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Nitrogen metabolism in sorghum under salinity and silicon treatments in Brazil

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The objective of the present research was to study nitrogen metabolism in sorghum plants subjected to salt stress and silicon concentration. The experiment was conducted at the Amazon Federal Rural University, Capitão Poço Decentralized Unit for 1 month, in 2013, using the cultivar BR 700 of forage sorghum plants (*Sorghum bicolor* [Moench.]). The experimental design was completely randomized, in a 5 × 3 factorial arrangement (0, 50, 100, 150 and 200 µM of silicon) and saline concentrations (0, 1.5 and 2.0 M), consisting of 4 replications. Analyses were conducted of amino acids, proteins, free ammonium, nitrate and nitrate reductase. Nitrate content increased in the leaves and root in the treatments 0 and 1.5 µM of Si, but decreased in treatments with the 0.5 and 1.0 µM doses of Si. In leaves and roots, the treatments 1.5 and 2.0 of SC caused reduction and increase, respectively, of ammonium levels. The silicon doses attenuated the negative effects of the treatments on the biochemical compounds caused by higher salt concentrations in sorghum plants.

Key words: Abiotic stress, *Sorghum bicolor*, salinity, silicon, nitrogen metabolism.

INTRODUCTION

The accumulation of salt in the soil solution causes salt stress because plants subjected to such stress cannot absorb water easily, especially the most sensitive plants. This is because excess salt in the soil solution can cause plasmolysis. In addition, with the expansion of irrigation throughout the world, the problem of secondary salinization has become severe, particularly in tropical regions where severe weather conditions prevail (for example, evaporation and high temperatures). These problems are often associated with inadequate water and soil management and use of water with a high salt content, which sorely aggravates the soil salinization

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problem (Silveira et al., 2010).

Salinity can cause two types of stress in tissues and organs of higher plants: water deficit, as a result of a high concentration of solutes in the root environment, and ionic stress, which stems largely from changes in the Na^+/ K^+ relationships and excessive concentration of salt ions (Na^+, Cl^-), which are detrimental to cell metabolism, especially in the leaves (Horie and Schroeder, 2004).

Knowledge of salt interactions with the plant and the soil and the effect of silicon can provide plant tolerance to salinity. According to Gunes et al. (2008), this chemical element plays a role in activities related to metabolism or to the physiology of plants under abiotic stresses, such as salt and water. Crusciol et al. (2013) observed that soybean, bean and peanut yields increased with foliar application of silicic acid. In addition, the beneficial effects of the use of silicon in plants subjected to salt stress have been reported in the literature (Dai et al., 2005; Liang et al., 2006), as in *Anacardium occidentale* and *Moringa oleifera* (Miranda et al., 2002), *Triticum aestivum* L. (Tuna et al., 2008), *Oriza sativa* L. (Kraska and Breintenbeck, 2010) and *Zea mays* L. (Lima et al., 2011).

Although silicon is not one of the essential elements for growth and development of plants (Lima et al., 2011), this chemical element promotes plant resistance under saline conditions, since it helps to maintain the integrity and stability of cell membranes (Zuccarini, 2008). Under high salinity conditions, the capacity of this chemical element to maintain cell-wall integrity is maintained by its efficiency in stimulating the antioxidant system (Rodrigues et al., 2011). Silicon can promote the growth and production of plants because it increases chlorophyll content in leaves and modifies plant structure, enabling plants to become more upright and avoiding excess self-shading and delaying senescence (Ma and Yamaji, 2008).

The mechanisms of action of salt stress in plants and tolerance in environments with silicon content are methods scarcely known in agriculture, and further research is required in this area of study (Lacerda et al., 2006). Moreover, this culture can exclude ions considered as toxic, reducing ion storage in the leaf (Trindade et al., 2006). This in-depth knowledge can establish soil and crop management strategies, to enable, for example, the selection of more salinity-tolerant cultivars, so that sorghum can express its productive potential even under salt stress. In Brazil, this culture is not widely used as food; its grains are produced for animal feeding purposes, in order to meet the demand of both the animal feed and the forage industries (Dykes et al., 2005; Tabosa et al., 1993).

However, studies related to Si contribution in reducing salinity are still incipient, especially as regards nitrogen metabolism in sorghum. Therefore, further studies are needed to demonstrate the efficiency of Si in order to mitigate this type of abiotic stress, thus contributing to increased sorghum production. Most studies, in order to evaluate final productivity, refer to the nutritional aspects and the beneficial role of silicon in abiotic stress resistance (Pozza et al., 2009).

The objective of this work was to study the nitrogen metabolism of sorghum plants subjected to salt stress and silicon concentration.

