changes in the SPAD index values following the application of saline treatments in both species of sorghum. This is in contrast to the findings of Willadino et al. (2011) and Khan et al. (2009), who reported reductions in chlorophyll content with increasing salinity in sugar cane and wheat, respectively.

Under the application of salt stress, the reduction in LA of plants of both species was more severe in Phase I (Figure 4A) than in Phase II of development (Figure 5A). In general, the reduction in leaf growth represents a defense mechanism of plants under stress conditions and high salinity because it reduces water loss by transpiration (Taiz and Zeiger, 2009). However, this also leads to changes in the partition of assimilates and a reduction in the area available for photosynthetic processes, which can result in productivity losses (Gomes et al., 2011). Despite the sharp reduction in LA in both species in Phase I of development, the photosynthetic rate was not affected by salinity (Figures 1 and 4). This result was contrary to that observed in Phase II *S. bicolor* plants, in which the LA was not affected by salt stress, but the photosynthetic rate was reduced. These results suggest that changes in LA did not correlate with changes in the photosynthetic rate of plants of both sorghum species studied.

As in LA, reductions in DMS and DMR were more severe in Phase I in both species; this suggests that plants in the early stages of development are very sensitive to salinity (Figures 4 and 5). One of the most

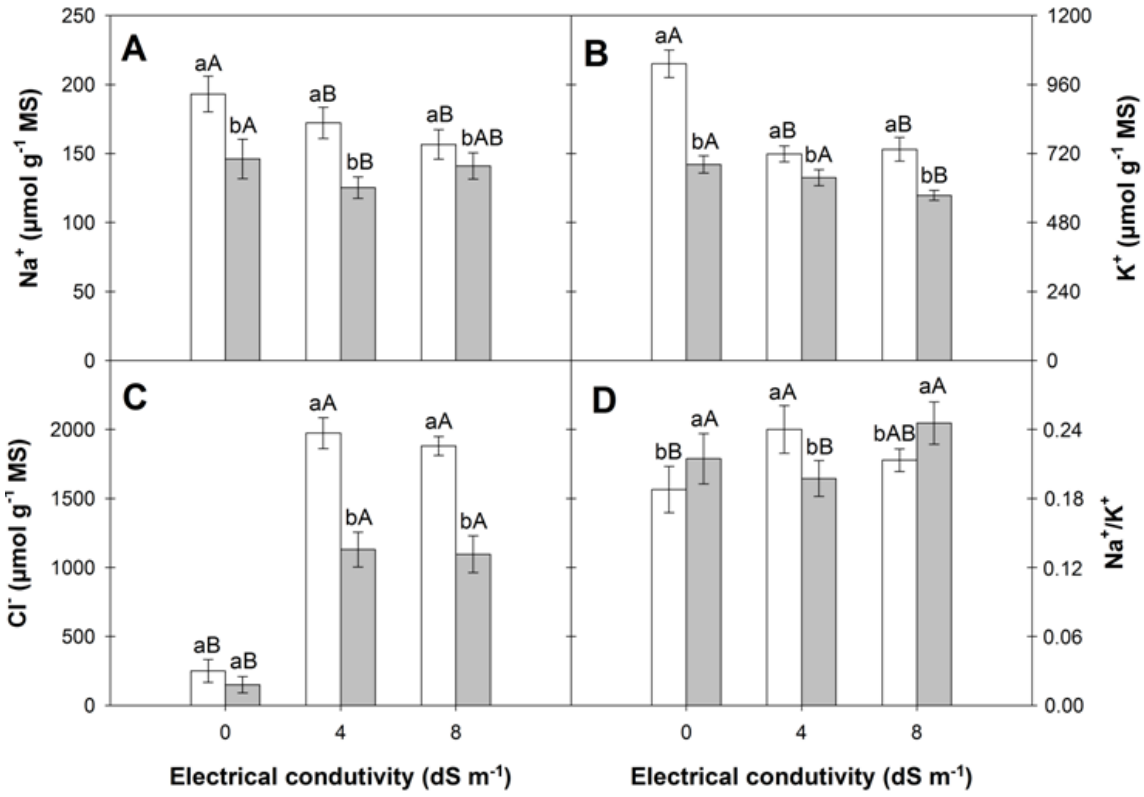

Figure 8. Inorganic solute content in leaves of plants of two species of sorghum, *S. bicolor* (□) and *S. sudanense* (▨), subjected to three saline treatments (0.0, 4.0 and 8.0 dS m^{-1}) applied from the 25th to the 49th day after sowing (Phase II). A, Na^+ content; B, K^+ content; C, Cl^- content; D, Na^+/K^+ ratio. Means followed by same lowercase letter for species and capital letter to saline treatments are not significantly different ($P \leq 0.05$). Error bars in each column represent the standard error.

accepted explanations for the growth inhibition by salt isthe diversion of energy from growth to maintenance of the plant (Greenway and Munns, 1980). Thus, the reduction in dry mass may reflect the metabolic cost of energy associated with a reduction in carbon gain and an adaptation to salinity (Azevedo Neto and Tabosa, 2000). As for the DMR/DMS ratio, significant changes in this variable were only observed in Phase II. The observed reduction in *S. bicolor* at this phase indicates that the effect of salinity was more evident in the roots than in the shoots. The roots may suffer the most damaging effect of salinity because that part of the plant remains in direct contact with the salts. Similar results were obtained by Rodrigues et al. (2005) in rice plants.

