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Full Length Research Paper

Application of multivariate analysis to evaluate the biochemical changes in sorghum (*Sorghum bicolor* L. Moench) after exposure to water stress and silicon applications

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The objective of the present work was to verify, through multivariate analysis, the behavior patterns of biochemical compounds in forage sorghum, submitted to different silicon applications and water stress. Experiment with forage sorghum (*Sorghum bicolor* L. Moench), variety BR 700, was conducted in a greenhouse. The experimental design was completely randomized in a 2×4 factorial arrangement with seven replicates, two hydric conditions (irrigated and water deficit) and four silicon applications (0.5, 1.0, 1.5 and 2.0 μM). The multivariate analysis showed that when there is no shortage of water and regardless of the silicon dose, nitrate levels were higher and carbohydrate, proline and sucrose levels were lower in leaves and roots. The quantity of biochemical compounds differed between sorghum leaves and roots. This condition also varied according to the soil water stress. Silicon application in sorghum plants mitigates the negative effect of drought stress, favoring this crop cultivation in areas of low water availability. Nonetheless, differences between silicon doses were not observed in this experiment. Therefore, it is recommended that this chemical should be applied in drought-ridden areas.

Key words: Water stress, proline, multivariate analysis, principal component analysis.

INTRODUCTION

Sorghum (*Sorghum bicolor L.* Moench) originates in the tropics and is cultivated in various regions of the world. In Brazil, sorghum is mainly used as a lower-cost alternative to corn in feed rations. It is also an important biomass producer in no-till farming and crop rotations (Landau and Guimarães, 2010).

Sorghum has a dense root system capable of breaking up soil and moving nutrients through different soil layers. It is a salt and aluminum-tolerant crop, making areas suitable for crop-growing, which would otherwise be

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considered marginal for agriculture (Prasad et al., 2007; Vasilakologlou et al., 2011). Besides, sorghum is also an important crop for ethanol production due to the high sugar levels in its stems (Zhao et al., 2009; Ratnavathi et al., 2011; Han et al., 2012; Zegada-Lizarazu and Monti, 2012).

Under soil water-stress conditions, sorghum can stunt its growth or decrease its metabolic activity and later, when water conditions improve, it resumes growth at rates that may be higher than those of plants that have not undergone water stress (Amaral et al., 2003). Insufficient water is considered one of the greatest worldwide limitations to agriculture because of its impact on species growth and development (Batista et al., 2008) and consequent reductions in relative water content, nitrate-reductase activity, proteins, ammonium, tissue nitrate and increased concentrations of amino acids (Souza et al., 2014).

Silicon is a nutrient that may improve water use in plants (Lima et al., 2011). However, in Brazil, there are limited studies on using silicon in sorghum crop management (Souza et al., 2011). Therefore, it is important to know water-deficit effects, in order to relate them to mechanisms providing tolerance in sorghum cultivation, such as silicon application, so the crop production is not adversely affected by water-deficit stress(Souza et al., 2013).

Information on behavior patterns and relations between these mechanisms,when silicon is applied to reduce the negative effects of water deficit, is still incipient.

The use of multivariate analysis is crucial for this study, since the application of univariate analysis, in which variables are verified separately, does not provide a broad and joint view of all variables, as is the case for the multivariate statistical method. The joint assessment of all biochemical variables helps reach a better understanding of the effects of silicon application in plants under drought stress, as this is a phenomenon that depends on several biochemical variables to obtain a more comprehensive answer (Vicini, 2005).

Thus, the present research starts from the hypothesis that silicon application mitigates the negative effects of water deficit that affect the levels of biochemical compounds in sorghum plants. In order to validate or disprovethis hypothesis empirically, multivariate analysis was applied to relate all biochemical compounds in only one response. Hence, this work aimed at verifying, through multivariate analysis, the behavior patterns of biochemical compounds of forage sorghum (*S. bicolor* L. Moench) submitted to different silicon doses and water stress.

