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Osmotic adjustment, toxic ion exclusion and oxidative protection as key traits for the tolerance to NaCl stress in forage sorghum

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Abstract

The physiological and biochemical responses to 120 mmol L^{-1} NaCl in CSF20 genotype and Catissorgo variety of forage sorghum were studied. The salinity did decrease the plant growth, but it was stronger in CSF20 than in Catissorgo. Under salinity, K^+ content was not changed in CSF20 and it was solely reduced in the stems and roots of Catissorgo. Na^+ and Cl^- accumulation in young leaves and stems was lower in Catissorgo than in CSF20. Roots of Catissorgo did retain more toxic ions than those of CSF20. Alterations in water status and oxidative damage were observed in CSF20 leaves. The chlorophyll content increased in CSF20, but it was unchanged in Catissorgo. Such responses were associated with carotenoid and glucose contents. Salinity promoted osmotic adjustment of leaves and roots. The reduction of leaf osmotic potential was due to the increase of Cl and Na^+ in Catissorgo, and Na^+ in CSF20. The contribution of organic solutes was similar among the control and salt-treated plants; glucose was the major organic osmolyte. The activity of guaiacol peroxidase and catalase was consistently higher in Catissorgo leaves than in CSF20 leaves. Salt stress induced the superoxide dismutase activity only in Catissorgo leaves, but it was weakly correlated with the low malondialdehyde accumulation. Isoforms of catalase and superoxide dismutase were increased in Catissorgo leaves. In summary, the systemic osmotic adjustment, the antioxidative protection by carotenoids and superoxide dismutase isoforms, and the toxic ion exclusion by roots are crucial to tolerate salt stress in forage sorghum.

1 Introduction

Salinization of arable lands owing to Na^+ and Cl^- abundance poses a serious threat to crop growth and yield. During the initial phase of salt stress, the plant's ability to absorb water and nutrients is harmed, resulting in osmotic stress and nutritional deficiency [1]. Later, the reduced photosynthesis associated with increased electron flux through photosystems lead to the formation of reactive species (ROS), which cause damage in cellular components and organelle activity [2]. These negative effects are further aggravated as Na^+ and Cl^- progressively accumulate in tissues, harming protein activity [1].

The salinity tolerance comprises numerous responses on morphological, physiological and molecular levels. A decreased leaf growth and transpiration rate are initial responses to maintain water status and minimize toxic ion transport to the photosynthetic organs [3]. Salt-tolerant glycophytes regulate ion transporters and channels present in root cell membranes for recovery of ionic and water homeostasis in the whole plant and accumulate compatible organic solutes in cytosol and other organelles for achieving cell turgor in aerial tissue and roots [3, 4].

To prevent oxidative stress, plants contain an antioxidative enzyme system, which is activated in sequential cascade to convert ROS into inert or harmless species. Superoxide dismutase converts superoxide radical into hydrogen peroxide and molecular oxygen, whereas ascorbate peroxidase, guaiacol peroxidase, and catalase convert hydrogen peroxide into water [2]. The failure in ROS scavenging mechanisms enhances the stress sensitivity.

Sorghum (*Sorghum bicolor* L. Moench) is a versatile grass crop, used also as forage for animal feed [5]. This species is efficient in using water and tolerant to heat. It is widely grown in world's arid and semiarid regions, where other cereals, such as maize, are impaired [6]. Sorghum is a moderately salt-tolerant crop and the well-adapted cultivars/genotypes to salinity display efficient mechanisms for Na^+ exclusion [7], ROS detoxification [8], and maintenance of water status [9].

This work aims to study the variety Catissorgo and the genotype CSF20 of sorghum. The main differences among them are the plant's height and the purpose of its production; while CSF20 is a truly forage and tall genotype, the Catissorgo variety is medium height and developed mainly for forage and seed production [10, 11]. Studies have highlighted the salt stress tolerance of genotype CSF20 at various stages of development and cultivation conditions [12], but little is known about the tolerance to salt stress in Catissorgo. Given the genotypic and agronomical differences among Catissorgo and CSF20, we hypothesize that both sorghum types can display differential adaptive responses under salinity. Understanding the performance of this crop in regions prone to salinization is an important issue for sustained forage production and integration of agriculture and livestock.

2 Materials and methods

2.1 Growth conditions and salt treatment

Sorghum seeds of the CSF20 genotype were gently provided by Instituto Agronômico de Pernambuco (IPA, Pernambuco, Brazil), and seeds of the Catissorgo variety (BRSEEDS Sementes LTDA, São Paulo, Brazil) were commercially purchased. Seeds were disinfected with 1% HClO for 5 min, thoroughly rinsed with tap water, and then sown in vermiculite moistened with 0.5 mmol L^{-1} $CaCl_2$. Seven days after sowing, twenty-five seedlings were transferred to plastic pots containing 5.0 L of Hoagland's nutrient solution at one-third ionic strength and grown for one week. Then, plants with uniform size were chosen and put in plastic pots containing 2.8 L of nutrient solution like the previous one. Two plants were grown in each pot. At this moment, the plants were submitted to control (without NaCl) and saline (with 120 mmol L^{-1} NaCl) treatments. The salinity was added in two fractions of NaCl at 60 mmol L^{-1} to avoid osmotic shock. The plants were exposed to these treatments for 10 days, and, during this period, the non-saline and saline solutions were renewed once. The experiment was installed in a naturally lit greenhouse with day/night air temperatures of 31.9/21.3°C and relative humidities of 58.1/98.0%. At harvest, the plants were collected for growth analysis and stored at − 20°C. Ion content was determined in all plant tissues, and physiological and biochemical analyses were made using the first fully expanded leaf.

2.2 Measurements of growth and morphological parameters

The lengths of shoot and roots were determined with a metric ruler. Then, the plants were divided into leaves, stem + sheath, and roots, and their fresh masses were determined. The leaf size (*A*) was estimated according to the following equation:

$$A = l \times w \times 0.72$$

Where *l* (m) = length and *w* (m) = width of the first completely expanded leaf [13].