MATERIALS AND METHODS

Location of the experiment

The experiment was conducted in a greenhouse at the Amazon Federal Rural University, Capitão Poço Decentralized Unit, geographic coordinates 01° 44' 04''S and 47° 03' 28''W, at an average altitude of 96 m (Figure 1), for 1 month in 2013. The study used forage sorghum (*Sorghum bicolor* [Moench.]), cultivar BR 700 obtained from the company Empresa Brasileira de Pesquisa Agropecuária (Embrapa Milho e Sorgo) from the 2010 season. The pots were arranged in a spacing of 0.60 m between rows and 0.40 m between plants in a random distribution. The sorghum plants were grown in Leonard fabric pots containing silica substrate: vermiculite (1:2) and irrigated with Hoagland and Arnon nutrient solution (1950).

Experimental design

The experimental design for plants subjected to salt stress was completely randomized (RCD) in a 5 × 3 factorial arrangement, referring to five doses of silicon (0, 50, 100, 150 and 200 µM of silicon) and three saline concentrations (0, 1.5 and 2.0 M) with 4 repetitions, totaling 60 experimental units, in which each experimental unit was composed of two plants/pot. The application of salt stress was carried out at 18 days after germination and the silicon concentrations were applied after seedling emergence (11 days after germination). The application of Si was performed daily, and the applications were performed in the afternoon (17 h). The nutrient solution was replaced at five days after application and pH was adjusted to 6.0 as necessary. Destructive sampling of plants at the vegetative stage (33 days after germination) was conducted at 9:00 am, when the plants were separated into roots and leaves. Samples of each were reserved for determination of moisture percentage by determining dry weight in a forced circulation air oven at 70°C (± 5°C).

Analyzed variables

Nitrate reductase activity was determined using the method described by Hageman and Hucklesb (1971). Hole punch leaf disks (0.5 cm^2 in diameter) were removed and then approximately 200 mg of these leaf discs were weighed. In order to obtain the extract, the leaf discs were transferred to test tubes and subjected to vacuum containing 5.0 ml of phosphate buffer (assay medium) for 2 min. The test tubes were then placed in a water bath at 300°C for 30 min and protected from light (kept in the dark). In order to obtain the soluble proteins, the method described by Bradford (1976) was used. Using 15 ml test tubes, lyophilized DM 100 mg/5.0 ml extraction buffer (25 µM Tris-HCl pH 7.6) were added and stirred for 2 h in a shacker (with properly sealed tubes) in order to obtain the extract. The soluble amino acids were obtained using the method of Peoples et al. (1989), when 50 mg of lyophilized DM were

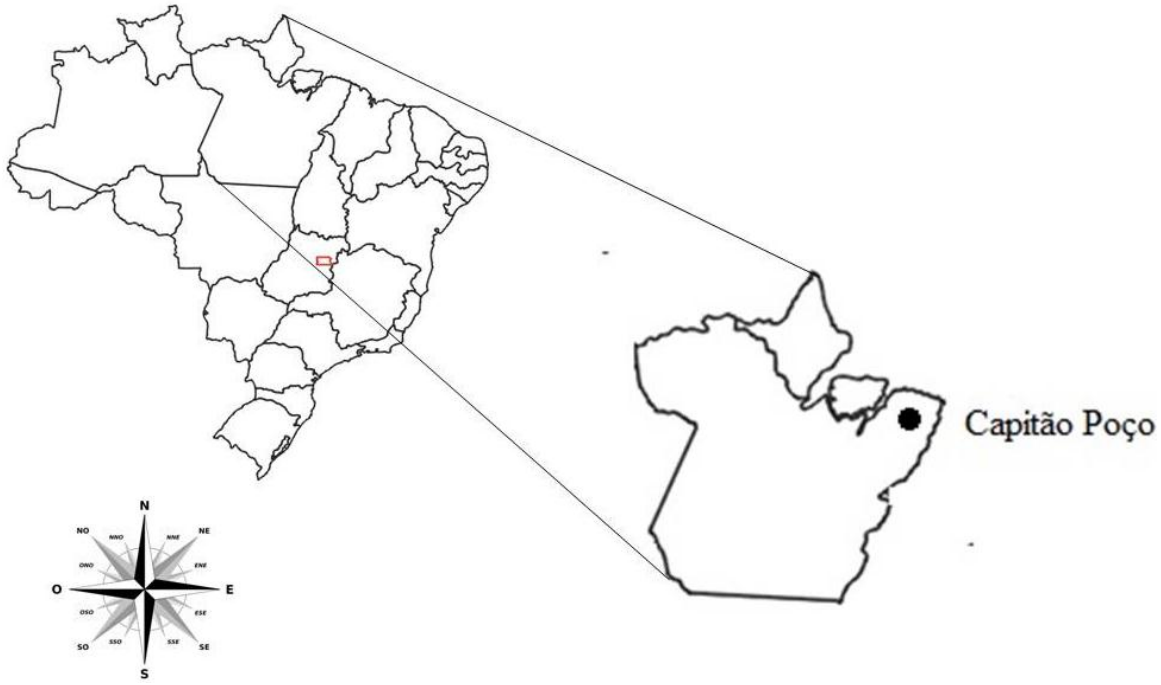

Figure 1. Location of the experiment at the Amazon Federal Rural University, Capitão Poço, Pará, Brazil.

