METABOLIC RESPONSE TO SALT STRESS IN SORGHUM PLANTS

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ABSTRACT: Salinity is one of the abiotic factors that most limits plant growth and development, causing metabolic, physiological, and molecular disturbances. Therefore, this study investigated metabolic variations that may confer salinity tolerance in sorghum. For this, seedlings were grown in Hoagland's solution and subjected to salt stress (75 mM NaCl) for 12 days, when they were harvested for physiological and metabolic analysis. Salinity reduced plant growth, with the most significant effect on leaf biomass accumulation. Under stress, plants accumulated more soluble carbohydrates in the leaves, which led to a reduction in leaf osmotic potential. In addition, salinity caused several changes in the metabolic profile of sorghum leaves, modulating mainly amino acids (asparagine, glutamine, threonine, aspartic acid) and antioxidants (ascorbic acid) compared to unstressed plants. However, although these mechanisms may have contributed to osmotic regulation and attenuation of oxidative stress, they were not efficient in maintaining plant growth and development under salinity.

KEYWORDS: Salinity; gas chromatography; amino acids.

INTRODUCTION

Salt stress is reported as severe stress that affects the growth and development of plants, imposing both osmotic complications associated with reduction of plant water status, and an ionic effect, involving the ion toxic concentration into the tissues. This growth reduction is a phenotype trait, impacting agricultural productivity, especially in arid and semi-arid regions (Bai et al., 2018). In addition, disturbed ionic homeostasis, impaired metabolism, oxidative stress and nutritional imbalance were also reported in many species under salt stress (Zhou et al., 2024). To cope with deleterious effects of stress, diverse strategies are triggered by plants,

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such as Na^+ exclusion from roots (Miranda et al., 2017), accumulation of osmolytes for osmotic adjustment (El-Badri et al., 2021), and enhancement of antioxidant system (Souza et al., 2021). The plant response to salinity is very complex. Phenotypic differences are the result of the interaction of different elements with stress, such as transcriptomic changes, proteomic variation and metabolic alteration (Formentin et al., 2018; Jia et al., 2019).

In this context, metabolomic studies recently have gained attention, because they allow an overview of complex metabolism, improving the understanding of the metabolic pathway regulation and alterations in key metabolites under stress conditions (Feng et al., 2020). Recently metabolomics studies report the salt impact on metabolism of plants, such as alteration in pathways involving energy metabolism, sugar metabolism, biosynthesis of phytohormones and amino acids profile (Chen et al., 2019; Araújo et al., 2021; El-Badri et al., 2021).

The sorghum (*Sorghum bicolor* L.) is the fifth cereal most cultivated in the world, been a relevant crop and presenting a versatile applicability, including nutrition, human feeding and ethanol production (Mathur et al., 2017). In Brazil, according CONAB (2025), the estimated sorghum production for the 2024/25 harvest should be 5.6 million tons. Nevertheless, many studies have contributed to knowledge concerning sorghum tolerance under salt stress, some effects on primary metabolism remain unclear. Therefore, we investigated the changes on the metabolomic profile of sorghum leaves under salt stress through gas chromatography coupled to mass spectrometry (GC–MS) to determine the possible metabolic responses related to acclimation mechanisms to salinity.

MATERIAL AND METHODS

Forage sorghum seeds [*Sorghum bicolor* (L.) Moench] cv. CSF 20, obtained from the Instituto Agronômico de Pernambuco (IPA, Pernambuco, Brazil), were sown in vermiculite moistened with distilled water. After four days of sown, uniform seedlings were transferred to Hoagland’s nutrient solution, which was renewed every three days. Salt treatments were imposed 12 days after sowing, by adding two daily doses of 37.5 mM NaCl until reach 75 mM NaCl. The experimental design was completely randomized in greenhouse with four replicates.

Twelve days after the salt addition, the plants were harvested to determine leaf osmotic potential (Ψs) (Bao et al., 2014), electrolyte leakage (EL) (Dionisio-Sese & Tobita, 1998) and soluble carbohydrates (CHO) (Dubois et al., 1956) in shoots and roots. Additionally, the plant material was divided into leaves, stem and roots and dried in air circulation oven at 60 °C for

72 h to provide the dry mass. The primary metabolites were extracted and derivatized from fresh leaves as described by Lisec et al. (2006).

