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Adaptive responses of millet (*Pennisetum glaucum*) and elephant grass (*Pennisetum purpureum*) at salinity

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Abstract. The Northeast region of Brazil is most affected by the reduction in water availability, contributing directly to the accumulation of salts at the soil surface. Therefore, this study aimed to evaluate the adaptive responses of *Pennisetum glaucum* and *Pennisetum purpureum* submitted to salinity. Seeds of two gramineous species, submitted to the saline treatment of 60 mM NaCl, were used in the nutrient solutions. K^+ absorption, accumulation of organic solutes and ions were measured as tolerance indicators. The experimental design was completely randomized, in a factorial 2x2 scheme (*P. glaucum* and *P. purpureum* and two control treatments and saline stress). The soluble carbohydrate content as a response to salinity had a greater effect on *P. purpureum* plants grown in solution. Regard to the stress, the species that most stood out was *P. purpureum*, presenting a lower accumulation of salts, and increasing the carbohydrate content in the plant tissues, making the *P. purpureum* plants have a tolerance greater than *P. glaucum*.
keywords: Íons, organic solutes, saline stress, grasses

Introduction

Salinity is a soil condition that occurs mainly in the arid and semi-arid regions of the world. Several factors can lead to these processes as the restricted rainfall, low bioclimatic activity, low degree of rock weathering, insufficient drainage, high applied fertilizer rates and the use of poor quality water (which in many cases are rich in salts). Such factors promote the formation of soils with high salt concentration which causes concern for current agriculture**.** In Brazil, a greater importance in relation to this subject is given to the Northeastern backlands, where evapotranspiration overcomes precipitation and, consequently, makes it impossible to percolate the water through the profile and, consequently, soil salts leaching (Pedrotti et al., 2015).

This represents a great challenge for agriculture. By reducing the areas available for planting and increasing the world's population, efforts should be devoted to increasing (or at least maintaining) essential food production in ever smaller areas. One of the alternatives proposed is the identification of crops or cultivars that are able to adapt or tolerate stress situations in which they can maintain their growth, development and production.

The plants develop mechanisms of tolerance to survive the saline stress, that can be: anatomical, biochemical and physiological. Among these mechanisms the accumulation of beneficial ions, such as potassium, and the synthesis of compatible solutes (soluble carbohydrates and amino acids), stomatal closure and alterations in the root / shoot growth pattern can be cited (Taiz; Zeiger, 2013).

The importance of the study of these solutes in stressed plants is due to its role in adaptation, besides many of these serves as "markers" for the selection of tolerant cultures. For example, amino acids are related to plant nutrition, absorption efficiency, transport, and assimilation; however, carbohydrates serve as metabolic reserves and plant protection substances due to its ability to retain water, allowing the enzymatic processes not to be interrupted, even under dehydration conditions (Prisco & Gomes Filho, 2010).

Another important characteristic of adaptation to environmental stresses is the maintenance of essential ions to the cellular metabolism, as is K^+. In saline soils, preferential absorption of potassium instead of sodium, allows the preservation of cell ionic homeostasis, characterized by the high concentration of potassium, which is essential to the activity of enzymes, osmoregulation and growth (Dias & Blanco, 2010).

A group of plants with great potential of cultivation in the northeast region is composed by forage grasses, possessing morphophysiological adaptations that allow their adequate growth in warm climate regions. Among this group we found several species of the genus Penissetum, highlighting the millet and the elephant grass; however, little is known about the tolerance of these species to salt stress. These species are of great importance in livestock, since they are widely used in animal feed, grazing, as a substitute for grains of higher cost (Bastos et al., 2006). Since these crops tolerate high temperatures and can grow on soils with low fertility, they represent a viable alternative for their introduction in the Brazilian Northeast. In this context, the objective of this work was to study the adaptive characteristics of two grasses of the genus *Penissetum* (*Pennisetum glaucum* and *Pennisetum purpureum*) submitted to saline stress.