transferred to a 15 ml test tube, adding 5 ml of distilled water. The tubes were then placed in a water bath for 30 min at 100°C in order to obtain the crude extract.

Free ammonia was obtained according to the method of Weatherburn (1967). A 50 mg sample of powdered root and leaf dry matter (DM) was weighed and placed in 15 ml test tubes, then 5 ml of distilled water was added, and placed in the water bath for 30 min at 100°C, in order to obtain the total extract. The nitrate concentration was obtained by the method described by Cataldo et al. (1975) in which 50 mg samples of previously freeze-dried leaves and roots were added to test tubes containing 5.0 ml distilled water and incubated in a water bath for 30 min at 100°C. This was then centrifuged at 3,000 rpm for 10 min In order to obtain the crude extract.

Statistical analysis

The results were submitted to normality tests (Shapiro-Wilk test, SPSS Inc., USA) and homogeneity of variances (Bartlett test, SPSS Inc., USA), and the significant H0 was obtained. The effect of doses of silicon (Si) and salt concentration (SC) were analyzed by adjusting regression to the equations to adequately express the behavior of the variables (Sisvar Inc., Brazil) and considering the regressions significant at p ≤0.01 (Ferreira, 2011).

RESULTS AND DISCUSSION

Nitrate content

Si levels influenced (p≤0.01) the biochemical variables, as well as the salt concentrations (SC), and showed different behavior towards the variables nitrate, nitrate reductase activity, ammonium, amino acids and proteins, since there was interaction between Si doses and salt concentrations for all variables in leaves and roots (Table 1). The 0 SC treatment showed higher nitrate content, both in the leaf and in the root (Figure 2). In the leaf, nitrate content in the 0 SC and 1.5 SC treatments increased at the 0.5 and 1.0 µM Si doses, respectively, and reduced at the Si doses of 1.5 and 2.0 µM, respectively. In the 2.0 µM SC treatment the nitrate levels became higher as the Si doses increased (Figure 2A). The 1.5 and 2.0 µM salt concentrations were attenuated by the 1.0 µM dose of Si and at all Si doses, respectively, which favored the increase in nitrate content. In the root, nitrate content was reduced in the SC 0 and 1.5 µM SC treatment for all silicon doses, while in the SC 2.0 µM treatment, the nitrate levels were higher as the silicon levels increased (Figure 2B).

The decrease in nitrate concentration in leaves and roots at doses of 0 SC (1.5 and 2.0 µM of Si) and 0 SC and 1.5 SC (0, 0.5, 1.0, 1.5 and 2.0 µM of Si), respectively, can be attributed to the antagonistic effect of salts on nitrate absorption, as they reduced with the intensification of NaCl doses for both the root and leaf. This occurs when the presence of large amounts of nitrate salts, particularly Cl^-, decreases their quantities, and a large amount of Cl^- will favor nitrate reduction. This

Table 1. Analysis of variance for nitrate, amino acid, ammonia, protein and NRA of sorghum leaf and root under salinity and silicon doses.

Source of variation	DF	Nitrate	
		Leaf	Root
Silicon doses (Si)	4	**	**
Salt concentration (SC)	2	**	**
Si × SC	8	**	**
CV (%)	-	7.24	15.30

	DF	Amino acid	
		Leaf	Root
Silicon doses (Si)	4	**	**
Salt concentration (SC)	2	**	**
Si × SC	8	**	**
CV (%)	-	0.44	0.07

	DF	Ammonium	
		Leaf	Root
Silicon doses (Si)	4	**	**
Salt concentration (SC)	2	**	**
Si × SC	8	**	**
CV (%)	-	3.56	0.51

	DF	Protein	
		Leaf	Root
Silicon doses (Si)	4	**	**
Salt concentration (SC)	2	**	*
Si × SC	8	**	**
CV (%)	-	2.52	5.36

	DF	NRA	
		Leaf	Root
Silicon doses (Si)	4	**	**
Salt concentration (SC)	2	**	**
Si × SC	8	**	**
CV (%)	-	3.48	10.17

CV = Coefficient of variation; DF: Degree of freedom; NRA = Nitrate reductase activity; ** = significant ($p \leq 0.01$).

result is in agreement with Ding et al. (2010) who worked sorghum plants and found that higher amounts of nitrate (NO_3^-) decreased the Cl^- uptake, which is considered a toxic ion, thereby increasing the resistance of plants to stress caused by excess salts. This effect is possibly due to direct competition between Cl^- and NO_3^- ions for the same carrier and/or alterations in membrane integrity (Mansour and Salama, 2004; Rubinigg et al., 2005; Aragão et al., 2010).