MATERIALS AND METHODS

Characterization and preparation of the experiment

The experiment was carried out in 2011 in a greenhouse at the CapitãoPoço campus of the Federal Rural University of Amazonia (UFRA - Universidade Federal Rural da Amazônia), in the municipality of CapitãoPoço, State of Pará, Brazil (Geographic coordinates: Latitude 01°44'47" South; Longitude 47°03'34" West).

Forage sorghum (*S. bicolor* L. Moench.) variety BR 700 was used and acquired from Embrapa Corn and Sorghum (Embrapa Milho e Sorgo - Empresa Brasileira de Pesquisa Agropecuária), 2010 harvest.

The experiment used a completely randomized design (CRD) with a 2×4 factorial arrangement and seven replicates: two hydric conditions (irrigated and water deficit) and four silicon application doses (0.5, 1.0, 1.5 and 2.0 µM) that were evaluated in the roots (R) and leaves (L) of sorghum plants. For treatment groups, silicon doses were applied in the roots (ER-Si) and in the leaves (EF-Si), and control groups included water stress without silicon application in the leaves (CWSL) and in the roots (CWSR), and water stress-free and without silicon application in the leaves (CWOSL) and in the roots (CWOSR).

For experiment installation, 1 L pots containing sand substrate:vermiculite (1:2) were used and irrigated twice a day (morning and afternoon) with Hoagland and Arnon nutrient solution (1950), from the third day after germination, until the third day after germination; only distilled water was added. The pots were spaced 0.60 m between rows and 0.40 m between plants and were randomly distributed.

In control groups under stress, the water deficit was applied from the 25th day after germination and the water suspension was maintained for 7 days. Stress-free control groups only received nutrient solution for 32 days.

Two plants were maintained in each pot after sowing and emergence. Seedling emergence and silicon applications occurred 3 days after sowing. From the 4 to 10th day after sowing, the plants were fed with half-strength nutrient solution and from the 11th day until the end of the experiment, the plants received the full-strength nutrient solution with different silicon concentrations (0.5 0, 1.00, 1.50, and 2.00 µM) in the form of sodium metasilicate ($Na_2SiO_3.9H_2O$). The silicon solutions were adjusted to a final pH of 5.8 (±0.2).

The plants were irrigated twice a day (morning and afternoon) with a nutrient solution (Hoagland and Arnon, 1950) containing the available macro and micronutrients shown in Table 1. The plants were irrigated only with distilled water until the third day after germination. Environmental conditions were not controlled during the experiment; however, temperature and relative air humidity were monitored using a digital thermo-hygrometer (5203 Model, Incoterm, RS, Brazil).

Evaluated variables

The analysis of relative water content in the roots and leaves was performed using the method described by Slavick (1979). Free ammonia rates were obtained using the method of Weatherburn (1967), nitrates were assessed using the method described by Cataldo et al. (1975), sucrose was determined using the method of Van Handel (1968) and the total soluble carbohydrate was obtained using the method described by Dubois et al. (1956).

The enzymatic activity of nitrate reductase was determined using the method described by Hageman and Hucklesby (1971), the activity of soluble proteins was determined by the method described by Bradford (1976) and soluble amino-acids activity was determined by the method recommended by Peoples et al. (1989).

Data processing and statistical analysis

The variability of the properties studied was evaluated by descriptive statistics (mean, standard deviation, minimum and

Table 1. Available nutrients in the nutrient solution added to the substrate for growth of forage sorghum in a greenhouse in CapitãoPoço, State of Pará, Brazil.