Methods

Sowing and planting conditions

The experiment was carried out in field coditions, with forage grasse cultivated under protected enviroment, in the Center of Agrarian Sciences and Biodiversity of the Federal University of Cariri (UFCA), located in the municipality of Crato, Ceará. The analyzes were conducted in the Laboratory of Biology, from March to June 2016. Seeds of *Pennisetum glaucum* (L.) R. Br. And *Pennisetum purpureum* Schum., Commercial, were used in the experiment. The seeds were stored in glass vials containing silica gel and stored at 4 ° C.

For sowing, 80 seeds of each species were sterilized with sodium hypochlorite solution diluted in distilled water in 1/3 ratio. They were then seeded in disposable plastic cups of a volume of 100 ml containing vermiculite moistened with a 0.5 mM calcium chloride solution ($CaCl_2$) to encourage germination. Eight cups were used, and 10 seeds were placed in each of them.

Then the cups were taken to the greenhouse where it remained under natural luminosity of the region, the average temperature of the day was 27.6º and the average relative air humidity was 56.6%, both values were obtained by of a portable thermohygrometer. 30 ml of distilled water were daily added to replenish the moisture lost by evapotranspiration and on the fifth day after sowing, the $CaCl_2$ solution was again added, replacing the use of the distilled water.

After ten days of sowing, the seedlings were homogeneously selected and then transferred to pots containing 2.3 L of nutrient solution from Hoagland, with 6 seedlings placed in each, and then 5 pots with 6 seedlings each, totaling 30 seedlings. The seedlings remained in this system for seven days.

With potted plants, root aeration was constant and maintained by a system of air pumps and porous stones. In the region of the intersection of the stem with the root plants were placed sponges to support the plants on the isopores that covered the vessels. The pH was monitored and maintained in the range of 5.5 to 6.5. For pH correction purposes, 1.0 M NaOH or HCl was used.

After this period, the plants were transferred and conditioned in containers made with plastic bottles with a capacity of 1 L solution, seven containers per treatment, each one received 1 plant, totaling 14 pots made with plastic bottles and 14 plants. The treatments used were control and saline. In the control treatment, 0.33 ml / L $MgSO_4$, 0.40 ml / L $NH_4H_2PO_4$, 1 ml / L Fe-EDTA, 1 ml / L Micronutrients, 2.8 ml / L $Ca(NO_3)$ and 0.1 ml / L KCl; the saline treatment was prepared before the same nutrient solution and added only 60 mM NaCl. The salt stress was applied fractionally, the NaCl amounts were: 35g / L, being 17.5g / L in the morning and 17.5g / L in the afternoon to avoid osmotic shock in the plants.

Collect of plants

At seven days of exposure to saline stress, the plants were collected. Where they were separated in aerial part and roots, for analysis of parameters such as, relative water content, preparation of the aqueous extracts that served to determine the levels of K^+ and Na^+ ions; and for the preparation of the ethanolic extracts for determination of the organic solutes.

Analysis of the contents of K^+ and Na^+

The content of the inorganic K^+ and Na^+ solutes accumulated in the aerial part new and intermediate leaves, sheaths and root, was determined from the use of the appropriately diluted aqueous extracts. The analyzes were performed in a flame photometer and the values were expressed in μmol g^{-1} of MF.

Preparation of ethanolic extracts

Ethanolic extracts were prepared from the fresh matter of leaves, scabbards and roots. Initially these were crushed with mortar and pistil for 1 minute. Thereafter, 1 ml of 80 % ethanol was added and the material was macerated for another five minutes. Subsequently, the extracts were shaken and centrifuged at 2500 rpm / min for 10 minutes. The supernatants were stored in a freezer while awaiting analysis.

Analysis of total soluble carbohydrates

Soluble sugars were quantified according to the method described by Dubois et al. (1956). In the aerial part and roots of *P. glaucum* was determined from conveniently diluted ethanolic extracts. The samples (20 μl) of the aerial part were mixed with 480 μl of 80% ethanol, 500 μl of 5.0 % phenol and 2.0 ml of concentrated sulfuric acid. After the reaction, the absorbance readings were determined in a spectrophotometer

(Shimadzu, UV-1800) at 490 nm. In the sheaths, 10 µl of the sample, 490 µl of 80 % ethanol, 500 µl of 5.0 % phenol and 2.0 ml of concentrated sulfuric acid were used. For the roots, the samples (10 µl) were mixed with 490 µl of 80 % ethanol, 500 µl of 5.0 % phenol and 2.0 ml of concentrated sulfuric acid. For P. purpureum, 50 µl of shoot samples were then mixed with 450 µl of 80 % ethanol, 500 µl of 5.0% phenol and 2.0 ml of concentrated sulfuric acid. After the reaction, the absorbance readings were determined as in the previous species. In the hems and roots the procedure was the same as previously followed.