The profile of metabolites was obtained using gas chromatography coupled to mass spectrometry (GC-MS, QP-PLUS 2010, Shimadzu, Japan). One microliter sample was injected in split mode (1:5 ratio). The metabolites were separated using a column RTX-5MS capillary column (30 m × 0.25 mm × 0.25 µm), being programed with initial temperature at 80 °C for 2 min, then ramped of 10 °C per min to 315 °C, and held for 8 min. Helium was used as the carrier gas with a flow rate of 1.2 mL min^{-1} and the injection and ion source temperature were kept at 250 °C and the MS interface temperature was set at 230 °C. The mass spectrometer operated at 70 eV (EI) and used scan range of 40-700 (*m/z*). Regarding physiological data, the mean values were compared by Tukey’s test ($p<0.05$), using Sisvar 5.6 program. Chromatography data were evaluated using Xcalibur^{TM} 2.1 software, being compared with mass spectrum of library NIST14 and metabolic profile were evaluated using MetaboAnalyst. We performed t-test for mean comparisons and volcano plot to visualize the differential metabolites.

RESULTS AND DISCUSSION

Salt stress severely affected plant growth, reducing the biomass accumulation (Fig.1A). Regarding biomass partitioning, only leaf mass were the organs significantly reduced by salinity compared to control plants (Fig. 1B). This may be related to osmotic and ionic factors of stress, which deleterious effects due to impairment metabolism and cell damage (Zhou et al., 2024), that was accompanied by significant increased electrolyte leakage (EL) in shoots and roots of plants under stress comparison to control conditions. The salinity-induced oxidative stress in plants may be assessed trough markers of oxidative damage of cell membranes, reflected in increase of electrolyte leakage (AbdElgawad et al., 2016). The increase in EL in response to salinity was also shown for sunflower cultivars under salt and drought stress (Umar et al., 2019).

Salinity induced an increase in accumulation of CHO in shoot and in the leaf Ψs (modulus value), but no significant difference in CHO content in root (Fig. 1B). It may be a strategy employed by plants to deal with salt-induced osmotic effect and to overcome the water status imbalance caused by salt stress (Bhattacharya & Kundu, 2020). Several studies have demonstrated the soluble sugars may be naturally accumulated under stress, functioning as osmolytes during the osmotic adjustment in crop plants (Jiao et al., 2018; Tang et al., 2019).

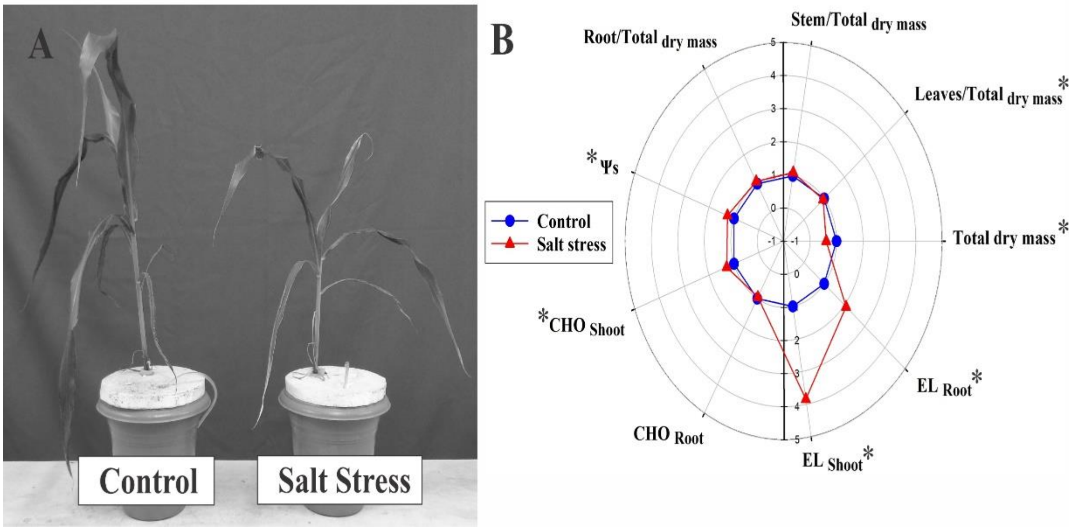

Figure 1. (**A**) Representative plants of sorghum in absence (control) and presence of 75 mM NaCl (salt stress). (**B**) Radar plot of the physiological responses from sorghum plants in control (blue circle) and salt stress conditions (red triangle). Using control as a reference, the data refers to relative alterations in the following parameters: Total dry mass, Leaves/total dry mass ratio, stem/total dry mass ratio, root/total dry mass ratio, leaf osmotic potential (Ψs, absolute values), soluble carbohydrates in shoot (CHO shoot) and root (CHO root), and electrolyte leakage in shoot (EL shoot) and root (EL root). Asterisk indicates significant difference due to salt stress by Tukey's test ($p<0.05$).