Macro	Conc.	ml L^{-1}	Micro	Conc.	ml L^{-1}	Silicon	Conc.	ml L^{-1}
KNO_3	1 M	5	H_3BO_3	0.04	1	$MoO_4.2H_2O$	0.0001 M	1
$Ca(NO_3)_2$	1 M	3	$MnCl_24H_2O$	0.009	1	$CoCl_2 . 6 H_2O$	0.004 M	1
NH_4NO_3	1 M	2	Na_2(EDTA)	0.08	1	$Na_2SiO_3.9H_2O$	0.5 μM	0.5
KH_2PO_4	1 M	0.1	Fe-EDTA		1	$Na_2SiO_3.9H_2O$	1.00 μM	0.5
$MgSO_4$	1 M	1	$CuSO_4.5H_2O$	0.0003	1	$Na_2SiO_3.9H_2O$	1.50 μM	0.5
$FeSO_4.7H_2O$		0.1	$ZnSO_4.7H_2O$	0.0007	1	$Na_2SiO_3.9H_2O$	2.00 μM	0.5

Macro: Macronutrient; Micro: micronutrient; conc.: concentration.

maximum). Afterwards, the variables were assessed by multivariate exploratory analyses (hierarchical cluster and principal component analyses). Grouping analysis was performed using a similarity matrix constructed from Euclidean distances and links between groups were determined using the method described by Ward (Sneath and Sokal, 1973). In this method, the distance between the two groups is defined as the sum of squares between all variables of both groups (Hair et al., 2005). The Euclidean distance between the access points of each variable set was calculated. This process distinguished each of the factors and allowed the illustration of the data group structure in a dendrogram form. The following variables were used for the principal component analysis (PCA): protein (PROT), amino acids (AMIN), ammonium (AMON), nitrate (NITR), carbohydrate (CARB), proline (PROL), sucrose (SUC), nitrate-reductase activity (NRA), glycine-betaine (GB), starch (STAR) and relative water content (RWC). Afterwards, the set of variables was dimensioned to visualize better the relationship between variables in coordinate axes. These new axes, the eigenvectors (new variables), defined as principal components (PC), were generated by linear combinations of the original variables constructed from the eigenvalues of the covariance matrix (Hair et al., 2005; Piovesan et al., 2009). Aiming at finding a simpler and more parsimonious model, criteria established by Kaiser (1958) were applied with eigenvectors greater than one. The STATISTICA 7.0 software package (StatSoft. Inc., Tulsa, OK, USA) was used to carry out the analyses. This is an integrated software used to perform statistical analysis and database, characterizing a broad selection of the analytic process (from beginner to advanced) for different areas (Ogliari and Pacheco, 2011).

Additionally, basic assumptions underlying analysis of variance, such as normality of errors and homogeneity of variance, were tested for all variables (data not shown).

RESULTS AND DISCUSSION

Grouping analysis of biochemical compounds

Proteins in the treatment under stress and silicon application displayed averages ranging between 0.48 and 2.52 mg g^{-1}, in the root portion (ER-Si) and in the leaves (EF-Si), respectively (Table 2).

The protein content in treatments without silicon application in the leaves displayed averages ranging between 1.35 and 4.16 mg g^{-1},under water stress (CWSL)and stress-free (CWOSL), respectively.In the root portion, the protein content showed lower levels, with an average of 0.27 (CWSR) and 1.83 mg g^{-1} (CWOSR) (Table 2).

The amino acids contents varied from 3.81 μmol g^{-1} (ER-Si) to 13.92 μmol g^{-1} (EL-Si), and in CWOSL, CWSL, CWOSRandCWSR treatments, the amino acids contents displayed the following results: 9.59, 21.4, 4.09,and 8.68 μmol g^{-1}, respectively (Table 2).

Cluster analysis showed three distinct groups: G1, G2 and G3 (Figure 1). The G1 represented treatments with silicon applications inthe roots. G3 represented silicon applicationsin the leaves as well as the control without water stress in the leaves. Group G2 represented the control treatments without silicon applications and under water stress and stress-free.

Differences of Si levels in leaves and roots are due to the characteristics of this nutrient. Specifically, silicon is absorbed as silicic acid (H_4SiO_4), either passively or actively, and accumulates primarily in leaves as amorphous silica (SiO_2nH_2O) (Ma et al., 2007).