2.3 Photosynthetic pigments

Chlorophyll (Chl_{total}) and carotenoids (Car_{total}) were extracted with 2.0 mL DMSO from 0.2 g leaf blades for 24 h under dark conditions. The homogenate was centrifuged at 3.000 × *g* for 10 min, and the absorbance of the supernatant was spectrophotometrically measured at 470, 645, and 663 nm. The content of photosynthetic pigments (mg g^{-1} FM) was estimated using the following equations:

$$Chl_{total} = (A_{645} \times 20.08 + A_{663} \times 8.02) \times M/V \times 1000\, Car_{total} = (A_{470} \times 1000 - 1.90\,(Chla) - 63.14\,(Chl_b)\,/214) \times M/V \times 1000$$

Where *A* = optical density at specific wavelengths, *M* (g) = fresh mass of tissue and *V* (mL) = final volume of DMSO [14].

2.4 Water relations

The transpirational flux was measured before harvest day using a leaf porometer (model SC-1, Decagon Devices, USA). The relative water content (RWC) was determined in leaf discs according to [15]. Osmolality of leaf and root saps was analyzed using a vapor pressure osmometer (model Vapro 5600, Elitech Wescor, USA) and expressed as mmol.Kg^{-1}. The tissue osmotic potential (Ψ_s) was calculated assuming that 400 mmol.Kg^{-1} ≈ −1.0 MPa.

2.5 Organic and inorganic solutes

Proline, total amino acids, and sugars were extracted with 5 mL 80%ethanol from 0.2 g of leaf blades. The samples were gently agitated in an orbital shaker for 1 h. Then, the homogenate was centrifuged at 5.000 × *g* for 15 min at 6°C. Proline and total amino acids were quantified according to [16] and [17], respectively. Fructose, glucose, and sucrose were quantified using the methodologies described by [18], [19], and [20], respectively.

Ions were extracted with 7.0 mL deionized water from 0.2 g leaf blades. The extraction was performed for 1 h in an orbital shaker at room temperature. Then, the homogenate was centrifuged at 3.000 × *g* for 15 min at 20°C. K^+ and Na^+ were quantified by flame photometry and chloride (Cl^-) following the method described by [21].

The contribution of analyzed solutes to Ψ_s of the first fully expanded leaf was calculated from the concentration of each solute, expressed as mmol.Kg^{-1}, in relation to leaf sap osmolality of the same sample.

2.6 Oxidative damages and antioxidative enzyme

Malondialdehyde (MDA) was extracted with 5% trichloroacetic acid from 0.2 g leaf blades and quantified by reaction with thiobarbituric acid. The preparation of enzyme extract was carried out at 4°C. Firstly, 0.4 g of fresh tissue of leaf blades was macerated with 2.0 mL of 0.05 mmol·L^{-1} potassium phosphate pH 7.2, and 1 mmol·L^{-1} ethylenediaminetetraacetic acid (EDTA) for 5 min using mortar and pestle. The material was then filtered through nylon cloth and centrifuged at 10.000 × *g* for 15 min. Soluble protein was quantified by reaction with Coomassie Brilliant Blue G-250 using bovine serum albumin as a standard. The supernatant was collected and stored at − 20ºC until further use.

For the determination of the catalase (CAT, EC 1.11.1.6) activity, the reaction mixture (0.8 mL) consisted of 50 mmol·L^{-1} potassium phosphate pH 7.0, 20 mmol·L^{-1} H_2O_2, and 0.1 mL enzyme extract containing 20 µg leaf protein. The enzyme activity of CAT was estimated using the H_2O_2 molar extinction coefficient (*e* = 36 M^{-1} cm^{-1}). For the determination of the guaiacol peroxidase (POX, EC 1.11.1.7) activity, the reaction mixture (1.0 mL) consisted of 50 mmol·L^{-1} potassium phosphate pH 7.0, 3.2 mmol·L^{-1} guaiacol, and enzyme extract containing 20 µg leaf protein. Then, the enzyme reaction was started by the addition of 0.1 mL of 4.0 mmol·L^{-1} H_2O_2. The tetraguaiacol formation was monitored through the increase of absorbance at 470 nm for 3 min. The enzyme activity of POX was estimated using the tetraguaiacol molar extinction coefficient (*e* = 26.6 M^{-1} cm^{-1}). For determination of the superoxide dismutase (SOD, EC 1.15.1.1) activity, the reaction mixture (1.0 mL) consisted of 50 mmol·L^{-1} potassium phosphate pH 7.0, Triton X-100 at 0.75%, 50 µmol·L^{-1} nitrotetrazolium blue (NBT), 10 mmol·L^{-1} methionine, and enzyme extract containing 20 µg leaf protein. The reaction was started by the addition of 20 µL of 0.12 mmol·L^{-1} riboflavin, and the samples were immediately incubated under fluorescent illumination for 7 min. One activity unit (AU) of SOD promoted a 50% inhibition of the NBT photoreduction.

2.7 Native electrophoresis of antioxidative enzymes

Non-denaturing continuous polyacrylamide gel electrophoresis (ND-PAGE) was done at 12% for analysis of isoforms and activity of POX and SOD and at 7% for analysis of isoforms and activity of CAT, according to [22]. Twenty micrograms of leaf proteins were loaded into the wells and run at 200 V at 4°C. The gels were stained with guaiacol and H_2O_2 for the detection of POX activity [23]; NBT, riboflavin, and methionine for SOD activity [24]; and $FeCl_3$ and $K_3Fe(CN)_6$ for CAT activity [25].

2.8 Experimental design and statistical analysis

The experimental design was completely randomized in a 2 × 2 factorial arrangement corresponding to a genotype and variety of forage sorghum (CSF20 and Catissorgo) and two salinity levels (0 and 120 mmol·L^{-1} NaCl). Each treatment was carried out with five repetitions, each repetition consisting of two

plants. The data were submitted to a two-way analysis of variance (ANOVA) by *F* test ($p < 0.05$), and the average values were compared by the Fischer test ($p < 0.05$). The Pearson's correlation coefficient (*r*) between different sets of biochemical and physiological parameters was worked out using the statistical software Jamovi v2.3 (The Jamovi Project, Sydney, Australia).

