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# Differential responses of antioxidative defense system to NaCl in grain and forage sorghum during germination and seedling establishment

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**Abstract**

Tolerance of crops to abiotic stresses can vary among cultivars and due to the phenotypic stage of development. The aim of this study was to evaluate the salinity effects on germination, seedling establishment and physiological changes on grain (BRS 310) and forage sorghum (BRS 800). Sorghum seeds were sown between two paper towels moistened with deionized water and also NaCl solution (50 mM) for 10 days. We evaluated the germination percentage, growth parameters, contents of inorganic and organic solutes and activity of antioxidant enzymes. The salinity did not affect sorghum seeds germination, however, it caused a reduction in the development of the seedlings. Both genotypes presented similar growth parameters under optimum and salt stress conditions, with exception for decreases in shoot length and shoot dry mass of BRS 800 genotype under salinity. Similar results for inorganic solutes accumulation in both genotypes suggest that osmotic adjustment cannot be considered as one of the strategies used by these genotypes to attenuate the NaCl effects. BRS 310 genotype is more tolerant to salinity than BRS 800 genotype and it is likely to be related to a better protection mechanism against oxidative damage by inducing activities of antioxidant enzymes, such as ascorbate peroxidase and catalase. This study highlights the importance of these enzymes in the establishment of salt-tolerant sorghum seedlings under salinity conditions.

**Key words:** Antioxidant enzymatic system; ions accumulation; organic solutes; reactive oxygen species; NaCl; *Sorghum bicolor* (L.) Moench.

## Introduction

Sorghum [*Sorghum bicolor* (L.) Moench] is a species which is often recommended as an alternative for generating income in arid and semi-arid regions. Thus, it is important to study the response of this crop to abiotic factors observed in these regions, such as water and salt stress (Silva et al. 2016). In fact, in arid and semi-arid environments due to intensity of evaporation and insufficient amount of rainfall for substantial leaching, saline soils are abundant (Dai et al. 2011). Salinity is known by promoting two rather peculiar characteristics: low osmotic potentials and high concentrations of cations, such as $Na^+$, $Ca^{2+}$, $Mg^{2+}$ and $K^+$, and anions, such as $Cl^-$, $SO_4^-$, $HCO_3^-$, $CO_3^{2-}$ and $NO_3^-$ (Munns 2002).

Recently, large genotypic differences have been reported among cultivars for salinity tolerance in sorghum (Shakery et al. 2017). In addition, the tolerance of crops to abiotic stresses can vary due to the phenotypic stage of development (Silva et al. 2016). Depending on the species or genotype, seedlings and young plants can be more sensitive to adverse climates than established plants, presenting irregular emergence and slower growth under harsh conditions (Oliveira and Gomes Filho 2009).

Many studies have been carried out looking for ways to provide tolerance to adverse conditions during germination and initial development of sorghum, such as salinity (Oliveira et al. 2012; Patanè et al. 2013; Shakery et al., 2017). In this context, Oliveira et al. (2011) reported enhanced growth and accumulation of organic and inorganic solutes in NaCl-stressed sorghum seedlings induced by different treatment applied to the seeds. It is related to the fact that plants under abiotic stresses may perform osmotic adjustments through the accumulation of osmotically active solutes, such as ions in the vacuole and organic solutes, which allow water uptake, cell enlargement and plant growth in adverse environments (Blum 2017).

Besides the adverse effects previously mentioned, it is well known that environmental stresses generate the production of reactive oxygen species (ROS) in plants (Møller and

Sweetlove 2012). These substances accumulation during an abiotic stress depends on the balance between ROS production and ROS scavenging (Mittler et al. 2004). In this context, Oukarroum et al. (2015) emphasized that enzymatic ROS scavenging mechanisms in plants include superoxide dismutase (SOD), ascorbate peroxidase (APX), guaiacol peroxidase (G-POD), and catalase (CAT). Therefore, increases in these enzyme activities, along with other acclimatization mechanisms, may be essential to attenuate the harmful effects of abiotic stresses, e.g. salinity, providing better seed germination and plant growth under those conditions.

The aim of this work was to evaluate the effects of salinity seed germination and seedling establishment of grain and forage sorghum genotypes, evaluating the initial growth, accumulation of organic and inorganic solutes, and enzymatic activity of ROS scavenging enzymes.