There was a difference between leaves and roots for the groups. However, within each group, differences betweensilicon application rates were found (0.5, 1.0, 1.5 and 2.0 μM silicon). Such contrasts were observedbecause the lowest application rateswere closer to the Y axis (Figure 1). Silicon probably did not contribute to the formation of nitrogen compounds, proteins and amino acids in the leaves and roots of the forage sorghum plants. Souza et al. (2014) worked with corn and showed similar results with reductions in total soluble proteins in roots and leaves atsilicon concentrations of 1.5 and 2.0 μM.

The control groups without water stress had higher nitrate levels in the leaves (0.46 μmol kg^{1}) and in the roots (0.58 μmol kg^{-1}) (Table 2). This occurred because nitrate-reductase activity decreases significantly in plants that are exposed to water stress as compared to plants that do not experience water stress (Souza et al., 2014). Under these conditions, carbohydrate, proline and sucrose levels are also lower (0.69 mmol g^{-1}, 4.67 mmol kg^{-1} and 35.4mmol g^{-1}in the leaves and 0.76 mmol g^{-1},4.32 mmol kg^{-1} and 15.72 mmol g^{-1}in the roots). This reduction is probably related to the fact that α and β-amylase degrade starch, contributing to the formation of new sugars. Proline accumulates in the cytosol when the

Table 2. Average protein (PROT), amino acids (AMIN), ammonium (AMON), nitrate (NITR), carbohydrate (CARB), proline (PROL), sucrose (SUC), nitrate-reductase activity (NRA), glycine betaine (GB), starch (STAR) availability and relative water content (RWC) in forage sorghum (*Sorghum bicolor* [Moench.]), variety BR in the roots (SR) and leaves (SF) in treatments that received silicon applications and control groups under water stress and without silicon in the leaves (CWSL) and in the roots (CWSR), and stress-free without silicon on the leaves (CWOSL) and on the roots (CWOSR).

Variable	PROT	AMIN	AMON	NITR	CARB	PROL	SUC	NRA	GB	STAR	RWC
Treatment without silicon in leaves											
Stress-free witness	4.16^a	9.59^c	19.03^c	0.46^b	0.69^b	4.67^e	35.40^e	1.48^a	5.90^e	0.24^a	91^a
Witness under stress	1.35^d	21.4^a	11.52^e	0.23^b	1.76^a	14.10^a	55.18^b	0.05^b	14.1^a	0.08^a	59^c
Treatment without silicon in roots											
Witness under stress	1.83^c	4.09^e	30.21^a	0.58^a	0.76^b	4.32^f	15.72^f	0.71^a	5.20^f	0.12^a	0^d
Witness under stress	0.27e	8.68d	15.27d	0.13b	1.23b	12.28b	64.31a	0.07^b	12.6^b	0.02^a	0^d
Treatment with silicon											
Leaves	2.52^b	13.92^b	19.16^c	0.35^b	0.78^b	6.65^d	37.8^d	0.49^{ab}	8.55^c	0.18^a	71^b
Roots	0.48^e	3.81^e	24.78^b	0.22^b	0.76^b	7.79^c	38.94^c	0.43^b	7.65^d	0.07^a	0^d
C.V.	2.98	2.27	5.76	6.87	4.45	6.53	7.03	6.61	6.21	24.26	2,04

Values of compounds are expressed as: nitrate (μmol kg^{-1}), ammonium (μmol kg^{-1}), nitrate-reductase activity (μmol g^{-1}), amino acid(μmol g^{-1}), protein (mg g^{-1}), carbohydrates (mmol g^{-1}), starch(mmol g^{-1}), sucrose (mmol g^{-1}), glycine-betaine (μmol g^{-1}), and proline(mmol kg^{-1}).