3 Results

The growth and biochemical and physiological parameters analyzed in sorghum plants under control and saline conditions are shown in Fig. 1 and Table 1. Under non-saline conditions, the fresh mass of stem + sheaths and roots was higher in CSF20 than in Catissorgo, whereas the leaf fresh mass and shoot/root ratio did not differ between both sorghum types. Salinity reduced the vegetative growth of forage sorghum studied. However, both sorghum types did not exhibit significant differences in fresh mass of leaves, stem + sheaths, roots, shoot/root ratio, and leaf area under salinity; only the shoot length of CSF20 was higher than that of Catissorgo. It is noteworthy that greater reductions in growth were induced by salinity in CSF20 than in Catissorgo as compared with the control treatment. For instance, the growth of leaves, roots and leaf size of Catissorgo under saline conditions was respectively lower at 43.9%, 26.7%, and 35.9%, whereas that of CSF20 was lower at 58.6%, 42.1%, and 46.3%, as compared with the control.

Salinity lowered the osmotic potential (Ψs) of roots and leaves in Catissorgo and only the Ψs of leaves in CSF20. Osmotic adjustment ($\Psi s_{control} - \Psi s_{saline}$) in leaves and roots of Catissorgo was 0.70 MPa and 0.32 MPa, respectively. In leaves of CSF20, the osmotic adjustment was deeper (0.99 MPa) than in leaves of Catissorgo. In CSF20 roots, there was not significant osmotic adjustment as exposed to salt stress. The transpiration rate did not differ between forage sorghum studied and was not affected by salinity. The relative water content was reduced by salinity only in leaves of CSF20 as compared to the control.

The total chlorophyll content was not affected by salinity in Catissorgo, but it did increase in CSF20 by about 50.0% as compared with the control. The carotenoid content did increase under salt stress, with increases of 20.6% and 32.2% in Catissorgo and CSF20, respectively. Salt stress did increase the MDA content by about 61.0% in CSF20 leaves, but it was not changed in Catissorgo leaves.

The content of total amino acids and soluble proteins in leaves of both forage sorghum did increase under salt stress. In these conditions, the content of total amino acids and soluble proteins was 31.3% and 22.1% higher in Catissorgo than in CSF20, respectively. Salinity induced proline accumulation only in Catissorgo leaves, but it did not differ among both forage sorghum under salinity.

Salt stress did not change the content of sucrose and fructose in leaves of both forage sorghum. However, the content of glucose significantly increased only in Catissorgo leaves (64.1%).

The activity of antioxidant enzymes studied was not changed by salinity in CSF20 (Table 1). On the other hand, enzyme activity of SOD in Catissorgo leaves was increased 46.3% as compared to the control, whereas POX and CAT activity were unchanged under salt stress. The POX and CAT activity of Catissorgo was 63.6% and 166.7% higher than that of CSF 20 under salinity conditions, respectively (Table 1).

The enzyme activities were corroborated on native electrophoresis gels (Fig. 2). Two isoforms of SOD were detected (S1 and S2, in Fig. 2a). Both isoforms were induced by salinity in Catissorgo leaves, but apparently S2 was induced in CSF20 leaves. On the other hand, an alone isoform of catalase (C1, in Fig. 2b) was observed on gels, which showed high activity under control conditions and was further induced under salinity (Fig. 2b). In both conditions, this isoform of catalase presented low activity in CSF20. Two isoforms of peroxidase were observed on gels (P1 and P2, in Fig. 2c). In both forage sorghum, P1 showed high activity and was slightly induced by salinity, whereas P2 seemed to be inhibited.

The accumulation of K^+, Na^+, and Cl^- in different tissues of forage sorghum is shown in Fig. 3. The K^+ content in young and first fully expanded leaves of both forage sorghum did not differ under non-saline conditions, and it was not affected by salinity (Fig. 3a,b). In stems + sheaths and roots, the K^+ content of Catissorgo was higher that of CSF20 under non-saline conditions, but it was reduced by salinity only in Catissorgo (Fig. 3c,d). With the salinity, the Na^+ and Cl^- content did increase in all plant tissues studied. However, Na^+ content was significantly higher in young leaves (82.6%) and stems + sheaths (56.3%) of CSF20 than of Catissorgo (Fig. 3e,g). Contrary, Na^+ content in roots of Catissorgo was 26.5% higher than in roots of CSF20 (Fig. 3h). Still under saline conditions, Cl^- contents in young leaves and stems + sheaths were 21.3% and 50.2% lower in Catissorgo than in CSF20 (Fig. 3i,k), while it in roots Catissorgo was higher in 50.5% than in CSF20 (Fig. 3l).

Potassium accounted for a major proportion of Ψs in fully expanded leaves of control plants, but this contribution fell 43.8% in Catissorgo and 40.4% in CSF20 under salt stress (Table 2). Na^+ and Cl^- contributed to Ψs lowering in stressed leaves of Catissorgo, and both ions accounted for 38.1% of leaf Ψs. On the other hand, the Cl^- contribution in leaf Ψs of CSF20 was not significantly different from that observed in leaves of control plants (roughly 17.6%). In this genotype, Na^+ contribution for the Ψs in fully expanded leaves reached 11.8% in relation to other analyzed solutes. Organic solutes contributed similarly to Ψs in fully expanded leaves of control and stressed plants of both studied sorghum, except the contribution of glucose in CSF20 stressed leaves, which was reduced by 26.9% in relation to control leaves (Table 3).

4 Discussion

In this work we studied the salt stress tolerance of two types of forage sorghum, which have different agronomic and genetic characteristics. The CSF20 genotype grow 2.5–3.0 m. tall, is mainly used for green mass production and is known by its tolerance to salinity; whereas the Catissorgo variety is low height and produced for seeds and forage furnishing. The tolerance to salt stress in Catissorgo has not been yet studied. The results show the shoot and

root growth of both forage sorghum were affected by salinity, achieving similar sizes (except the shoot length) under such conditions (Table 1). However, such reductions were higher in CSF20 than in Catissorgo, which suggest the induction of tolerance mechanisms more efficient in the latter to minimize the negative impact on cellular homeostasis and thus, its better performance under salinity.