In the present study, we observed an increase in the soluble carbohydrates in Phase I *S. bicolor* leaves following salinity treatment (Figure 6). Specifically, these plants were more sensitive to the deleterious effects of salinity (Figure 4). According to Lacerda et al. (2003), the accumulation of soluble carbohydrates under high salt concentrations may result from the plant's reduced carbohydrate usage rather than an increase in its biosynthesis to compensate for changes in Ψ_s during salt stress. The soluble carbohydrates have an important biological role in protecting membranes and proteins in cells exposed to salt stress (Garcia et al., 1997). The accumulation of these carbohydrates in plants was reported as a response to salinity even when significant reductions in net assimilation rates of CO_2 were observed (Murakeözy et al., 2003). *S. sudanense*, in turn, showed a decrease in the content of soluble carbohydrates in the leaves of plants in Phase II (Figure 8A). Silva et al. (2009) also observed a reduction of soluble carbohydrates with salinity in jatropha plants. This reduction may be related to the higher migration rates of these compounds through the phloem to other parts of the plant (Taiz and Zeiger, 2009).

In *S. bicolor*, there was an increase in proline content in plants in Phase I of development and a reduction in Phase II. *S. sudanense* had an inverse response; its proline content was reduced in Phase I, but increased in Phase II (Figure 7C and D). These results may indicate that the content of proline did not change specifically as a mechanism of salt tolerance but only as a physiological response of the species of sorghum under salt stress. It is known that proline occurs widely in higher plants (Ashraf and Harris, 2004). In addition, it regulates the accumulation of usable nitrogen and is osmotically very

active (Ashraf, 1994). Proline also contributes to the stability of the cell membrane (Gadallah, 1999), however, its role in osmoregulation and salt tolerance has been questioned (Ashraf and Harris, 2004). Lutts et al. (1996) observed that proline was not involved in osmotic adjustment in rice plants stressed by excess salts, and its accumulation seemed more a symptom of injury rather than an indicator of salt tolerance. Costa et al. (2003) also suggested that proline did not contribute to osmotic adjustment in *Vigna unguiculata* cultivars.

The total free amino acids in *S. bicolor* were not changed with salinity in either phases of development. This result is similar to that found by Costa et al. (2003), who observed no differences in content of these osmolytes in cultivars of *V. unguiculata* in response to salinity. In *S. sudanense*, the increase of free amino acids was observed only in plants in Phase II. This result was also observed by Silva et al. (2009) in jatropha plants under salt stress.

Under salt stress, plants accumulate large quantities of toxic ions, especially Na^+ and Cl^-. Therefore, mechanisms that lead to exclusion of these ions in metabolically active tissues of the shoot may be responsible for the tolerance of crops to salt stress (Azevedo Neto and Tabosa, 2000). In Phase I plants, the high content of Na^+ and Cl^- in the leaf tissues of species of sorghum exposed to salt stress may have had a major stressful effect on the metabolism of plants. This effect may have contributed to the reduction in growth because these ions, at toxic levels, cause irreparable damage to the cellular structures and affect metabolic functions (Munns and Tester, 2008). Unlike the results seen in Phase I plants, the content of Na^+ in Phase II plants of both species was slightly reduced or remained unchanged, compared with controls (Figures 7A and 8A). It is possible that, at this stage of development, plants have a more efficient mechanism for retention of this toxic ion in the stems and roots, which prevented excess Na^+ accumulation in the leaves (Netondo et al., 2004; Bavei et al., 2011). The steep increase in the concentration of Cl^- as a function of salinity in Phase II plants from in both species (Figure 8C) can be explained by the high proportion of Cl^- present in the irrigation water.

The K^+ content in both development phases was reduced by salinity, but these reductions were greater in Phase I. This effect may be due to competition between Na^+ and K^+ sites that are part of the same absorption system in the plasma membrane of the root cells (Marschner, 1995). An increase in K^+ selectivity may represent an important mechanism of plant tolerance to salt stress. The content of K^+, similarly, has been reduced due to increased doses of NaCl in maize plants (Azevedo Neto and Tabosa, 2000), jatropha plants (Silva et al., 2009) and *V. unguiculata* (Costa et al., 2003).

The Na^+/K^+ ratio is important in assessing the degree of plant tolerance to salinity because Na^+ inhibits many enzymes that require K^+ as a cofactor (Greenway and Munns, 1980). In this study, the Na^+/K^+ was increased in Phase I of development, but in Phase II, it remained unchanged with increasing salinity in both species (Figures 7D and 8D). This result is consistent with the fact that the plants in Phase I of development were more affected by salinity than were those in Phase II (Figures 4 and 5).

With these results of our physiological and biochemical analysis, it was possible to understand the effects of salt stress in two stages of development of the sorghum plants and to identify the stage of development most sensitive to salinity. From the results of gas exchange and growth analysis, it was concluded that plants in Phase I of development were more sensitive to the effects of salt stress than were plants in Phase II. We did not observe, however, major differences in salinity tolerance between *S. bicolor* and *S. sudanense*. The lower accumulation of organic solutes and the higher toxic ion concentration in the Phase I sorghum plants also confirmed that plants in Phase I were more sensitive to salinity than were those in Phase II.

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