**Results**

***Germination and growth parameters***

In general, salinity conditions reduced the growth of forage sorghum seedlings (BRS 800) and decreased the root dry weight in both grain and forage genotypes (BRS 310 and BRS 800), but it did not affect these genotype seeds germination. We observed in both genotypes that salt stress induction with 50 mM NaCl did not affect significantly ($p>0.05$) the percentage of sorghum seeds germinated at 10 DAS (Fig 1A). The shoot and length of the grain sorghum, cv. BRS 310, also showed similar behavior, being not influenced by NaCl (Fig 1B and 1C). On the other hand, salinity conditions reduced the shoot length of the forage sorghum, cv. BRS 800 (Fig 1B). Salinity did not affect the shoot fresh weight and shoot dry weight of grain sorghum, BRS 310 genotype (Fig 2A and 2B). Nonetheless, in forage sorghum seedlings (BRS 800) both fresh and dry weight of shoot were negatively influenced by salt stress, being significantly reduced ($\alpha<0.05$) by 50 mM NaCl (Fig 2A and 2B).

NaCl had no significant influence on root fresh weight of grain sorghum (BRS 310), whereas the forage sorghum (BRS 800) presented higher mean values for this variable due to salinity (Fig 2C). The root dry weight of both genotypes was significantly reduced by NaCl treatment (Fig 2D). Nevertheless, both fresh and dry weight of seeds presented statistically higher mean values for both genotypes due to salt stress (Fig 2E and 2F).

Summing up these growth parameters, we observed that in general, salinity conditions reduced the growth of forage sorghum seedlings (BRS 800) and decreased the root dry weight in both grain and forage genotypes (BRS 310 and BRS 800), but it did not affect these genotype seeds germination.

***$K^+$ and $Na^+$ accumulation***

In this study, the $K^+$ content in roots of both genotypes was statistically influenced by NaCl ($\alpha<0.05$), with a strong accumulation of this ion in these organs due to salt stress (Fig 3A and 3B). On the other hand, opposite behavior was observed for this characteristic in grain sorghum (BRS 310) seeds (Fig 3A). In both genotypes, no significant effect ($\alpha>0.05$) was confirmed in shoot, so in this case $K^+$ content was not responsive to salinity.

$Na^+$ content, nonetheless, was strongly influenced by NaCl, once under this condition there was high amount of this ion accumulated in all organs evaluated, regardless of the genotype (Fig 3C and 3D). Therefore, under salt stress we observed accumulation of both inorganic solutes evaluated (ions $K^+$ and $Na^+$).

***Organic solutes***

Only the BRS 800 genotype had its soluble carbohydrates content influenced by salinity ($\alpha<0.05$), which presented significant increases in seeds due to NaCl (Fig 4B). Similar behavior was observed for this genotype in both shoot and seed soluble amino-N contents, being the salinity responsible for increases in these amino acids in both organs (Fig 4C and 4D). However, for grain sorghum (BRS 310) shoots we observed the soluble amino-N content was negatively affected in the presence of NaCl (Fig 4C).

Soluble proteins content of both genotypes was strongly influenced by salt stress conditions ($\alpha<0.05$), where most organs presented higher mean values as a function of NaCl, except for grain sorghum (BRS 310) shoot, in which soluble proteins were not affected ($\alpha>0.05$) by salinity (Fig 4E and 4F).

***ROS scavenging enzymes***

The enzymatic antioxidant system of grain sorghum (BRS 310) was highly responsive to salinity conditions ($\alpha<0.05$), in a way that NaCl resulted in increases in APX activity for shoot, root and seed (Fig 5A). In forage sorghum (BRS 800) similar behavior was visualized only for shoot, once there was no statistically difference between treatments ($\alpha>0.05$) for root and shoot (Fig 5B).

Unlike APX activity, the presence of 50 mM NaCl resulted in lower G-POD activity, not only in roots of BRS 310 genotype, but also in all parts of BRS 800 genotype seedlings (Fig 5C and 5D). Similar behavior was observed for CAT activity in shoot of grain sorghum, whereas this enzyme activity was increased in its roots due to salinity (Fig 5E). However, shoot and seeds of forage sorghum presented higher CAT activity under salt stress condition, with no significant influence ($\alpha>0.05$) of this factor on roots (Fig 5F). SOD activity was reduced due to salinity effects on roots and seeds of grain sorghum, cv. BRS 310 (Fig 5G). Similarly, NaCl provided reduction of SOD activity in forage sorghum shoots, cv. BRS 800, but seeds and roots were not influenced by salt stress (Fig 5H).

**Discussion**

Seeds germination (Fig 1A) and root length (Fig 1C) was not influenced by NaCl in our study ($\alpha>0.05$). Similar results were found by Patanè et al. (2013), who reported that salinity only prolonged germination time of two sweet sorghum cultivars. On the other hand, Shrestha et al. (2016) observed that germination was reduced by 50% in forage sorghum (SS405) at 19.3 dS $m^{-1}$, but a grain sorghum (NK5418) had 70% germination even at 25 dS $m^{-1}$. These discrepancies may be related to the level of salinity applied in our research (50 mM NaCl), which was way lower than this one verified in the research mentioned before.