Salinity did affect the RWC in CSF20 leaves but not in Catissorgo (Table 1). In sorghum plants under salinity, the stomatal conductance (g_s) and osmotic adjustment of tissues are crucial responses to maintain the water status [9]. Here, both forage sorghum presented a reduced g_s, but it did not account for the observed differences in regulation of RWC. In accordance with our results, no difference in values in g_s among sorghum genotypes and varieties exposed to salt stress were observed by [26] and [27]. Thus, g_s seems not to be a relevant parameter for discriminating among salt-tolerant and salt-sensitive forage sorghum. On the other hand, there was a significant relationship between the Ψ_s in leaves with the RWC (r = -0.506, p = 0.03), suggesting studied forage sorghum meet salt-induced water shortage through the osmotic regulation. In this study, the osmotic adjustment in mature leaves (also in roots) of studied sorghum was not made to the same degree. The degree of osmotic adjustment in Catissorgo leaves was lower than it was in CSF20 leaves; however, a coordinated regulation with Ψ_s of Catissorgo roots was evident (Table 1). The systemic osmotic regulation in Catissorgo may have guaranteed a continuous water flux for maintenance of the water status of leaves under osmotic stress. In accordance with this assumption, osmotic adjustment in leaves and roots was a crucial mechanism to create a gradient essential for water uptake from hypertonic medium in salt-tolerant wheat genotypes [28]. The absence of a systemic osmotic regulation may entail a reduction of water relative content in mature leaves of CSF20 (Table 1).

Under saline stress, Ψ_s in sorghum mature leaves was reduced by massive accumulation of Na^+ and Cl^- ions (Table 2). In fact, an inverse relationship among the content of these ions and the Ψ_s reduction of fully expanded leaves (for Cl^-, r = -0.869, p = 0.00; for Na^+, r = -0.799, p = 0.00) was found. Although there were no significant differences in the content of Na^+ and Cl^- in mature leaves (Fig. 3f, j), its relative contributions to osmotic adjustment in these tissues varied in both types of sorghum. Na^+ and Cl^- significantly contributed to the Ψ_s reduction in mature leaves of Catissorgo, whereas only Na^+ acts as the main inorganic solute in CSF20 under salinity stress (Table 2). Therefore, the use of chloride in osmotic adjustment constituted a distinctive characteristic between both studied sorghum and suggests, at least in part, differences in ability for its sequestration in vacuoles. The chloride channels (CLC) play a key role in vacuolar sequestration of chloride in all plant tissue, and its expression in leaf blade of sweet sorghum is upregulated after NaCl treatment [29]. Likely, the expression of these chloride channels in mature leaves of Catissorgo would contribute to the maintenance of leaf water status and reduction of chloride toxicity on enzyme activity in cytosol. Significant correlation between K^+ content and Ψ_s in leaves was not found.

Among the soluble sugars analyzed (Table 1), glucose and sucrose were strongly correlated with lowering of Ψ_s (for fructose, r = -0.524, p = 0.02; sucrose, r = -0.693, p = 0.00; glucose, r = -0.767, p = 0.00). Nevertheless, no change in soluble sugar contents were observed, except increase in glucose content in Catissorgo leaves under stress. Analyses of metabolic profiles in leaves of sorghum exposed to NaCl stress at 75 mM reveal the accumulation of D-glucose and fructose in tolerant genotypes; likely associated to breakdown of sucrose [30]. In this study, unaltered content of sucrose in mature leaves suggest that its metabolism remains stable under salt stress; while increased content of glucose could result from inactivation of oxidative metabolic routes. In line with this statement, low activity of glucokinase, phosphoglucoisomerase and pyruvate kinase was observed in kukuyu grass leaves under salt stress [31]. Hence, accumulation of glucose in sorghum leaves would be an essential condition for controlling cell osmotic potential. Considering soluble amino acids, proteins and proline (Table 1), this latter was slightly associated with Ψ_s in fully expanded leaves (for total amino acids, r = – 0.262, p > 0.05; for soluble protein, r = -0.272, p > 0.05; for proline, r = -0.455, p = 0.04). Proline and the free amino acid pool are considered essential solutes for the osmotic regulation and adaptation to salt stress [32, 3]. However, our results attributed to proline a secondary role. Previous work also described great accumulation of free amino acids in sorghum leaves with salt increase, but it was not enough to regulate internal osmotic potential of leaf cell [33]. The contribution of organic solutes to the leaf osmotic potential was not different among the control and stressed plants, except that of glucose, which was reduced by salinity in CSF20 mature leaves. The absence of a significant accumulation of glucose could have resulted in a reduced contribution to osmotic potential in CSF20 leaves.

Under salinity, the secondary oxidative stress is alleviated by an antioxidant enzyme network and low-molecular antioxidant compounds, which are constitutively higher or induced by stress [2]. In this work, the enzyme activity of POX and CAT was consistently higher in Catissorgo leaves than in those CSF20 under both growth conditions (Table 1 and Fig. 2), suggesting the presence of an innate mechanism to control toxic H_2O_2 level in Catissorgo leaves. On the other hand, the SOD activity was induced by salinity only in Catissorgo leaves (Table 1) and probably, the isoform named S1 on native gels (Fig. 2a) to be responsive under such condition. Responses of antioxidant enzymes in sorghum have shown a complex regulation depending on genotype, salt stress level and interaction between both factors. The tolerant genotypes are capable of increasing the enzyme activity of various antioxidant enzymes as salinity level increases. Notably, the salt tolerance in sorghum has been associated to an elevated SOD activity yet if the enzyme activity of ascorbate peroxidase and catalase are stable or reduced [8]. Thus, the induced SOD activity in Catissorgo leaves under elevated salinity level emphasize this pattern of enzyme responses and would suggest the activation of an efficient mechanism to avoid a substantial elevation of oxidative damages. Despite role of SOD in antioxidative protection in sorghum, the activity of superoxide dismutase was weakly associated (r = -0.438, p = 0.06) with the content of peroxidate lipids (MDA). Moreover, no significant relationship between the MDA level and the enzyme activity of POX and CAT was found.