The 0.5 and 1.0 µM concentrations of Si provided greater resistance to the decrease of nitrate concentration in leaves. For Lima et al. (2011), silicon added directly in the nutrient solution at a dose of 1 µM mitigated the negative effects on the growth parameter of corn seedlings (*Zea mays*) submitted to salt concentration (NaCl), while there were no beneficial effects for cowpea.

Ammonium content

Ammonium levels were higher in the 0 SC treatment in the leaf than the other treatments. In this treatment, ammonium levels increased until the 1.5 µM Si dose and

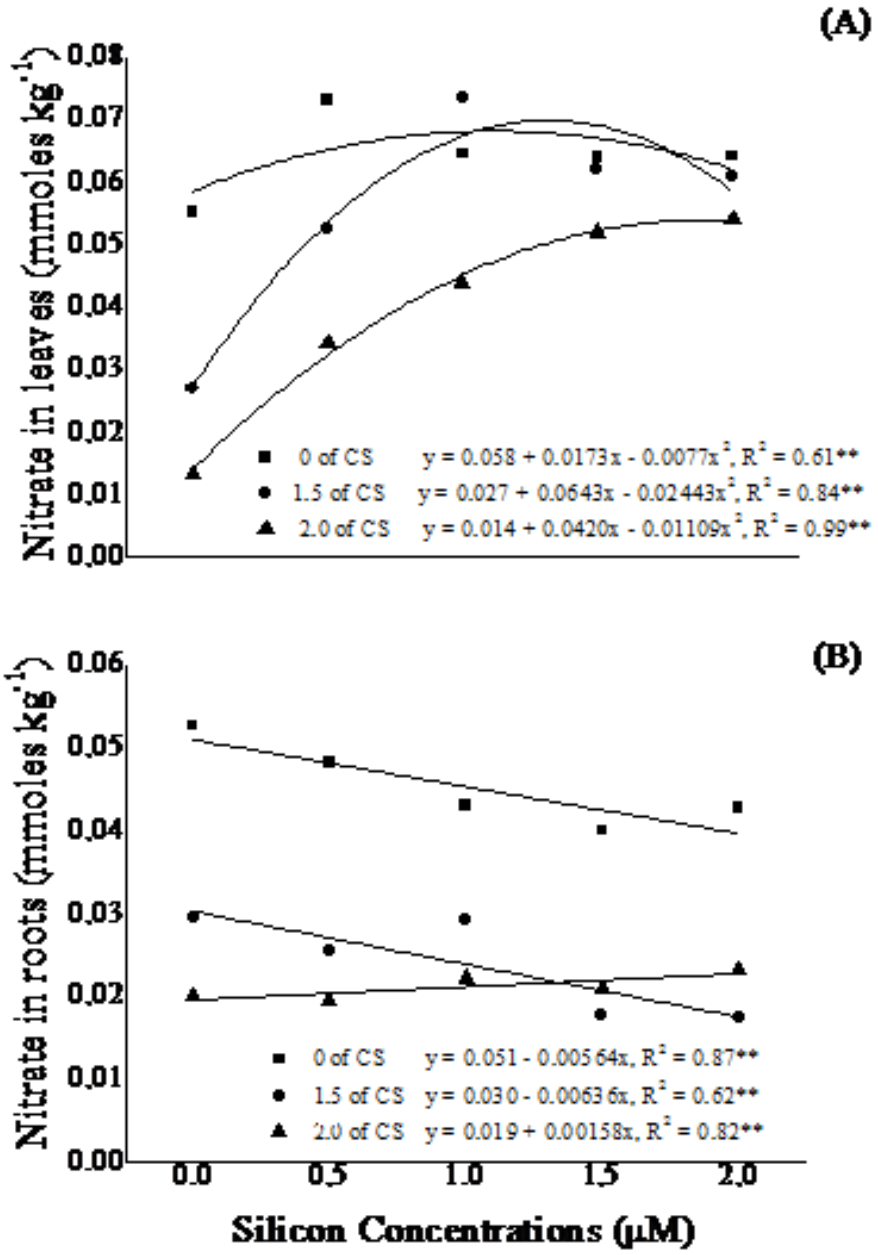

Figure 2. Nitrate concentrations in leaves (A) and roots (B) of sorghum under salinity and silicon concentrations. **Significant ($p \leq 0.01$) by the F-test.