Analyzes of reducing sugars

Reducing sugars were estimated according to the method described by Miller (1959). For the preparation of Nelson A (20 ml) was used anhydrous sodium sulfate (20 % Na_2SO_4), 2.5 % anhydrous sodium carbonate (Na_2CO_3), 2.0 % sodium bicarbonate ($NaHCO_3$) and sodium tartrate and 2.5 % potassium. To prepare the Nelson B reaction (60 ml) was used copper sulphate pentahydrate ($CuSO_4.5H_2O$) 15 % and sulfuric acid (H_2SO_4) 0.01 %. Subsequently molybdic acid (dissolving in 50 ml) was prepared: 5 % ammonium molybdate, 4.25 % sulfuric acid (H_2SO_4) and 0.6 % sodium dibasic arsenate (HNa_2AsO_4).

The quantification of the reducing sugar content in the aerial part new leaves (FN), intermediates (FI), in the sheath and roots of Pennisetum glaucum and P. purpureum were determined from ethanolic extracts. Samples (50 µl) of aerial part (AP) and roots were mixed with 200 µl solution of 80 % ethanol, 250 µl of the Nelson A + B mixture and then stirred and heated in a 95 ° C water bath. 10 minutes. After cooling in a vessel containing ice water, 250 µl of molybdic acid and 1.75 ml of distilled water were added. Absorbance readings were determined in a spectrophotometer (Shimadzu, UV-1800) at 540nm. The calibration curve was elaborated with a standard solution of glucose in different concentrations, expressed in mg g^{-1} of MF.

Curve pattern of reducers sugars

The standard solution of glucose (500 mg L^{-1}) was diluted with distilled water in test tubes, where these values were converted to mg g-1 of MF for the required calculation. The tubes were then shaken for homogenization and a 1.0 mL aliquot was removed for the SN test (Somogyi-Nelson).

Designing experimental and statistical analysis

The experimental design was a completely randomized design, in a 2x3 factorial scheme (*P. glaucum* and *P. purpureum* and two control treatments, and saline stress), with seven replications. The data were submitted to analysis of variance (ANOVA) and the means were compared by the Tukey test at a significance level of 5% using the Sigmaplot program.

Results and discussion

The levels of K^+

The plants that are able to maintain the relationship K^+/Na^+ in the interior of its cells have a greater willingness to develop in saline environments, because K^+ competes with Na^+ and reduces the deleterious effect of this (Flowers & Läuchli, 1983). The levels of K^+ of the new leaves and intermediate, of the sheaths and roots of *P. glaucum* and *P. purpureum* grown under conditions of control and stress saline are presented in Figure 1.

In the FN of *P. glaucum*, the levels of K^+ did not differed statistically in the two treatments (Figure 1A), this shows that the same plants subjected to stress saline maintained their levels of K^+, therefore the treatment saline compared with the control maintained a medium almost equal (Figure 1B).

In relation to the sheaths (Figure 1C), the levels of K^+ in stress saline as well as in the aerial part presented no statistical differences compared with the control in the species P. *glaucum* and *P. purpureum*. In roots, the accumulation of K^+ a function of stress saline, showed the almost equal to the control in the two species (Figure 1D). Therefore in the roots was not observed effect of the salinity in the species studied.

It is well known that K+ has an important role in the stress tolerance of salt spray (Kerbauy, 2012). This cation is essential in regulating the osmotic cell as well as in the activation of enzymes citosólicas that require this ion as a cofator. Studies have shown that the accumulation or maintenance of the levels of K^+ in plant tissues under stress conditions would indicate a greater tolerance when they are in conditions of stress. In the present work, it was observed that the two species have not accumulated K^+ in most of the tissues analysed under stress conditions saline. On the other hand, the maintenance of the levels of K^+ under conditions of salinity, and not its accumulation can be attributed to the competition that exists between the Na^+ and K^+ by the sites of absorption of the latter. The Na^+ absorbed by the plants when in high concentrations in the soil can remain in the root or be transported via the xylem to the tissues and air (Munns & Tester, 2008).