Carotenoids are secondary harvesting light pigments and play also in photo-protection and detoxification of ROS caused by stressful conditions. These pigments are able to dissipate the excitation energy of triplet state of chlorophyll and the singlet state of oxygen as heat [2]. Previous works showed negative effect of salinity on the content of photosynthetic pigments in sorghum leaves, being the strongest effect in sensitive cultivars than tolerant ones [34]. In this work, salt stress with 120 mM NaCl stimulated the carotenoid accumulation in both genotypes, likely as a protective non-enzymatic mechanism to combat the excessive production of free radicals and it was directly correlated with the chlorophyll content (r = 0.800, p = 0.00). Studies showed the low and medium expression of carrot phytoene synthase 2 (*DcPSY2*) codifying phytoene induces the accumulation of luteins with a

concomitant increase of total chlorophyll in transgenic *Nicotiana tabacum* under salt stress [35]. Likely, salinity would have stimulated the metabolic pathway of carotenoids biosynthesis at levels that promoted the increase or maintenance of chlorophyll as observed in CSF20 and Catissorgo, respectively.

The high contents of Na^+ and Cl^- in forage sorghum did significantly impair the growth of all plant tissues (Table 1, Fig. 2), being the leaf size of first fully-expanded leave severely reduced by Na^+ (r ≈ -0.804, p ≈ 0.00) and Cl^- (r ≈ -0.822, p ≈ 0.00), whereas root length was slightly affected. Moreover, the reduced transpiration was associated with Na^+ and Cl^- accumulation in leaves (r > -0.600, p < 0.05). The distribution of Na^+ and Cl^- ions among root and aerial tissues suggest that both forage sorghum display a different ability to exclude toxic ions from leaves; whereas CSF20 appears to accumulate it in your stem + sheaths (Fig. 2g.k), Catissorgo retain it in your roots (Fig. 2h, l), but this latter appear be more efficient since lower accumulation of Na^+ and Cl^- were observed in its young leaves (Fig. 2e,i). The toxic ion retention in stem and roots is a pivotal mechanism displayed by salt tolerant lines of sorghum, depending upon a complex regulation of several Na^+ and K^+ transporters and signaling route to limit Na^+ accumulation in photosynthetic tissues under salt stress [7]. The ionic homeostasis regulation through transport control of Na^+ and Cl^- to shoot is an important attribute of salt stress tolerant grasses to improve the growth under saline conditions [36].

5 Conclusion

In this work, we verified that sorghum plants exposed to salinity respond through adaptive mechanisms underlying by control of ionic, osmotic and oxidative homeostasis. In summary, retention of Na^+ and Cl^- in roots; osmoregulation mainly mediated by glucose accumulation; and a constitutive and inductive enzymatic system are important mechanisms displayed by this species under salt stress. The efficient activation of these responses did determine the greater ability of Catissorgo than CSF20 to tolerate salt stress. Our results are especially relevant since there is a need to use crops with adequate performance or survival in salinization-prone soils.

Declarations

Author contribution

JJGC performed the experiment installation and ion analysis. All authors have contributed to data collection and analysis of other physiological and biochemical parameters. JCAP contributed to study's design and wrote the manuscript. All authors checked and approved the final manuscript.

Conflict of interest

The authors of this work declare that they have no conflicts of interest.

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Ethics statements

Not applicable

Consent to publish declaration

Not applicable

Consent to participate declaration

Not applicable

Permissions to collect the plants/plant parts

Sorghum seeds of genotype CSF20 were provided by Dr. José Nildo Tabosa of Instituto Agronômico de Pernambuco (IPA, Pernambuco, Brazil – Lat. 8°04'07" S, Long. 34°55'05" W), who authorized its use for research purposes. The forage sorghum variety Catissorgo was produced by Diretoria de Assistência Técnica Integral (CATI, São Paulo, Brazil – Lat. 22°53'16" S, Long. 47°04'31" W) and seeds of this variety were purchased from the brazilian company BRSeeds Sementes LTDA (www.brseeds.com). The plants were cultivated in the greenhouse of Centro de Ciências Agrárias e da Biodiversidade of Universidade Federal do Cariri (CCAB, UFCA, Ceará, Brazil – Lat. 7°14'04" S, Long. 39°22'09" W). The use of this plant material complied with relevant institutional and national guidelines

Ethics and consent to participate

The use of seeds of forage sorghum variety/genotype complied with relevant institutional and national guidelines

Data Availability Statement

All data generated or analyzed during this study are included in this published article

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Tables

Table 1 Growth, water relations, photosynthetic pigments, organic solutes and activities of antioxidative enzymes of forage sorghum plants, variety Catissorgo and genotype CSF20, growing in nutrient solution and submitted to control treatment (without NaCl) and salt stress with 120 $mmol.L^{-1}$ NaCl for ten days