reduced under the 2.0 µM Si dose, while in the 1.5 and 2.0 SC treatments, ammonium levels reduced as the Si dose increased (Figure 3A). The ammonium concentration in leaves was lower when there was an increase in salt concentration, which can be attributed to the positive effect of silicon. The roots presented the lowest ammonium content under the 0 SC treatment. In the 1.5 and 2.0 SC treatment, ammonium levels were higher when Si doses increased, but at a dose of 2.0 µM, these levels began to decrease (Figure 3B). In the root, there was an increase of ammonium content at higher salt concentrations. This is probably due to possible problems caused by the enzyme glutamine synthetize, which transforms ammonium to glutamate, because with a decrease in the activity of this enzyme, ammonium buildup may have occurred, which can cause problems

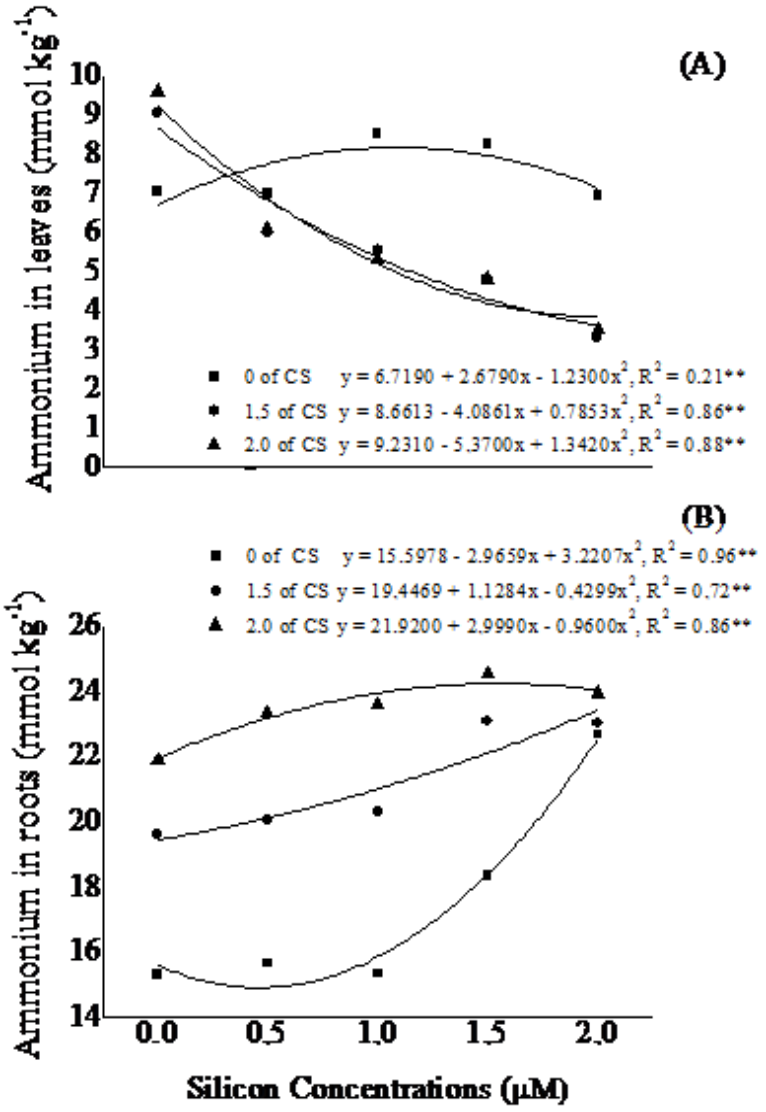

Figure 3. Ammonium concentrations in leaves (A) and roots (B) of sorghum under salinity and different silicon concentrations. ** Significant ($p \leq 0.01$) by the F-test.

for plants, since large amounts of ammonium can cause toxicity in plants.