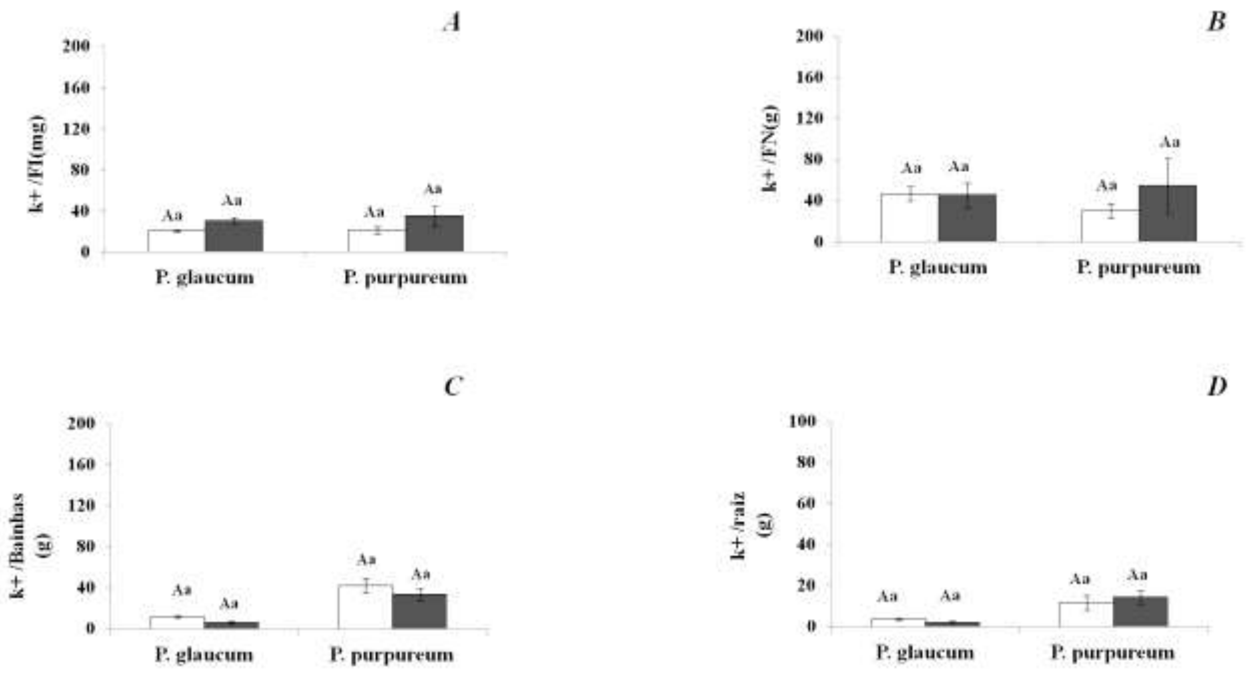

Figure 1. Potassium in the aerial part leaves new (The), sheets interim (B) Hem (C) and roots (D) of plants of Millet (P. *glaucum*) and grass elephant (P. *purpureum*) grown in solutions nutritional of Hoagland and submitted to the treatments control (white bar) and stress-buffered saline with NaCl to 60 mM (bars in dark gray). Uppercase letters compare the means of treatments within the same species. Lowercase letters compare the means of treatments between both species. Averages followed by same letter do not differ statistically by the test of Tukey 5% of probability.

Levels of Na^+

The data of sodium from the aerial part leaves new and interim, of the sheaths and roots of *P. glaucum* and *P. purpureum* grown under conditions of control and stress saline are shown in (Figure 2).