Growth and physiological and biochemical variables	Sorghum variety / genotype			
	Catissorgo		CSF20	
	Control	Salt stress	Control	Salt stress
Leaf mass (g plant^{-1})	9.8±1.1^{Aa}	5.5±0.4^{Ab}	11.6±0.^{Aa}	4.8±0.3^{Ab}
Stem+Sheats mass (g plant^{-1})	10.7±1.1^{Ba}	5.4±0.4^{Ab}	13.8±0.8^{Aa}	6.1±0.5^{Ab}
Root mass (g plant^{-1})	10.1±1.0^{Ba}	7.4±0.3^{Ab}	13.3±0.4^{Aa}	7.7±1.0^{Ab}
Shoot /root (g g^{-1})	2.0±0.1^{Aa}	1.5±0.1^{Ab}	1.9±0.2^{Aa}	1.3±0.1^{Ab}
Leaf area (m^2 leave^{-1})	0.131±0.012^{Aa}	0.084±0.003^{Ab}	0.148±0.005^{Aa}	0.080±0.005^{Ab}
Shoot length (m plant^{-1})	0.71±0.04^{Ba}	0.55±0.01^{Bb}	0.85±0.02^{Aa}	0.64±0.01^{Ab}
Root length (m plant^{-1})	0.44±0.04^{Aa}	0.32±0.03^{Ab}	0.34±0.01^{Ba}	0.23±0.02^{Ab}
Root Ψs (MPa)	-0.53±-0.03^{Ba}	-0.85±-0.01^{Bb}	-0.47±-0.03^{Aa}	-0.70±0.03^{Aa}
Leaf Ψs (MPa)	-1.04±-0.04^{Aa}	-1.74±-0.21^{Ab}	-1.02±-0.09^{Aa}	-2.01±-0.19^{Bb}
g_s (mmol m^{-2} s^{-1})	476±51^{Aa}	412±55^{Aa}	553±32^{Aa}	489±40^{Aa}
RWC (%)	86.5±1.9^{Ba}	86.5±1.0^{Aa}	90.1±1.0^{Aa}	83.5±2.1^{Ab}
Total chlorophyll (g kg^{-1}FM)	1.4±0.1^{Aa}	1.6±0.2^{Ba}	1.6±0.2^{Ab}	2.4±0.1^{Aa}
Carotenoids (g kg^{-1}FM)	0.29±0.0^{Ab}	0.35±0.0^{Ba}	0.31±0.0^{Ab}	0.41±0.0^{Aa}
MDA (nmol g^{-1}FM)	116.2±5.8^{Aa}	130.8±9.2^{Aa}	81.6±8.2^{Bb}	131.2±14.0^{Aa}
Proline (g kg^{-1}FM)	0.17±0.1^{Ab}	0.23±0.2^{Aa}	0.17±0.2^{Aa}	0.21±0.1^{Aa}
Total amino acids (g kg^{-1}FM)	3.1±0.4^{Ab}	4.2±0.2^{Aa}	2.4±0.2^{Ab}	3.2±0.3^{Ba}
Soluble protein (g kg^{-1}FM)	13.6±1.1^{Ab}	17.1±0.6^{Aa}	10.9±0.4^{Ab}	14.0±1.3^{Ba}
Frutose (g kg^{-1}FM)	4.0±0.6^{Aa}	5.2±0.8^{Aa}	6.1±1.2^{Aa}	6.9±1.2^{Aa}
Glucose (g kg^{-1}FM)	6.4±1.0^{Ab}	10.5±0.6^{Aa}	9.7±1.2^{Aa}	13.0±1.4^{Aa}
Sucrose (g kg^{-1}FM)	2.3±0.6^{Aa}	2.9±0.8^{Aa}	1.7±0.1^{Aa}	3.1±1.0^{Aa}
SOD (AU mg^{-1} min^{-1})	4.1±0.2^{Bb}	6.0±0.3^{Aa}	5.3±0.4^{Aa}	5.3±0.4^{Aa}
POX (μmol mg^{-1} min^{-1})	1.03±0.07^{Aa}	0.72±0.06^{Aa}	0.47±0.07^{Ba}	0.46±0.05^{Ba}
CAT (μmol mg^{-1} min^{-1})	2.2±0.3^{Aa}	3.2±0.5^{Aa}	0.9±0.1^{Ba}	1.2±0.4^{Ba}

The values represent mean ± SE from five repetitions (n = 5). ^{A,B} indicate significant differences among cultivar/variety at the same salt level and ^{a,b} indicate significant differences among the treatments for the same cultivar at $p < 0.05$ (LSD test).

Table 2 Contribution of inorganic and organic solutes to osmotic potential in first fully expanded leaf of variety Catissorgo and genotype CSF20 of forage sorghum growing in nutrient solution and submitted to control treatment (without NaCl) and salt stress with 120 mmol.L^{-1} NaCl for ten days

Relative contribution (%)	Sorghum variety/genotype			
	Catissorgo		CSF20	
	Control	Salt stress	Control	Salt stress
	First fully expanded leave			
Potassium	28.8±1.2^{Aa}	16.2±2.4^{Ab}	24.5±3.4^{Aa}	14.6±1.0^{Ab}
Sodium	0.6±0.4^{Ab}	15.4±2.3^{Aa}	0.0±0.0^{Ab}	11.8±1.8^{Aa}
Chloride	13.2±1.1^{Ab}	22.7±1.6^{Aa}	15.9±1.1^{Aa}	19.3±1.7^{Aa}
Fructose	6.2±1.1^{Aa}	4.8±0.3^{Aa}	9.7±2.4^{Aa}	8.2±2.0^{Aa}
Glucose	10.1±1.8^{Ba}	10.0±0.6^{Aa}	14.5±1.3^{Aa}	10.6±0.4^{Ab}
Sucrose	1.9±0.5^{Aa}	1.9±0.4^{Aa}	1.4±0.1^{Aa}	1.2±0.3^{Aa}
Proline	0.4±0.1^{Aa}	0.4±0.1^{Aa}	0.4±0.1^{Aa}	0.3±0.0^{Aa}
Amino acids	6.5±0.5^{Aa}	5.5±0.7^{Aa}	4.8±0.2^{Ba}	3.6±0.5^{Ba}

The values represent mean ± SE from five repetitions (n = 5). ^{A,B} indicate significant differences among cultivar/variety at the same salt level and ^{a,b} indicate significant differences among the treatments for the same cultivar at $p < 0.05$ (LSD test).

Table 3 is not available with this version.