Nitrate reductase activity - NRA content

The 0 SC treatment showed higher nitrate reductase activity (NRA) content, both in the leaf and in the root, when compared to 1.5 and 2.0 SC treatments. However, in the leaf, the 0 SC treatment decreased the NRA content as the Si doses increased (Figure 4A), while in the root, for the same treatment, there was an increase until the 1.5 µM Si dose (Figure 4B). In treatments with 1.5 and 2.0 SC, the NRA levels increased and decreased at the 2.0 µM Si dose, respectively. When there is a decrease in RNA, the formation of amino acids, proteins and chlorophyll is compromised, thus affecting the growth of plants (Souza et al., 2014). The root system tends to keep the Na^+ and Cl^- levels constant during stress exposure time, through the export of these ions into the soil or to the aerial part in studies with sorghum (Willadino and Camara, 2010). This result is due to the NRA reduction at higher salt concentrations, and the 2.0 µM silicon dose showed no positive effect because this dose is toxic to plants. In general, the reduction in nitrate reductase activity in the leaf may have been caused by

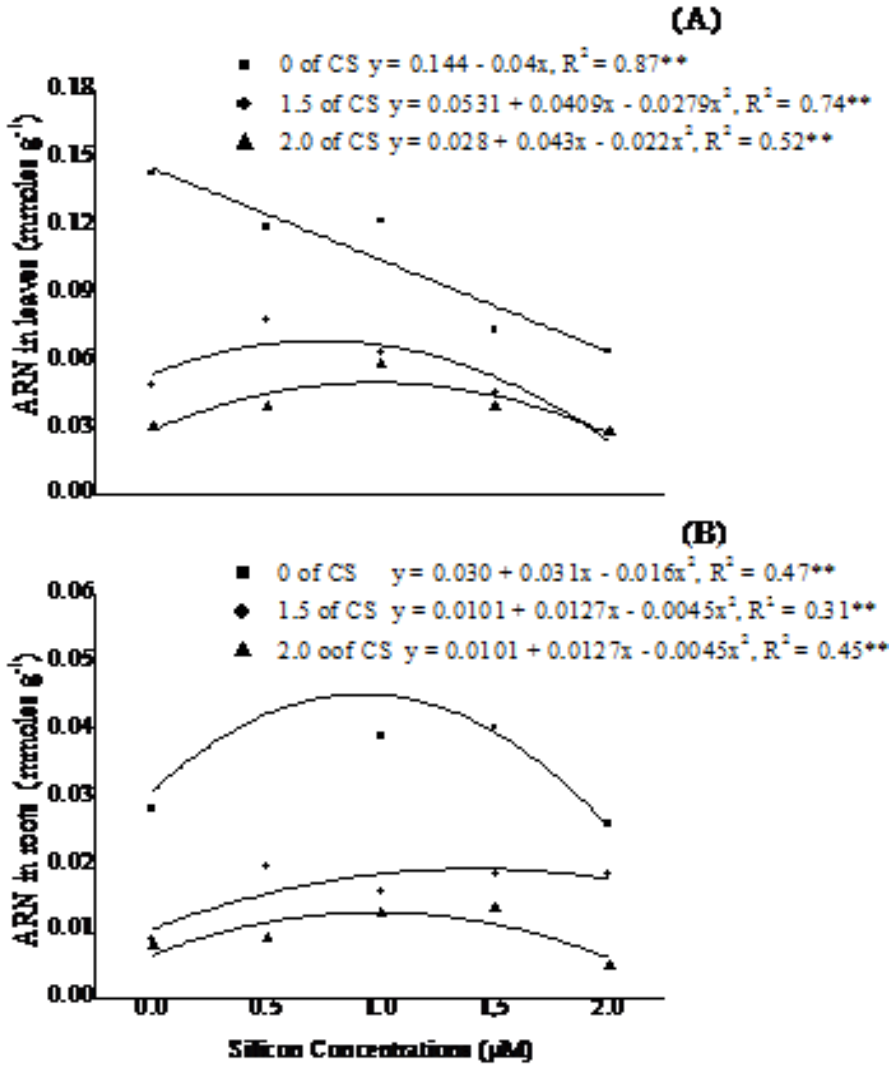

Figure 4. Nitrate Reductase Activity (ARN) in leaves (A) and roots (B) of sorghum under salinity and different silicon concentrations. **Significant ($p \leq 0.01$) by the F-test.

the imbalance of salts that may have occurred in cells, promoting a reduction in the activity of this enzyme.