Metabolic profile analysis identified a total of 67 metabolites from leaves of sorghum under salinity and non-salinity condition, including amino acids (16), sugar and derivates (26), amines (1), organic acids (19), phenolic compounds (3), and vitamins (2) (Table 1). However, only 37 compounds were significant by t-test ($p<0.05$). PCA analysis of metabolic profile exhibited a good separation of the treatments (79.7%), with PC1 and PC2 accounted for 71.9 and 7.8% of total variance, respectively (Fig. 2).

The volcano plot showed 11 differentially produced metabolites (10 upregulated and 1 downregulated) between salt stress and control conditions (Fig. 3). The accumulation of amino acids (asparagine, threonine, glutamine, glutamine, glutamine, glutamic acid, valine and aspartic acid) may be associated with elevated protein and chlorophyll degradation induced by salt stress (Fan et al., 2019), or as an acclimation response to carbon and nitrogen reassimilation to other biological process, such as osmotic adjustment observed in Glycine max under salt stress (Jiao et al., 2018). In cultivars of *Brassica napus* L., El Badri et al. (2021) observed that the accumulation of amino acids derivates might be related with osmoregulation under salt stress, which contributes to better growth and development of salt-tolerant cultivars.

Table 1. Fold changes of metabolites of leaves of sorghum cv. CSF 20 after salt stress. Values of log2(salt stressed/non-stressed and significant difference by T-test with $p < 0.05$ (*) and $p < 0.01$ (**).

Sugar and derivates	**Fold change (log$_2$)**	**Organic acids**	**Fold change (log$_2$)**	**Amino acids**	**Fold change (log$_2$)**
Xylose	0.21*	Glycolic acid	1.08**	Valine	1.08**
Ribose	-0.01	Pyruvic acid	0.53	Leucine	0.08
Galactonic acid	0.87**	Lactic acid	0.05	Proline	0.40*
Gluconic acid	1.00**	Oxalic acid	0.63	Glycine	0.76*
Fructose	-0.14	Phosphoric acid	0.21*	Serine	0.16
Sorbose	-0.19	Succinic acid	0.58*	Alanine	-1.00**
Galactose	-0.12	Glyceric acid	0.30	Threonine	2.03**
Glucose	-0.01	Fumaric acid	-0.14	Beta-Alanine	0.62*
Methyl alpha-D-glucopyranoside	0.28*	4-Hydroxybutyric acid	0.34**	Aspartic acid	1.65**
Sucrose	0.09	Malonic acid	0.68**	Pyroglutamic acid	0.62*
Cellobiose	0.16	Malic acid	0.06	Glutamic acid	1.38**
Threalose-alpha	0.46*	Erythronic acid	0.26	Phenylalanine	0.73*
Maltose	-0.05	Threonic acid	0.35*	Glutamine	1.85**
Palatinose	0.37*	Glutaric acid 2-oxo	0.59*	Asparagine	4.39**
Raffinose	0.71**	Cis-aconitic acid	2.04**	Lysine	0.75**
Maltotriose	-0.18	Shikimic acid	0.22	Tyrosine	0.58*
Glycerol-3-phosphate	0.93**	Tartaric acid	0.49*		
Glyceric acid 3-phosphate	0.45	Citric acid	0.36	**Amine**	**Fold change (log$_2$)**
Ribulose-5-phosphate	0.24	Dehydroascorbic acid	1.11**	Putrescine	0.06
Fructose-6-phosphate	0.83**				
Glucose-6-phosphate	0.76**	**Phenolic compound**	**Fold change (log$_2$)**	**Vitamin**	**Fold change (log$_2$)**
Mannitol	0.17	Quinic acid	0.17	Ascorbic acid	1.68**
Myo-inositol	0.04	Caffeic acid	0.10	Pantothenic acid	0.24
Lactitol	-0.30	Caffeyolquinic acid	-0.88*		
Maltitol	-0.06				
Galactinol	0.51**				

Organic acids are important C skeletons source for biochemical processes. They can also enter in TCA cycle to produce energy for plant metabolism, as well as supplying other metabolic pathway, including amino acids biosynthesis (Quan et al., 2016). Our data showed changes in the organic acids levels with increased punctual metabolites (phosphoric acid, succinic acid, threonic acid, glutaric acid and glycolic acid) involved in the charge balance (Chang et al., 2019). In *Poa pratensis*, the accumulation of organic acids acted in the regulation of charge imbalance induced by alkalinity and salinity stress (Hu et al., 2015). Das et al. (2019)

reported that salinity promotes accumulation of organic acids, it might be related to growth maintenance in salt-tolerant rice cultivars.