Figures

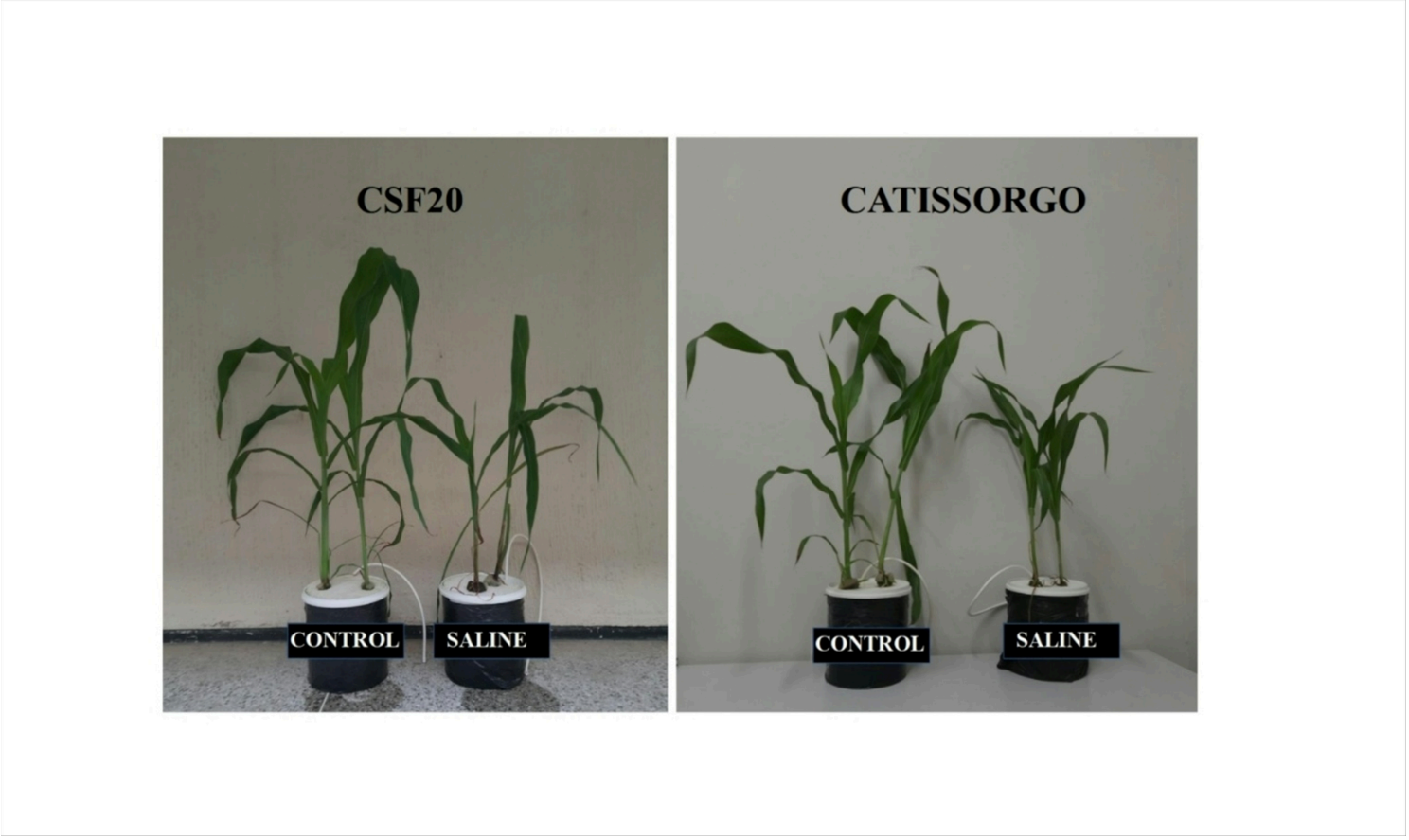


Figure 1

Twenty four days-old plants of forage sorghum growing under non-saline (control) and saline (120 mM NaCl) conditions. The plants were expose to salt stress for ten days