Amino-acid content

For Amino-acid content, the 0 SC treatment showed higher contents in both the leaf and the root for the 1.5 and 2.0 SC treatments. Furthermore, the Amino-acid content decreased with the increase in the Si dose in the 0 SC treatment. In the 1.5 and 2.0 SC treatments, the amino-acid levels in the leaf and root increased until the 1.0 µM Si dose and reduced at the 2.0 µM Si dose (Figure 5). Sodium content of the plant leaf is reducewhen silicon is applied in substrates that lack this element (Faria, 2000). In soil salinity conditions (substrate) without the presence of silicon, there is a reduction in osmotic potential that causes water deficiency and subsequent toxicity to plants (Debouba et al., 2006; Munns and Tester, 2008). Silicon becomes effective in minimizing the effect of salinity on several plant species (Tuna et al., 2008), because Si acts by reducing the permeability of the plasma and the lipid membranes and keeps these membranes active for integrity and functionality (Zhu et al., 2004). The reductions in the amino-acids concentrations can be the result of inhibition or decrease of the deamination processes, which transformed these amino acids present in the plant parts. Under saline conditions, there was the breakdown of water and ionic homeostasis. This disruption of homeostasis occurs at the cellular level and

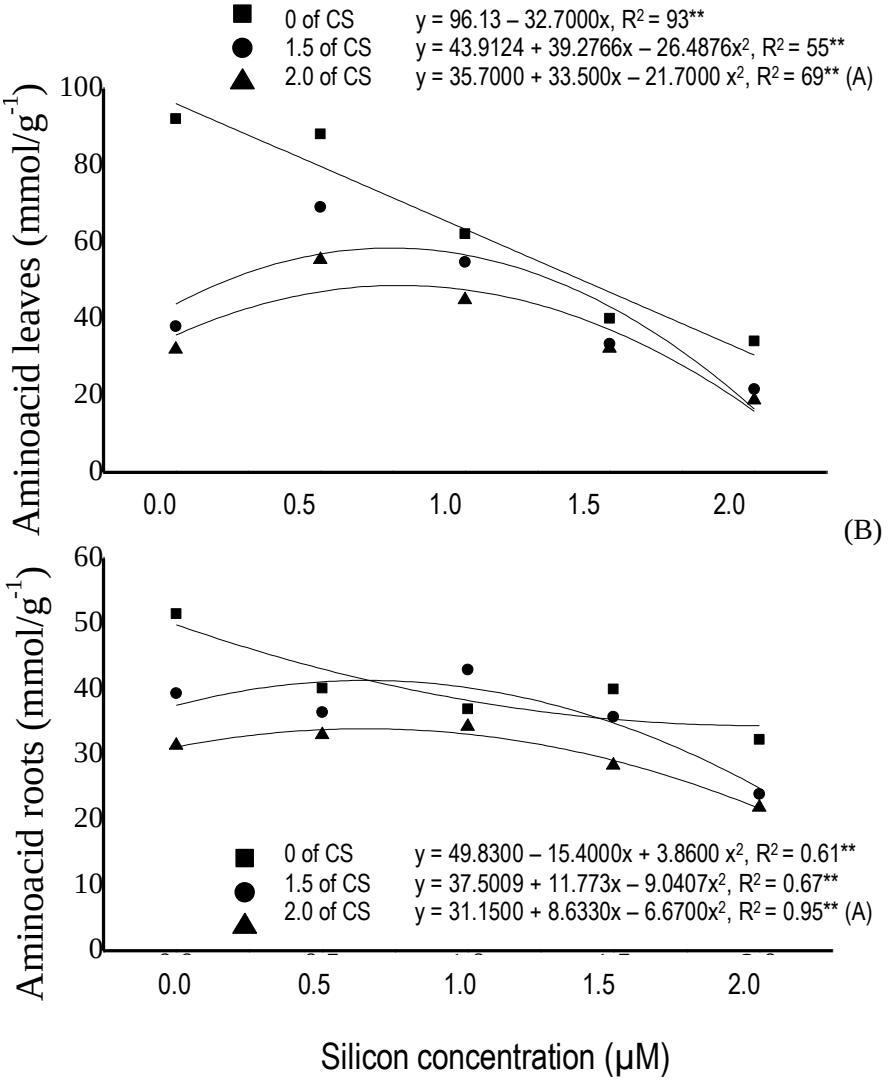

Figure 5. Total soluble amino-acids concentrations in leaves (A) and roots (B) of sorghum under salinity and different silicon concentrations. ** Significant ($p \leq 0.01$) by the F-test.

throughout the whole plant, causing molecular damage, restricting growth and perhaps even leading to plant death (Willadino and Camara, 2010).

Protein content

The 0 SC treatment showed higher protein content in the leaf as well as in the root (Figure 6). In the 1.5 and 2.0 SC treatments, there was an increase in protein in the leaf and root up to a Si dose of 1.5 µM and then a reduction at the 2.0 µM Si dose. The increase in the salt quantity reduces the amount of protein, and this may be due to the transformation of these proteins into amino acids and possibly ammonia, or even as a consequence of the protein denaturing when in the presence of large amounts of salts. The maximum silicon dose reduced protein concentrations. This occurred because very high doses of this element can promote stress rather than act as a controller (Debouba et al., 2006). In general, the addition of N improves the production and the growth of plants, whether or not submitted to salt stress (Barhoumi et al., 2010).