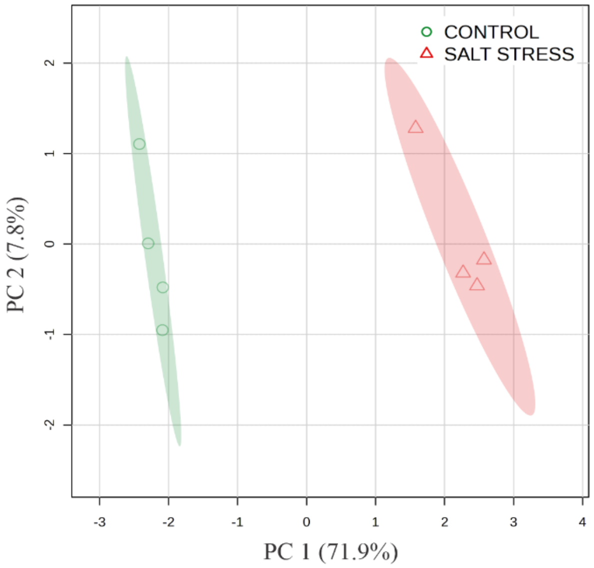

Figure 2. Principal Component Analysis (PCA) of metabolic profiling of leaves of sorghum, cv. CSF 20, grown under absence (0 mM, Control) and presence of 75 mM NaCl (Salt stress).

Additionally, increased ascorbic acid can be related to a strategy to mitigate oxidative stress under salinity. More specifically, ascorbate is an important antioxidant molecule to regulate the redox status and act as cofactor in biochemical pathway (Foyer & Noctor, 2011). Its role to salt stress acclimation has been reported in Arabdopsis (Makavitskaya et al., 2018) and Hordeum vulgare (Hassan et al., 2021), reporting its relation to salt tolerance in plants. In wheat, the enhancement of ascorbate and glutathione contents due to salicylic acid and cold pre-treatment alleviated oxidative damages under subsequent cold stress (Wang et al., 2020).

CONCLUSION

Salinity affected the performance of sorghum plants, reducing significatively the plant growth. Although metabolic adjustment and osmotic regulation have been triggered to salt acclimation, these responses were not sufficient for withstand deleterious effects of salinity in plant growth and development. However, this study promotes new insights into modulation of primary metabolism in sorghum plants under salt stress.

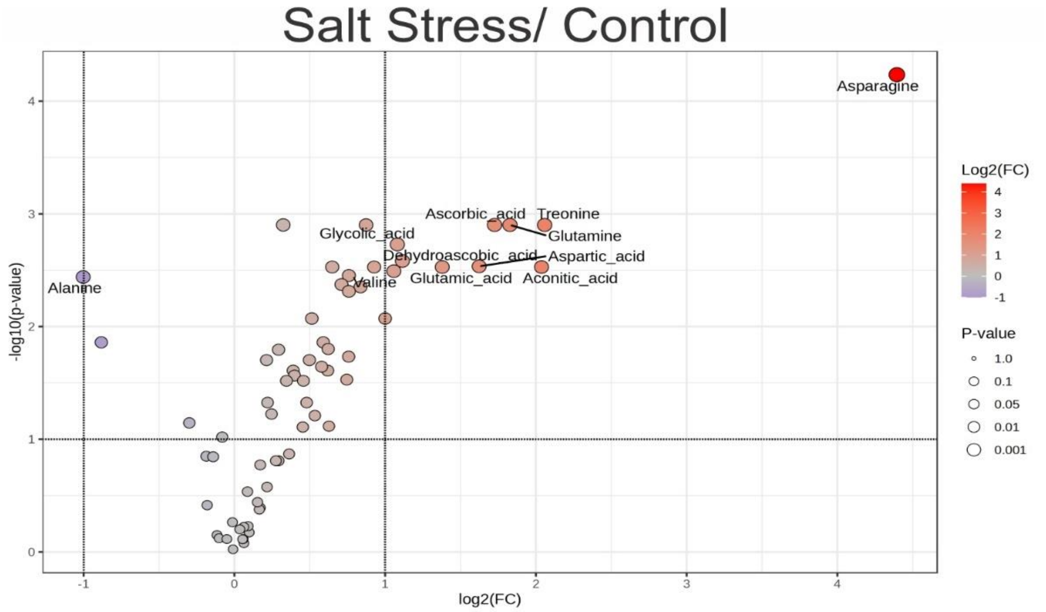

Figure 3. Volcano plot demonstrates the important and statistically different metabolites (i.e., at least a fold change of 2 and a $p<0.05$) between salt stress and control conditions at positive mode (red) and the negative mode (blue).

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