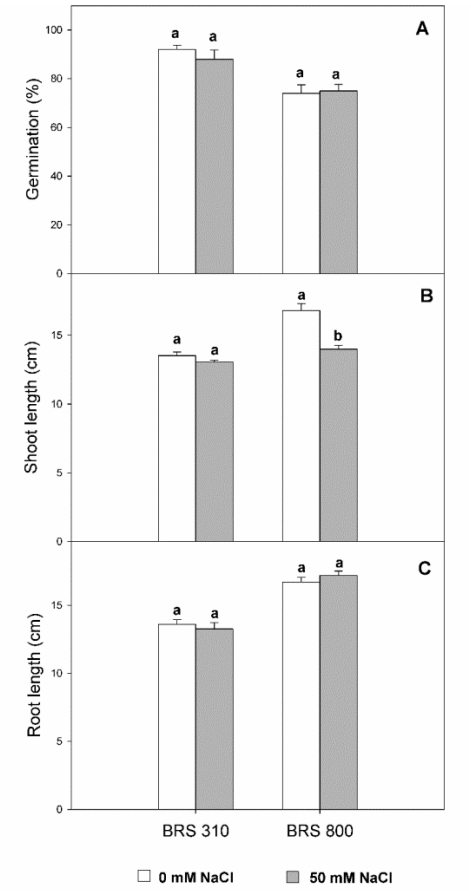


**Fig 1.** Seed germination, A (%), shoot length, B (cm) and root length, C (cm) of grain (cv. BRS 310) and forage (cv. BRS 800) sorghum seedlings at 10 days after sown under optimum conditions (0 mM NaCl) and salt stress (50mM NaCl). Means followed by same letter at same genotype do not differ statistically by *t* Student Test at 5% of significance. Vertical bars represent the means standard error.

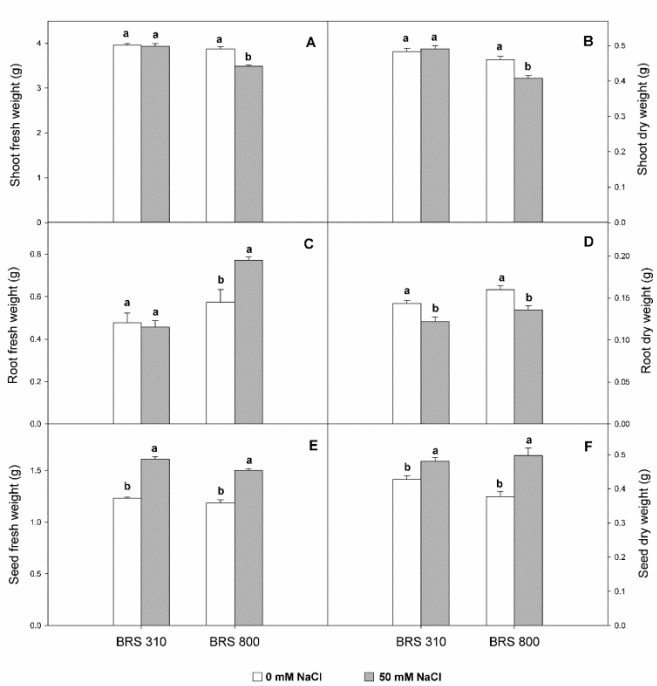


**Fig 2.** Shoot, A (g), root, C (g) and seed, E (g) fresh weight, and shoot, B (g), root, D (g) and seed, F (g) dry weight of grain (cv. BRS 310) and forage (cv. BRS 800) sorghum seedlings at 10 days after sown under optimum conditions (0 mM NaCl) and salt stress (50mM NaCl). Means followed by same letter at same genotype do not differ statistically by *t* Student Test at 5% of significance. Vertical bars represent the means standard error.