In general, the accumulation of Na^+ in the plants was relatively high in the FN of plants of *P. glaucum* subjected to salinity, differing statistically from the control (Figure 2A). This increase was 940,3 % higher than the control. Similar results were observed also in the FI, with an increase of 829,3 % greater than the control. In the species *P. purpureum* in FN there was no statistical difference in the treatment saline when compared with the control (Figures 2A and 2B). This suggests that the last species would be a mechanism to level of roots that controls the transport of Na^+ to the shoot. In several species of grasses the retention of Na^+ in the roots prevents the accumulation on the leaves to prevent damage to the apparatus the photosynthetic plant.

This feature of tolerance was observed in the species studied here. The accumulation of Na+ in the roots shows that the plants of *P. purpureum*, prevent the transport of this ion to be made for the aerial part, making with that the same are not harmed as they carry out photosynthesis. The plants of *P. purpureum* accumulated 558,6 % more Na^+ compared to the control, so these plants tend to accumulate more Na^+ in the roots, preventing the build-up is higher in the aerial part and in the hems, for mass production is a relevant factor, because the plant shows that the productivity of the mass will not be affected in saline soils.

In *P. purpureum* FI there was no statistical difference in the treatment saline when compared with the control, this shows that the plant be more adapted to a soil type with high levels of salinity, without accumulations high in the air.

In the sheaths the accumulation of Na+ was relatively high (Figure 2C). In the plants of *P. glaucum*, the treatment saline not differentiated statistically from the control, being that it was observed that there was a higher accumulation of Na^+ in the plants, however, was not sufficient to be considered statistically (Figure 2C). In the plants of *P. purpureum*, treatment saline differentiated statistically from the control, with an increase of 888,3 % greater than the control (Figure 2B), so these plants tend to accumulate more Na^+ in the sheaths, preventing the build-up is higher in the aerial part, mass production is a relevant factor, because the plant shows that the productivity of the mass will not be affected in saline soils. These data are particularly relevant, since there are no reports that the hems act as organs of retention to the stress of saline.