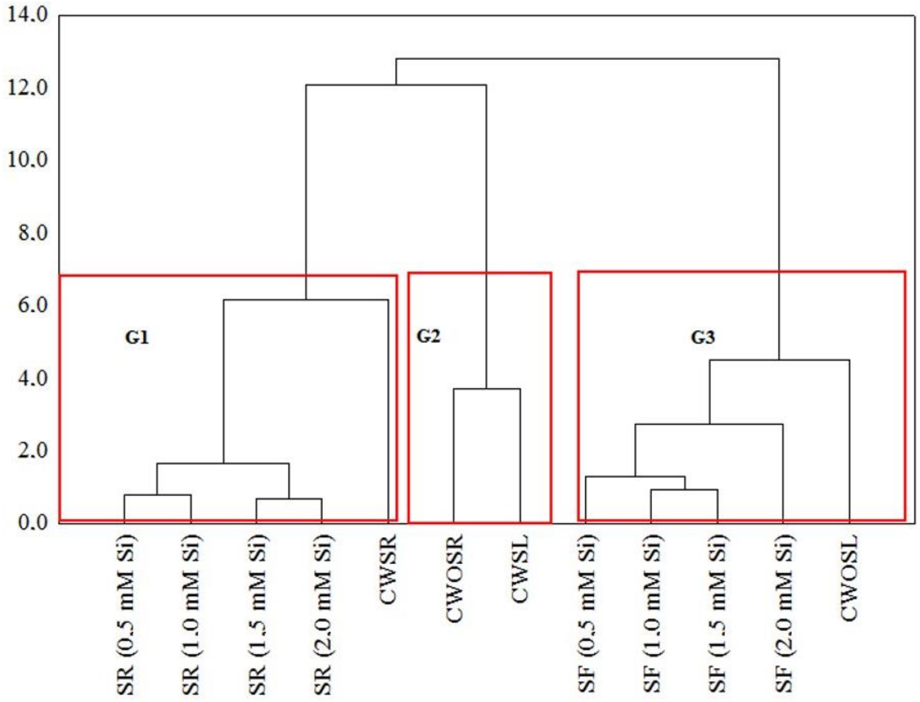

Figure 1. Grouping analysis of nitrogen, protein and amino acids in the roots (SR) and leaves (SF) of forage sorghum after silicon applications (0.5, 1.0, 1.5 and 2.0 μM of Si), and controls under water stress and without silicon in the leaves (CWSL) and in the roots (CWSR) and water stress-free and without silicon on the leaves (CWOSL) and on the roots (CWOSR).

plant undergoes water stress, resulting in osmotic adjustment and plant tolerance towater deficit (Ashraf and Foolad, 2007). These compounds are stress-sensitive, particularly to water stress. Similar results were found by Souza et al. (2014).

Plants leaves that did not undergo water stress had higher aminoacids and protein contents when compared with plants receiving lower silicon application rates (Table