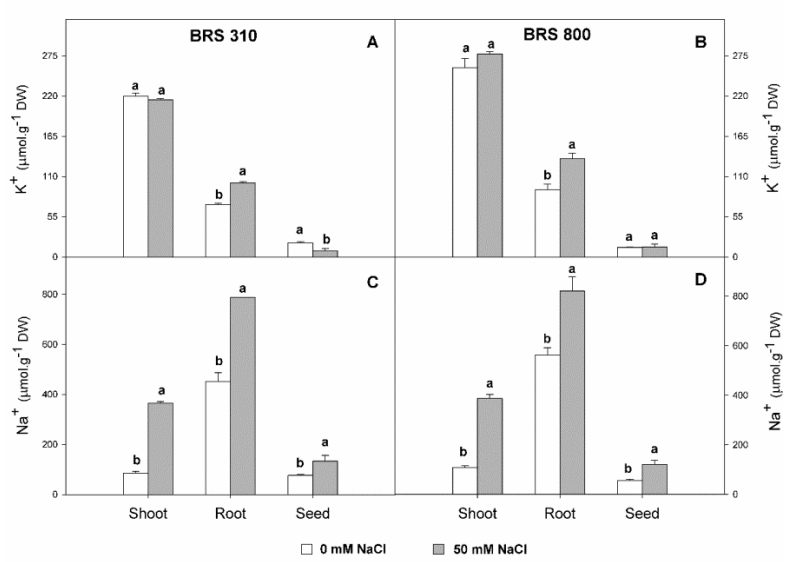


**Fig 3.** $K^+$, A and B (µmol.$g^{-1}$ DW) and $Na^+$ (µmol.$g^{-1}$ DW) contents in shoot, root and seed of grain (cv. BRS 310) and forage (cv. BRS 800) sorghum at 10 days after sown under optimum conditions (0 mM NaCl) and salt stress (50mM NaCl). Means followed by same letter at same genotype do not differ statistically by *t* Student Test at 5% of significance. Vertical bars represent the means standard error.

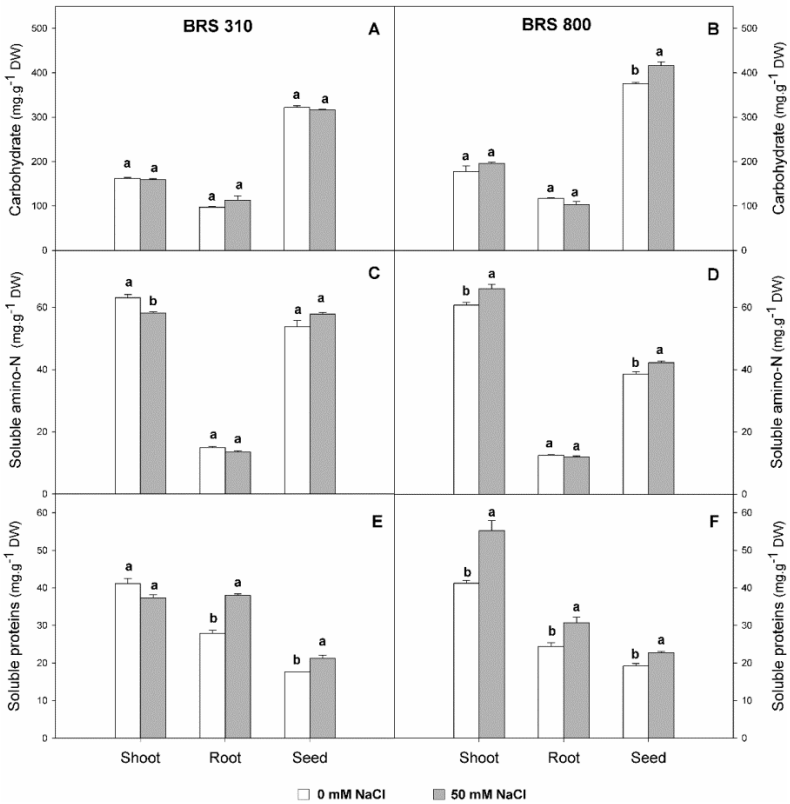


**Fig 4.** Carbohydrate, A and B (mg.$g^{-1}$ DW), soluble amino-N, C and D (mg.$g^{-1}$ DW) and soluble proteins, E and F (mg.$g^{-1}$ DW), contents in shoot, root and seed of grain (cv. BRS 310) and forage (cv. BRS 800) sorghum at 10 days after sown under optimum conditions (0 mM NaCl) and salt stress (50mM NaCl). Means followed by same letter at same genotype do not differ statistically by *t* Student Test at 5% of significance. Vertical bars represent the means standard error.