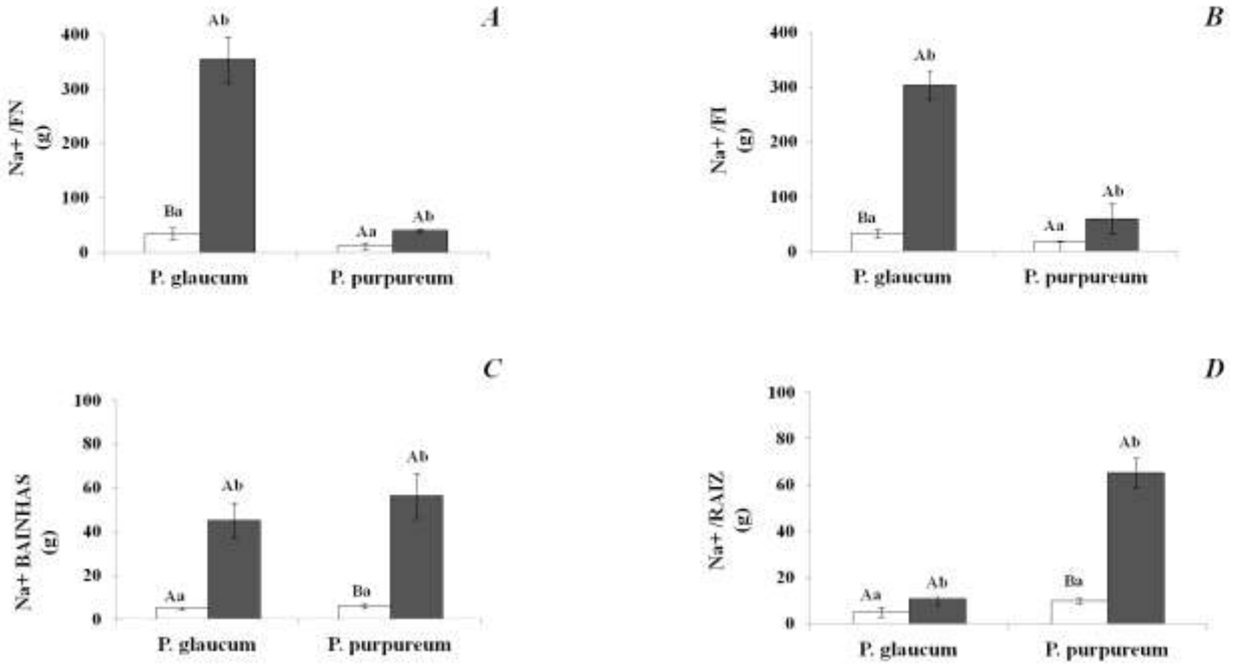

Figure 2. Levels of Na+ in the shoot out new leaves (The), sheets interim (B) Hem (C) and roots (D) of plants of Millet (*P. glaucum*) and grass elephant (*P. purpureum*) grown in solutions nutritional of Hoagland and submitted to the treatments control (white bar) and stress-buffered saline with NaCl to 60 mM (bars in dark gray). Uppercase letters compare the means of treatments within the same species. Lowercase letters compare the means of treatments between both species. Averages followed by same letter do not differ statistically by the test of Tukey 5% of probability.

Carbohydrates Total Soluble

The data of carbohydrates soluble sheets new and interim, of the sheaths and roots of plants of *P. glaucum* and *P. purpureum* grown under conditions of control and stress saline, are shown in Figure 3.

The carbohydrate soluble in the species *P. glaucum* in the FN, there were no statistical differences between the treatment of saline and the control, this shows that the plants of *P. glaucum,* even in conditions of stress managed to accumulate almost the same quantities of carbohydrates soluble in comparison with the control plants (Figure 3A).

In relation to carbohydrates soluble FN *P. purpureum* (Figure 3A), the concentration of these recorded large increases in the aerial part when subjected to salinity. The carbohydrates soluble FN *P. purpureum* (Figure 3A), the concentration of these recorded large increases in the aerial part when subjected to salinity, being that the treatments differed among themselves, the treatment of saline differentiated statistically from the control, increasing 218,0 % compared to the control. In FI in the species *P. glaucum*, although the analyses of data showed that these values do not have statistical significance, according to the analysis in the plants you will note that both FN and FI the results were almost the same.

The carbohydrates soluble *P. purpureum* (Figure 3B), the concentration of these did not register differences in statistics when compared with the control. In the species *P. glaucum.*

The analyses of the data showed that these values do not have statistical significance, because it has accumulated a low amount of carbohydrates soluble.

In the plants of the species *P. purpureum*, in the analysis of the sheaths was observed if the concentration of high levels of carbohydrates that are soluble when subjected to salinity, and thus, an increase of 680,0 % compared to the control. The plants in the treatment saline have achieved high levels of accumulation. (Figure 3C).

In the roots of the two species according to the analysis there were no statistical differences, being that the results correspond to the previous data, as if the plants have been able to have an accumulation greater carbohydrate soluble in the aerial part and in the sheaths, thus their accumulation will be less in the roots, showing so that it does not have statistical differences within treatments or between species. In general the species *P. glaucum*, accumulates much less carbohydrates soluble in all tissues of the plants that the species *P. purpureum*, where the plants have a significant increase of the carbohydrates soluble.

Despite this accumulation of carbohydrates under stress conditions saline in *P. purpureum*, there was a reduction in content relative to water in these species (Figure 3A and 3B). According to Kramer and Boyer (1995), the addition of solutes osmoticamente assets to the cells is more effective in promoting the lowering of the water potential that the loss of water.