Conclusion

The silicon doses attenuated the negative effects of the

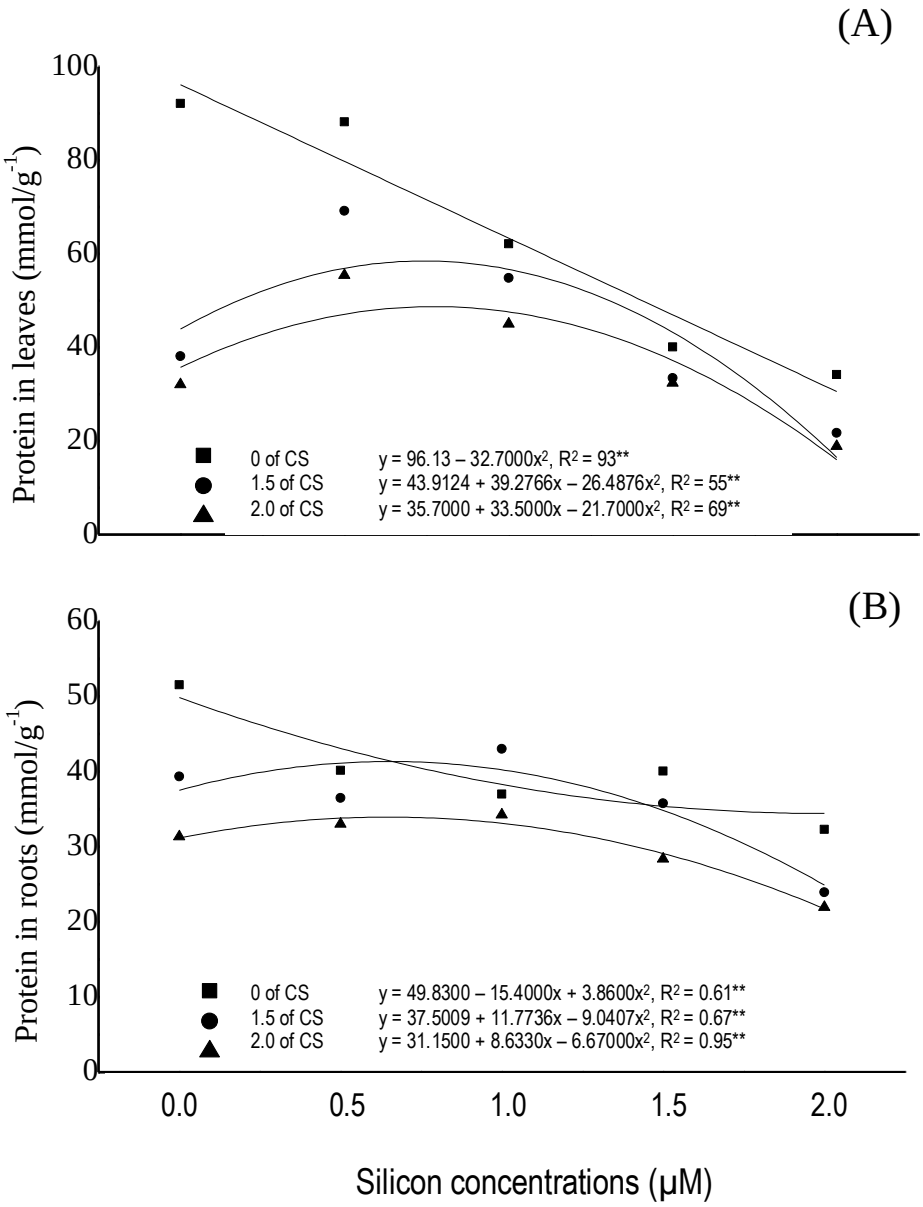

Figure 6. Total soluble protein concentrations in leaves (A) and roots (B) of sorghum under salinity and different silicon concentrations. ** Significant ($p \leq 0.01$) by the F-test.

treatments on the biochemical compounds caused by higher salt concentrations in sorghum plants. Nitrate content increased in the leaves and root in the treatments 0 and 1.5 µM of Si, but decreased in treatments with the 0.5 and 1.0 µM doses of Si. The treatment 2.0 uM of SC, nitrate levels had higher concentrations of both in the leaf and in the root with increasing doses of Si. Leaves and roots, the treatments 1.5 and 2.0 of SC caused reduction and increase, respectively, of ammonium levels. Thus, the dose of 1.0 µM of Si is recommended to lessen the effect of salt concentrations of 1.5 and 2.0 µM.

Conflict of Interests

The authors have not declared any conflict of interests.

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