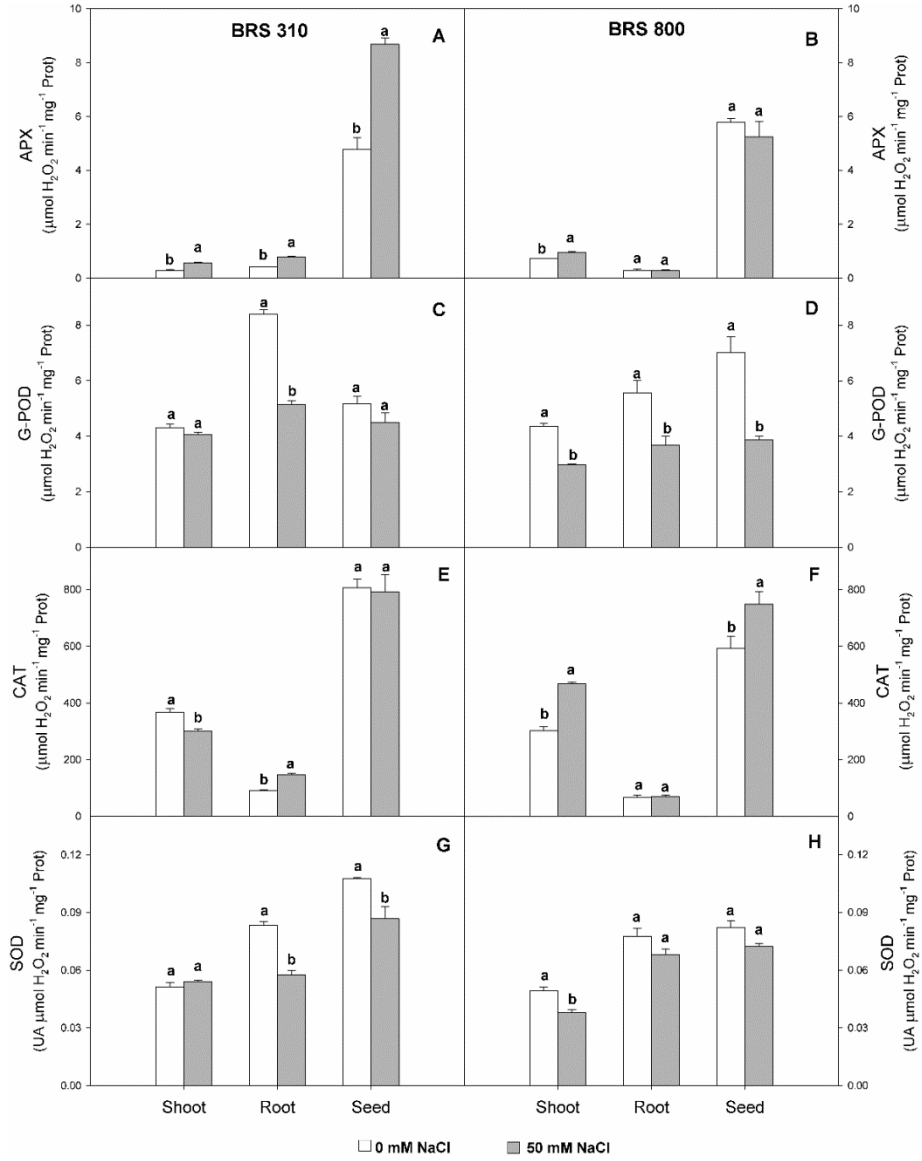


**Fig 5.** APX, A and B (µmol $H_2O_2$ $min^{-1}$ $mg^{-1}$ Prot), G-POD, C and D (µmol $H_2O_2$ $min^{-1}$ $mg^{-1}$ Prot), CAT, E and F (µmol $H_2O_2$ $min^{-1}$ $mg^{-1}$ Prot), and SOD, G and H (UA µmol $H_2O_2$ $min^{-1}$ $mg^{-1}$ Prot), contents in shoot, root and seed of grain (cv. BRS 310) and forage (cv. BRS 800) sorghum at 10 days after sown under optimum conditions (0 mM NaCl) and salt stress (50mM NaCl). Means followed by same letter at same genotype do not differ statistically by *t* Student Test at 5% of significance. Vertical bars represent the means standard error.

Shoot length (Fig 1B), shoot fresh weight (Fig 2B) and shoot dry weight (Fig 2C) of forage sorghum (BRS 800) presented lower mean values due to salinity exposition, showing that this genotype is less tolerant to salt stress during early development phases than the grain sorghum (BRS 310). Shrestha et al. (2016), however, reported similar behavior for both grain and forage sorghum, who reported that biomass of both hybrids of grain and forage sorghum was reduced by 50% at 16.8 dS $m^{-1}$. In this context, the tolerance to salt stress is highly correlated to intrinsic factors, such as the genetic material evaluated (Munns 2002), as observed by Oliveira and Gomes-Filho (2009) in grain and forage sorghum seeds germinated under water and salt stress.

Genetic differences in tolerance to abiotic stresses have been reported in sorghum seedlings, which can be useful in identifying genotypes more adapted salt-affected areas. In this context, salt stress effects upon seed germination in sorghum have been documented in the literature and confirmed this differential tolerance due to the genotype factor (Oliveira and Gomes-Filho 2009; Patanè et al. 2013; Shrestha et al. 2016).