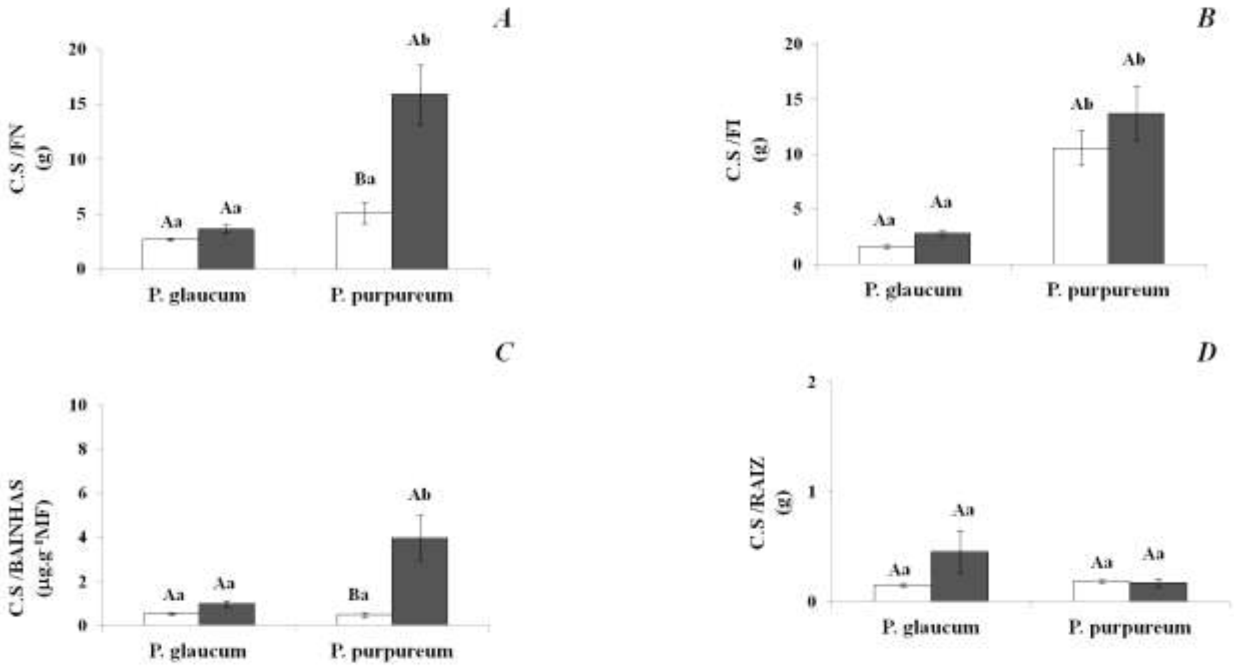

Figure 3. Carbohydrates soluble in the air new leaves (A), sheets interim (B) Hem (C) and roots (D) of plants of Millet (*P. glaucum*) and grass elephant (*P. purpureum*) grown in solutions nutritional of Hoagland and submitted to the treatments control (white bar) and stress-buffered saline with NaCl to 60 mM (bars in dark gray). Uppercase letters compare the means of treatments within the same species. Lowercase letters compare the means of treatments between the two species. Averages followed by same letter do not differ statistically by the test of Tukey 5% of probability.

Reducing Sugars

The data of carbohydrates-reducing of the new leaves, and interim, of the sheaths and roots of plants of *P. glaucum* and *P. purpureum* grown under stress conditions saline and control, are presented in Figure 4.

The levels of sugars in the species *P. glaucum* on FN, showed no statistical differences between the treatments, being that in the saline, this reduction was of 82,4 % (Figure 4A).

Different species earlier in the FN of *P. purpureum* showed that there were no statistical differences between the treatment saline when compared with the control (Figure 4A). This shows that the treatment has little effect on the synthesis of carbohydrates reducing, from which will be formed other carbohydrates such as sucrose, the main sugar transported in the plant. Plants of *P. purpureum*, even in the stress conditions were able to earn almost the same amounts of sugars compared with the control plants, (Figure 4A).

In FI and in the sheaths of the species *P. glaucum*, although the analyses of data showed that these values do not have statistical significance, we can note that both FN and FI the results were almost the same.

The levels of sugars in the sheaths of *P. purpureum*, do not showed statistical differences, the plants accumulated low amounts of sugar reducer. (Figure 4C).

By analyzing the roots of the two species in the treatment saline there were no statistical differences when compared with the control (Figure 4D).

The reducing sugars are capable of reducing the oxidizing agents present in the plant, distinguishing it from other sugars as oligosaccharides and polysaccharides, which do not have this feature without suffering hydrolysis of connection glicosídica, are referred to as sugars non-reducing (Maldonade et al., 2013).