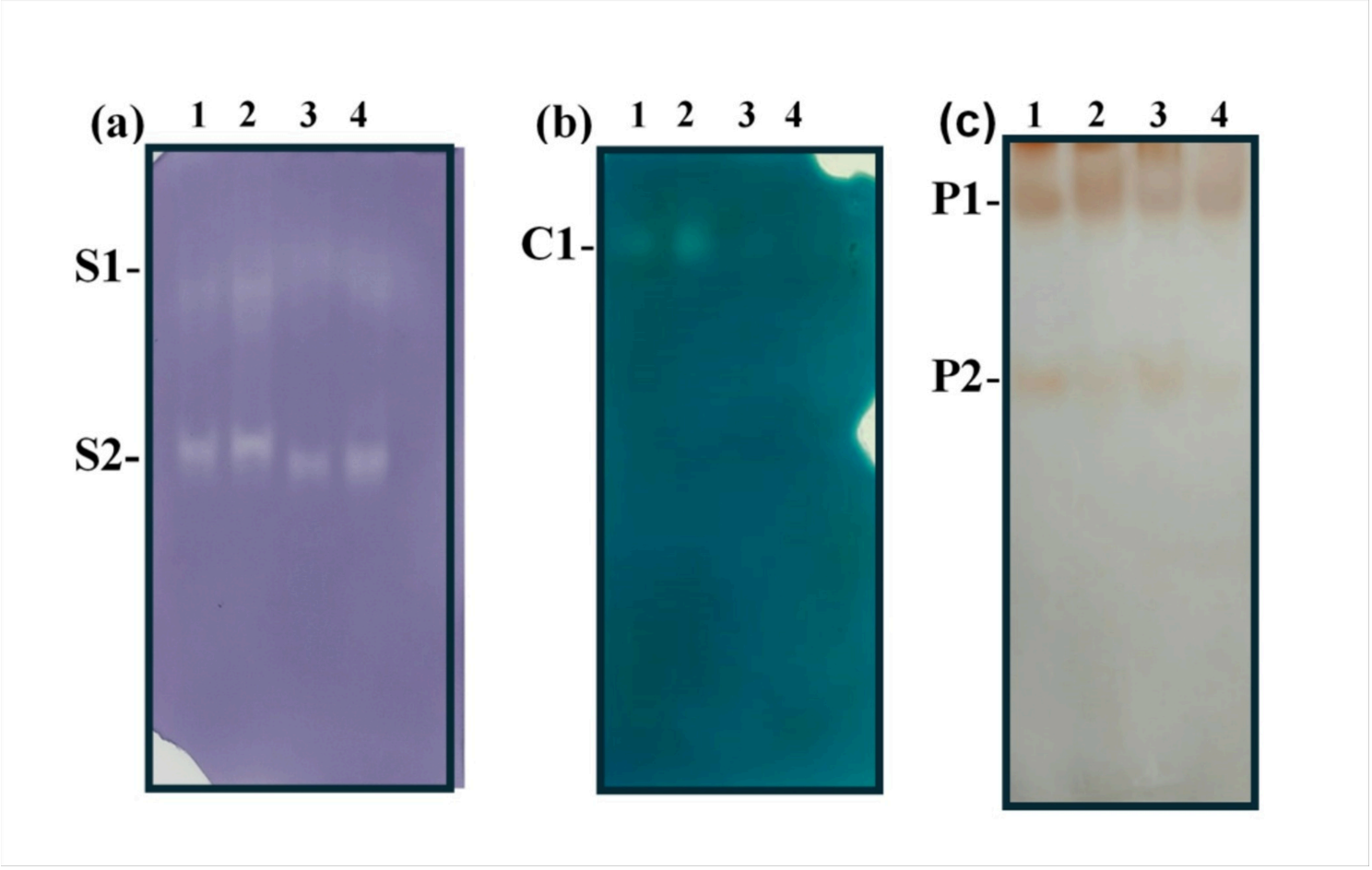


Figure 2

Activity in gel of the antioxidative enzymes, superoxide dismutase (a), catalase (b) and guaiacol peroxidase (c) in first fully expanded leaf of variety Catissorgo and genotype CSF20 of forage sorghum, growing in nutrient solution and submitted to control treatment (without NaCl) and salt stress with 120 mmol.L^{-1} NaCl for ten days. 1: Catissorgo, control; 2: Catissorgo, salt stress; 3: CSF20, control; 4: CSF20, salt stress. Letters S, C and P followed by numbers indicate isoforms detected for SOD, catalase and POX, respectively

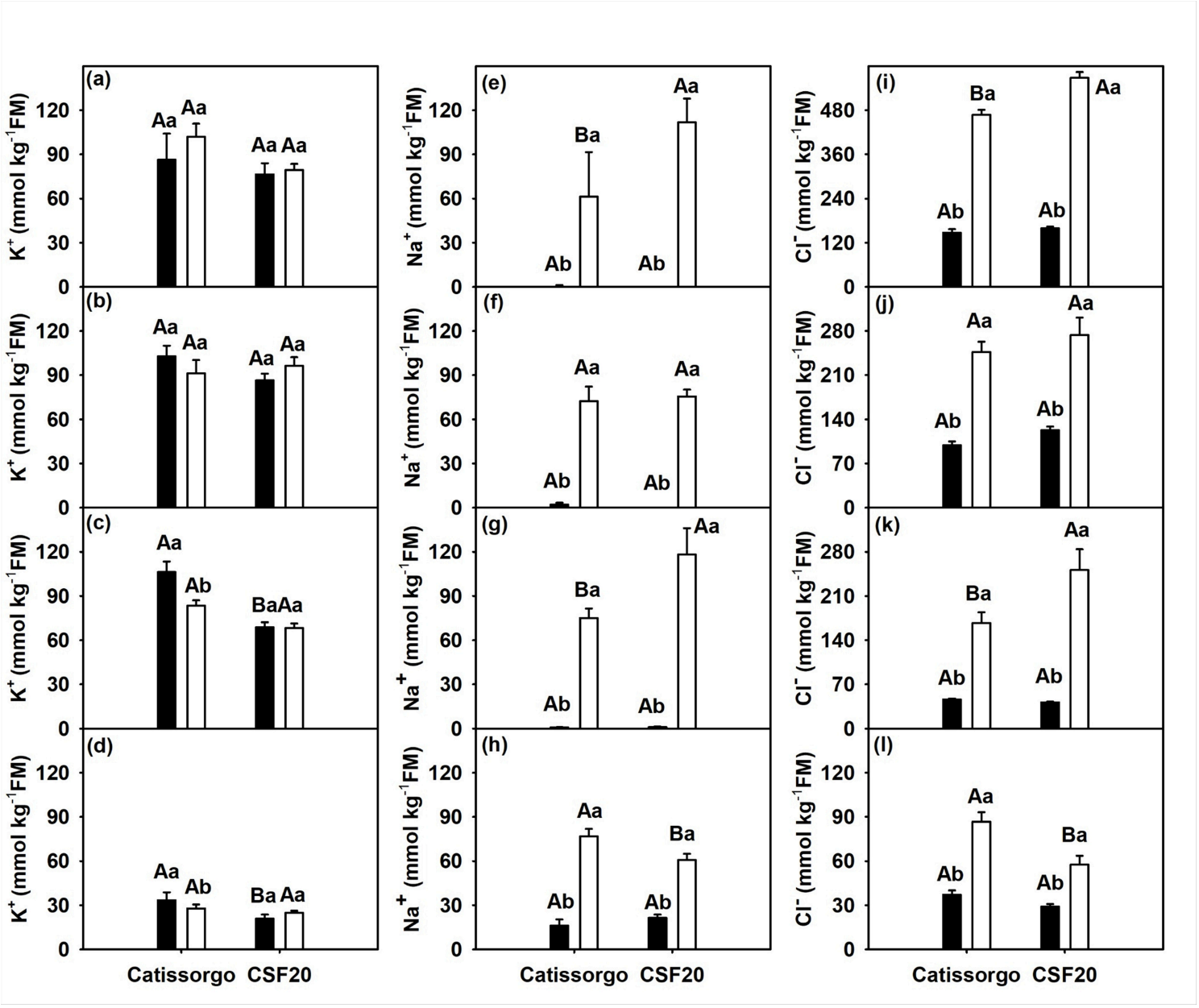


Figure 3

K^+, Na^+ and Cl^- contents in young leaves (a, e and i), first fully expanded leaf (b, f and j), stems + sheaths (c, g and k) and roots (d, h and l) of variety Catissorgo and genotype CSF20 of forage sorghum, growing in nutrient solution and submitted to control treatment (without NaCl) and salt stress with 120 $mmol.L^{-1}$ NaCl for ten days