### *$K^+$ and $Na^+$ accumulation*

The present research also studied inorganic solute contents, such as $Na^+$ and $K^+$, being this first ion usually the major cation accumulated in seedlings under salt stress conditions, as we observed in shoots, roots and seeds of both genotypes evaluated in this study (Fig 3C and 3D). At least partially, this problem may be related to the fact that $K^+$ homeostasis is severely affected by salinity due to the competition between $K^+$ and $Na^+$ for common absorption sites present in the

plasma membrane (Almeida et al., 2017). Sustaining this hypothesis, salt-stressed plants presented increase in $K^+$ content only in roots (Fig 3A and 3B), indicating also a possible issue transporting this element to other organs. Lower $Na^+$ accumulation and the maintenance of $K^+$ levels, primarily in the photosynthetic tissues, can be considered one of the most important salinity-tolerance mechanisms of glycophytes (Almeida et al., 2017).

According to Oliveira et al (2011), $Na^+$ and $Cl^-$ accumulation in shoots of NaCl-stressed sorghum seedlings indicated an osmotic adjustment, which was efficient in reducing the osmotic stress caused by salinity. Thus, the similar results for inorganic solutes accumulation in both genotypes evaluated in our study suggests that osmotic adjustment cannot be considered one of the strategies used by these genotypes to attenuate the salt stress effects.

***Organic solutes***

The increase in organic solute concentration in the cytoplasm of plants subjected to salt stress has been considered as a mechanism used by the plants to balance the osmotic potential between cytoplasm and vacuole, and avoid enzymatic damage (Munns 2002). However, this fact was not verified in our work, once there was not a clear behavior regarding organic solutes accumulation in both genotypes evaluated, except for soluble proteins, which increased due to salt stress. Freitas et al. (2011), on the other hand, found that carbohydrate levels increased in response to salinity, and that these were the main organic solutes responsible for osmotic adjustment in the leaves of young sorghum plants. Similar behavior was observed by Oliveira et al. (2011) in grain sorghum seedlings grown under salt stress.

The increased soluble amino-N content in salt-stressed shoots and seeds of forage sorghum (Fig 4D) in this study was in concordance with amino acid accumulation that has been seen in previous studies in plants exposed to abiotic stress (Lugan et al. 2010; Freitas et al. 2011; Oliveira et al. 2011). Similarly, Bezerra et al. (2010) also emphasized an increase in concentrations of total free amino acids in sunflower plants for increases in the NaCl concentration of the nutrient solution. However, higher accumulation of amino acids under stress conditions may indicate cell damage in some species (Widodo et al. 2009). Hence, we can infer that this may be one of the reasons why the BRS 800 genotype presented lower tolerance to salt stress in this study.

The higher contents of soluble carbohydrates, soluble amino-N and soluble proteins observed in seeds of forage sorghum (BRS 800) indicates a possible delay in reserve mobilization during germination of seeds from this cultivar, primarily in presence of NaCl. Similar findings were reported by Oliveira and Gomes-Filho (2011), who observed the germination speed index and germination average time of forage sorghum were affected by NaCl, having the CSF 18 genotype greater tolerance to water and salt stress during the germination phase than CSF 20. This corroborates with a studied carried out by Patanè et al. (2013), whose authors reported that salt stress prolonged the germination time in sweet sorghum.

In concordance with our findings in this experiment for both sorghum genotypes, Bezerra et al. (2010) found that the presence of NaCl induced an increase in total soluble protein content in sunflower plants. It is likely that the increase of soluble protein contents in salt-stressed plants may be related to the contribution of these macromolecules to produce ROS scavenging enzymes in order to enhance plant's ability to tolerate salt stress, once we observed the increases in soluble protein contents (Fig 4E and 4F) were correlated to the increases in antioxidant enzyme activities (Fig 5).

***ROS scavenging enzymes***

In the present work, enzymatic ROS scavenging mechanisms in both genotypes were evaluated by measuring the activity of major enzymes evolved with this process, such as APX, G-POD, CAT and SOD. The strong increase in APX activity observed in grain sorghum (BRS 310) suggests that this enzyme played a relevant role ameliorating the negative effects of salt stress (Fig 5A). Similar results have been found in the literature for sorghum (Oliveira et al. 2012) and corn (Azevedo Neto et al. 2006). Despite what was observed for BRS 310 genotype, APX activity in forage sorghum (BRS 800) was not significantly influenced by salinity, except for a slight increase observed in shoots. This behavior could indicate that low APX activity in this genotype may have affected negatively the tolerance to salt stress.