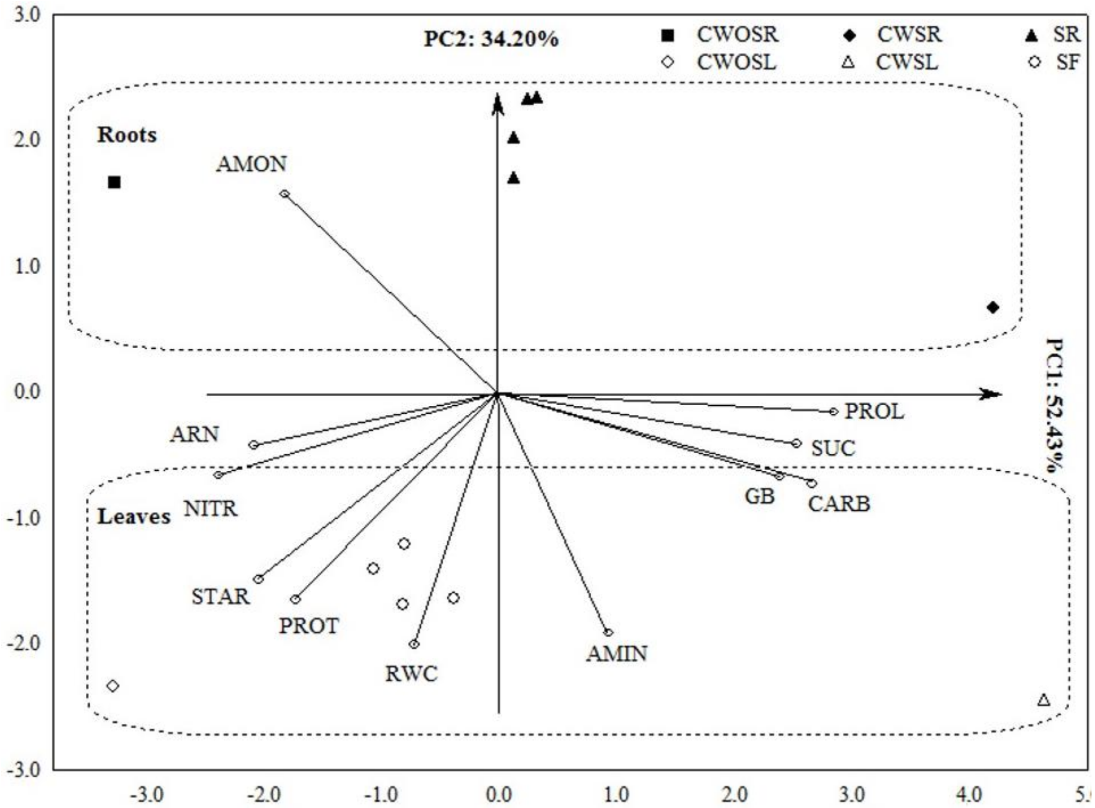

Figure 2. Principal component analysis (PCA) of PC1 and PC2 with the variables: protein (PROT), amino acid (AMIN), ammonium (AMON), nitrate (NITR), carbohydrate (CARB), proline (PROL), sucrose (SUC), nitrate-reductase activity (NRA), glycine-betaine (GB), starch (STAR) and relative water content (RWC) in the roots (R) and leaves (F) of forage sorghum plants, in treatments that received silicon applications and control groups under water stress and without silicon in the leaves (CWSL) and in the roots (CWSR), and stress-free without silicon on the leaves (CWOSL) and on the roots (CWOSR).

2). Of all the application rates, the 2.0 µM rate led to the highest aminoacid and protein levels. According to Takahashi (1995), silicon absorption is not inhibited when water supplies are temporarily interrupted, because respiratory inhibitors affect absorption.

PCA of biochemical compounds

A two-dimensional plane was formed by PCA (Figure 2), which was, on its turn, generated from the first two components PC1 and PC2 that corresponded to 86.63% of theoriginal information: 52.43 and 34.20%. This result is consistent with criteria established by Sneath and Sokal (1973), who affirm that the PC used for interpretation must explain at least 70% of the total variance.

Regarding control treatments, the same behavior found in the grouping analysis was also observed in PCA, considering that the same were positionedat the extremities of the biplot and displayed low correlation with the analyzed variables.

The PC1attributes that presented the highest significant positive correlation coefficients were: PROL (0.98), GB (0.91), SUC (0.87) and CARB (0.82). The highest significant negative correlation coefficients were:NRA (-0.71), AMON (-0.61), NITR (-0.80), STAR (-0.69) and PROT (-0.58). Table 3 shows these correlations, while Figure 2 represents each attribute by an arrow and its projection.

The PC2 contained fewer variables. As in PC1, PROT, AMON and STAR were also highly significant in PC2. In order of importance, PC2variables that exhibited the highest positive correlation coefficient was AMON (0.75) and the highest negative correlation coefficient were observed inRWC (-0.94) AMIN (-0.90), PROT (-0.77), and STAR (-0.69) (Table 3).

The PROL, GB, SUC and CARB levels increased in the plants, whereas NRA, AMON, NITR, STAR and PROT decreased (Table 3). The NRA, RWC, NITR and AMON decreased and AMIN levels increased in sorghum plants exposed to water stress. These results are due to the fact that water deficits promote the degradation of proteins through proteolytic enzymes and, consequently, increase

Table 3. Coefficient correlation of principal components for the variables.