Gutierrez et al. (1976), studying the species of *P. purpureum* with the title variations of daylight in carbohydrate content soluble stem and leafs of grass, Napier, observed that the content of reducing sugars in the stem was maximum at 15 hours, and in the leaves were not detected significant differences. The ratio sugars/carbohydrate total, practically has not suffered variations during the harvest, both for rod as for the leaves, comparing with the present work we can observe differences, because unlike the cited work, there was a higher accumulation of soluble sugar than sugars

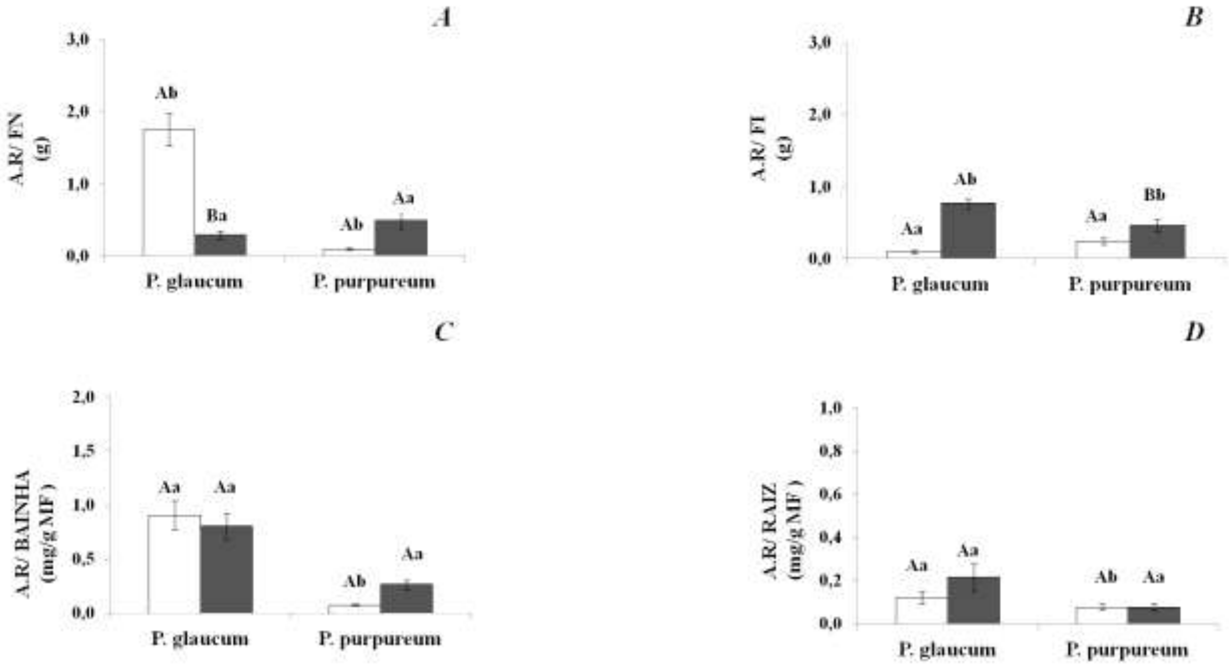

Figure 4. Reducing sugars in the aerial part leaves new (The), sheets interim (B) Hem (C) and roots (D) plants of millet (*P. glaucum*) and grass elephant (*P. purpureum*) grown in solutions nutritional of Hoagland and submitted to the treatments control (white bar) and stress-buffered saline with NaCl to 60 mM (bars in dark gray). Uppercase letters compare the means of treatments within the same species. Lowercase letters compare the means of treatments between both species. Averages followed by same letter do not differ statistically by the test of Tukey 5% of probability.

Conclusions

The stress-saline influenced mainly due to the growth of the aerial part of P. purpureum; also in P. purpureum fresh pasta was influenced, presenting smaller in plants grown with the stress saline, the root system was less developed than the aerial part.

The carbohydrate content soluble as a response to salinity had a greater record in the plants of *P. purpureum* grown in solution and in the sheets interim of *P. glaucum*, was obtained, a value quite high in relationship to the other variables.

Plants of *P. glaucum*, cultivated in solution with the stress salt have accumulated more ions inorganic in the air, already *P. purpureum* the highest levels were recorded in the roots. With respect to adaptation to stress-saline species that most stood out was the *P. purpureum*, featuring a smaller accumulation of salts, and increasing the carbohydrate content in the tissues of plants, causing the plants of *P. purpureum* has a higher resistance than *P. glaucum*.

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