Unlike the APX behavior, G-POD activity dropped in salt-stressed plants, especially in forage sorghum (BRS 800), indicating this may be caused by a negative effect of salinity, e.g. degradation of this enzyme. Increases in this enzyme activity, along with other enzymatic ROS scavenging mechanisms may be essential to attenuate the harmful effects of abiotic stresses, e.g. salinity, providing better seed germination and seedling establishment under those conditions. In this way, the lower salinity-tolerance observed in BRS 800 genotype may be related to the ineffective responses of this enzyme to NaCl.

In disagreement with the results of this work, a study carried out by Vaidyanathan et al. (2003) compared two rice cultivars that differed in their degree of tolerance to salt stress and found that G-POD activity increased in both cultivars. This corroborates with the study of Oliveira et al. (2012), who reported an increase in G-POD activity and CAT, attributing the higher activity of these enzymes to enhanced protection of grain sorghum seedlings against oxidative damage caused by NaCl.

Reduction of CAT activity in shoots of salt-stressed plants from BRS 310 genotype was an unexpected result, since, as a rule, the activity of this enzyme increases in plants under various adverse conditions (Azevedo Neto et al. 2006; Møller and Sweetlove 2012). These results may indicate that grain sorghum (BRS 310) provided other physiological mechanisms to ameliorate the harmful effects of salinity, in a way that these seedlings did not experimented an intensive oxidative stress, and no increase in CAT activity was verified. Shoots and seeds of forage sorghum presented opposite behavior. Once this genotype (BRS 800) presented lower tolerance to NaCl than the BRS 310 genotype, it is likely that higher activity of this enzyme alone could not protect these seedlings from salt stress and oxidative stress caused by ROS. Our analysis showed lower values of SOD activity in both roots and seeds of BRS 310 genotype, as well as in shoots of BRS 800 genotype, indicating lower participation of

this enzyme on ROS scavenging than APX and CAT. Thus, some authors have emphasized the importance of coordinated activity between SOD and CAT, APX and G-POD activities in processes of elimination of $O^{-}_{2}$ and $H_2O_2$, and consequently, improve tolerance to salt stress, mainly because of the maintenance of low levels of lipid peroxidation under this adverse condition (Azevedo Neto et al. 2006; Oliveira et al. 2012). In general, among the enzymes evaluated in this study, both APX and CAT presented high activities in seeds, indicating a possible relevant role played by the enzymatic antioxidant system on germination of both genotypes, primarily under salt stress conditions. SOD converts superoxide radicals ($O_2^{-}$) into hydrogen peroxide ($H_2O_2$), POD reduces $H_2O_2$ to water using various substrates as electron donors, APX uses ascorbate as an electron donor to reduce $H_2O_2$ to water, and CAT dismutates $H_2O_2$ into water and oxygen (Wang et al. 2009; Oukarroum et al. 2015). In concordance with this fact, analyzing antioxidant enzyme activity during germination of alfalfa under salinity, Wang et al. (2009) indicated that tolerance to salt or drought stresses during germination is associated with enhanced activity of anti- oxidant enzymes. Hence, our study highlights the importance of antioxidant enzymes in the establishment of salt-tolerant sorghum seedlings under salinity conditions, which is one of the major issues reported currently in many agricultural lands.

## Materials and methods

### *Experimental conditions and plant material*

In this study, we used sorghum seeds [*Sorghum bicolor* (L.) Moench] of two genotypes, a grain cultivar, BRS 310, and a forage cultivar, BRS 800. Eighteen replicates of 50 seeds for each genotype were sown between two neutral pH paper towels (Germitest®) moistened with distilled water or 50 mM NaCl solution equivalent to 2.5 times the substrate dry mass. This material was kept in seed germination chamber at 25 $^{o}$C and 12h photoperiod (Oliveira et al., 2012).

### *Seed germination and growth parameters*

After 10 days of sown (DAS), we evaluated the percentage of germinated seeds and the growth, physiological and biochemical parameters. These plants were randomly reassembled in four replicates of 50 seedlings for each genotype and separated in shoots, roots and seeds. Then, we measured the length of shoots and roots, as well as the fresh and dry mass of shoots, roots and seeds. This material was frozen and stored at -25 $^{o}$C; subsequent lyophilization resulted in dry mass of shoot, root and seeds.

### *Physiological and biochemical parameters evaluated*

The freeze-dried material was macerated and used for extracts preparation. Subsequently, we performed the determination of organic solutes (soluble carbohydrates, soluble amino-N and soluble proteins) and inorganic solutes ($K^+$ and $Na^+$ ions). Finally, we measured the enzymatic activities of some enzymes that belong to the antioxidant enzymatic system, such as ascorbate peroxidase (APX), guaiacol peroxidase (G-POD), catalase (CAT) and superoxide dismutase (SOD) in shoots, roots and seeds of both genotypes.