Variable	PC1 (52.43%)	PC2 (34.20%)
PROT	-0.58	-0.77
AMIN	0.32	-0.90
AMON	-0.61	0.75
NITR	-0.80	-0.31
CARB	0.82	-0.31
PROL	0.98	-0.07
SUC	0.87	-0.18
ARN	-0.71	-0.19
GB	0.91	-0.33
STAR	-0.69	-0.69
RWC	-0.24	-0.94

*Values refer to the percentage of variability of the original data set generated by the respective principal components. Correlations in bold type (>0.50, absolute value) were considered highly significant in the interpretation of principal components. PROT: Protein; AMIN: amino acid; AMON: ammonium; NITR: nitrate; CARB: carbohydrate; PROL: proline; SUC: sucrose; NRA: nitrate-reductase activity; GB: glycine betaine; STAR: starch; RWC: relative water content.

AMIN levels. In addition to water stress, amounts of nitrogen (N) should be observed, given that this nutrient normally restricts plant growth in low-rainfall areas. Thus, decreases in NRA reduce AMIN, PROT and chlorophyll formation, which in turn affects corn growth and development (Souza et al., 2014).

Low water levels in plants alter their metabolism. Therefore, plants respond osmotically to increased carbohydrate levels by adapting to water scarcity in the environment (Alves, 2010; Costa, 2010).

Positive correlations between PC1carbohydrate and sucrose parameters (Table 3 and Figure 2) in the root system are responses to water stress and consist of osmotic adjustmentsfor plant metabolism that reduces osmotic potential, or differences in concentration gradients, which in turn maintain turgidity and consequently delay dehydration of plant tissues (Salisbury and Ross, 2012).

Starch concentrations decreased in plants under water stress and without silicon (Table 2). This response is probably due to the primary activity of α and β amylase enzymes, which transform starch into carbohydrates and sucrose (Oliveira Neto et al., 2009). Proline and glycine-betaine were also positively correlated (Figure 2) due to specific enzymes breaking down proteins into aminoacids. This occurs because water stress increases proteolytic enzyme activity, which breaks down the reserved proteins in plants there by reducing the synthesis of new proteins. Water stress essentially interferes with plant biochemical metabolism and, as a defense against water scarcity, causes metabolic behavior, among other things, breaking down proteins into amino acids such as proline (Oliveira Neto, 2010).

These strong correlations between aminoacids may indicate their participation not only in osmotic adjustment and osmoregulation, but also in stabilizing the structures and enzyme activity of protein complexes and protecting the integrity of membranes against the damaging effects of various types of water stress (Sakamoto and Murata, 2002).

The negative correlation coefficients in the PC1 nitrate parameter (Table 3 and Figure 2) are mainly due to low water content in the soil, given that nitrate (NO_3^-) is the main form that nitrogen is absorbed from the soil, followed by ammonia (NH_4^+) (Malavolta, 2006).

However, when quantities of $NO_3^-$are insufficient, absorption by the roots is hindered, decreasing transport through the shoots via the xylem-transpiration pathway (Shaner and Boyer, 1976). This, in turn, decreases nitrate-reductase activity, which was negatively correlated in this experiment (Table 3 and Figure 2). Low water flow in the transpiration system may have decreased the activity of this enzyme in plants undergoing water stress (Oliveira Neto, 2010).

Conclusion

Multivariate statistical analysis showed that treatments water stress-free, regardless of silicon dose applied, presented higher nitrate levels and lower carbohydrate, proline and sucrose levels in both leaves and roots. Differences were observed in the amount of biochemical compounds in sorghum roots and leaves,and this quantity also varied according to soil water-stress conditions.

Silicon application in sorghum plants mitigates the negative effects of drought stress, favoring this crop cultivation in areas of low water availability. Therefore, the application of this compound is highly recommended, especially in regions undergoing dry conditions.

Conflict of Interests

The authors have not declared any conflict of interests.

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