### *Determination of organic and inorganic solutes*

Lyophilized material (shoot and root) was extracted with 15 mL of deionized water and placed in a 100 $^{o}$C bath for 1 h. The extracts were filtered and stored at -20 $^{o}$C for later analyses. The organic solutes measured in this study were, soluble amino-N (Yemm and Cocking 1955), soluble carbohydrates (Dubois et al. 1956) and proteins (Bradford 1976). The inorganic solutes that we evaluated in this experiment were the ions $Na^+$ and $K^+$, both determined by flame photometry (Malavolta et al. 1997).

### *Enzymes extract preparation*

Lyophilized shoots, roots and seeds powder (0.20 g) was homogenized in a mortar and pestle with 4 mL of ice-cold extraction buffer (100 mM potassium phosphate buffer, pH 7.0, 0.1 mM EDTA). The homogenate was filtered through muslin cloth and centrifuged at 16,000 $\times$ *g* for 15 minutes. The supernatant fraction was used as crude extract for enzyme activity assays. All operations were carried out at 4ºC.

### *Enzyme assays*

Total SOD (EC 1.15.1.1) activity was determined by measuring its ability to inhibit the photochemical reduction of nitroblue tetrazolium chloride (NBT), as described by Giannopolitis and Ries (1977). The reaction mixture (1.5 mL) contained 50 mM phosphate buffer (pH 7.8), 0.1 μM EDTA, 13 mM methionine, 75 μM NBT, 2 μM riboflavin and 50 μL enzyme extract. Riboflavin was added last and the tubes were shaken and illuminated with a two 20-W fluorescent tubes. The reaction was allowed to proceed for 15 minutes, after which the lights were switched off and the tubes covered with a black cloth. Absorbance of the reaction mixture was read at 560 nm. One unit of SOD activity (U) was defined as the amount of enzyme required to cause 50% inhibition of the NBT photoreduction rate. Total CAT (EC 1.11.1.6) activity was measured through the method of Beers Jr and Sizer (1952), with minor modifications. The reaction mixture (1.5 mL) consisted of 100 mM phosphate buffer (pH 7.0), 0.1 μM EDTA, 20 mM $H_2O_2$ and 50 μL enzyme extract. The reaction was started by adding the extract. The decrease of $H_2O_2$ was monitored at 240 nm and quantified using its molar extinction coefficient (36 $M^{-1}$ $cm^{-1}$). Total APX (EC 1.11.1.1) activity was assayed according to Nakano and Asada (1981). The reaction mixture (1.5 mL) contained 50 mM phosphate buffer (pH 6.0), 0.1 μM EDTA, 0.5 mM ascorbate, 1.0 mM $H_2O_2$ and 50 μL enzyme extract. The reaction was started with the addition of $H_2O_2$ and ascorbate oxidation, measured at 290 nm for 1 minute. Enzyme activity was quantified using the molar extinction coefficient for ascorbate (2.8 $mM^{-1}$ $cm^{-1}$), taking into consideration that two mols of ascorbate are required for the reduction of one mol of $H_2O_2$ (McKersie and Leshem 2010). Total G-POD (EC 1.11.1.7) activity was determined as described by Urbanek et al. (1991) in a reaction mixture (2.0 mL) containing 100 mM phosphate buffer (pH 7.0), 0.1 μM EDTA, 5.0 mM guaiacol, 15.0 mM $H_2O_2$ and 50 μL enzyme extract. The addition of enzyme extract started the reaction

and the increase in absorbance was recorded at 470 nm for 1 minute. Enzyme activity was quantified by the amount of tetraguaiacol formed using its molar extinction coefficient (26.6 $mM^{-1}$ $cm^{-1}$), taking into consideration that four mols of $H_2O_2$ are required to produce one mol of tetraguaiacol (Plewa et al. 1991). ROS scavenging enzymes activities were calculated on protein basis and the results were expressed as enzymatic activity unit, in μmols mg $protein^{-1}$ $min^{-1}$ (specific activity). Total soluble proteins content was obtained from same extract used to lipid peroxidation and enzyme assays. The proteins were determined at 595 nm by method of dye binding Coomassie® Brilliant Blue according to Bradford (1976) using a bovine serum albumin reference curve.

**Conclusions**

Similar results for inorganic solutes accumulation in both genotypes suggest that osmotic adjustment cannot be considered as one of the strategies used by these genotypes to attenuate the NaCl effects. The grain sorghum (cv. BRS 310) presented slightly higher tolerance to salinity than the forage sorghum (cv. BRS 800), and it is likely to be related to an enhanced enzymatic antioxidant defense system, e.g. higher activities of ascorbate peroxidase and catalase. This study highlights the importance of these enzymes in the establishment of salt-tolerant sorghum seedlings under salinity conditions.

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