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Adaptive Response to Salt Stress in Sorghum (*Sorghum bicolor*)

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Abstract: Sorghum (*Sorghum bicolor*) plants were treated with 50, 100 and 150 mM NaCl. Growth parameters (length, dry weight, fresh weight and water content), biochemistry parameters (proline, proteins, amino acids, total soluble sugars and chlorophyll content), activities of antioxidative enzymes (superoxide dismutase (SOD), catalase (CAT), guaïacol peroxidase (POD) and glutathione reductase (GR)) as will as the activities of nitrate reductase (NR), glutamine synthetase (GS) and phosphoenolpyruvate carboxylase (PEPC) were studied in the leaves and roots after 25 days of NaCl exposure. The results showed that growth parameters were not affected by salinity in both organs. Therefore biochemistry parameters and activities of antioxidative enzymes generally were increased in roots and leaves with increasing salinity levels up to 150 mM NaCl. Exogenous NaCl in culture medium no induced significant difference in the leaves and roots about nitrate reductase activity. In contrast, an increase in glutamine synthetase activity was noticed in these organs. Activity phosphoenolpyruvate carboxylase was significantly increased by 50 mM NaCl in the leaves while in roots, it was increased only at 50 mM and 100 mM NaCl. These results suggested that the sorghum showed a better protection mechanism against oxidative damage caused by salt stress by its higher induced activities of antioxidant enzymes,GS, PEPC, accumulation of compatible organic solutes and chlorophyll.

Key words: Sorghum bicolor · Salt stress · Antioxidative enzymes · Compatible organic solutes · Glutamine synthetase and Phosphoenolpyruvate carboxylase

INTRODUCTION

Sorghum (*Sorghum bicolor* (L.) Moench) (C4 plants) is the fourth most cultivated cereal in the world, being produced most frequently in high temperature and low rainfall areas and in soil with salinity problems [1]. It is a crop fairly tolerant to salinity [2]. Although there are significant differences between genotypes of sorghum grown in salt stress conditions [3]. Salinity effects on plants include ion toxicity, osmotic stress, mineral deficiencies, physiological and biochemical perturbations [4], and like other abiotic stresses, salinity can also lead to oxidative stress through the increase in reactive oxygen species (ROS), such as superoxide (O_2•–), hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH•), which are highly reactive and may cause cellular damage through oxidation of lipids, proteins and nucleic acids [5]. In order to prevent or alleviate the damage that ROS may cause in plant tissues, these ROS are detoxified by the sequential and simultaneous action of a number of enzymes including SOD, CAT, POD and GR, that are able to metabolise them to less toxic compounds. Superoxide dismutase (SOD; EC 1.15.1.1) is located in various cell compartments, is a major scavenger of superoxide (O_2•-) and its enzymatic action results in the formation of H_2O_2 and O_2 [6]. The hydrogen peroxide produced is then scavenged by catalase (CAT; EC 1.11.1.6.) and a variety of peroxidases (POD; EC 1.11.1.7). Catalase dismutates mostly photorespiratory/respiratory H2O2 into water and molecular O_2 [7], whereas POD decomposes H_2O_2 by oxidation of co-substrates such as phenolic compounds and/or antioxidants. Glutathione reductase (GR; EC 1.6.4.2) that catalyses the NADPH-dependent reduction of the oxidized form of glutathione (GSSG) to reduced glutathione (GSH) [8]. Most of studies suggest a correlation between stress tolerance and the presence of an efficient antioxidant system. Accumulation of compatible organic solutes, so called osmolytes: Soluble

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carbohydrates, amino acids, organic acids and proline are also a common response to salt stress in plants. In the cells, the toxic ions usually accumulate in the vacuoles and the osmotic equilibrium between vacuoles and cytoplasm may be reached by an increase in the synthesis and accumulation of compatible organic solutes in the later [9]. These osmolytes may also contribute to the stabilization of protein molecules and membranes [10] or may serve as reserve for plant metabolism [9].

In Sorghum (C4 plant), the phosphoenolpyruvate carboxylase (PEPC) (EC.4. 1. 1.31) plays a key role in primary fixation of atmospheric CO_2 [11]. PEPC has been implicated in various physiological contexts, but most important: the anapleurotic pathway i.e. replenishment of citric acid cycle intermediates when carbon skeletons are removed for other metabolic functions like nitrogen assimilation and amino acid biosynthesis. Therefore, PEPC may be an important interface between carbon and nitrogen metabolism. Nitrate is the main nitrogen source in agricultural soils. However, the reduced nitrogen form available to plants for assimilation into amino acids and proteins is ammonium; nitrate is converted to ammonium by sequential actions of two enzymes, nitrate reductase (NR; EC 1.7.1.1) and nitrite reductase (NiR; EC 1.7.1.4) [12] with NR catalyzing the limiting step [13]. Ammonium is mainly assimilated through the concerted action of glutamine synthetase (GS; EC 6.3.1.2) and glutamate synthase (GOGAT; EC 1.4.1.14).

Although some papers concerning the impact of NaCl on antioxidative and osmoprotective responses have been published, but few of them were investigated simultaneously these changes en relation with nitrogen and carbon metabolism in roots and leaves sorghum. The purpose of the present study was to investigate effects of the salt stress on plant sorghum, by studying parameters of growth, in addition, we examined antioxidant enzyme activities (SOD, POD, CAT and GR), Nitrate reductase, Glutamine synthetase and Phosphoenolpyruvate carboxylase (PEPC). Our goal was in order to better understand the mechanism of saline tolerance in sorghum plant.

MATERIALS AND METHODS

Plant Material and Growth: Sorghum seeds (*Sorghum bicolor*) were sterilized with 5% of NaOCl for 15 minutes and washed thoroughly with sterile water and germinated on filter paper (Whatmann paper) in a petri dish soaked in distilled water for 4 days under dark condition at 26°C and planted in a pots filled with the vermiculite and then grown in a growth chamber. The environmental conditions in the growth chamber were 25°C and light intensity of 1000 Lux with a 16 h photoperiod. The plants received (twice a week (100 ml/pot)) a nutrient solution of 0.5 mM KNO_3, 0.375 mM KH_2PO_4, 0.125 mM K_2HPO_4, 0.375 mM $MgSO_4$, 1.25 mM $CaSO_4$, 10 mg/L Fe-ethylene-diamine tetraacetate (EDTA) and micronutrients [14], pH 6.2±0.2. For the treated cultures, the nutrient solution was supplemented by different concentrations of NaCl; 50 mmole (moderate concentration), 100 or 150 mM (sublethal concentration). Plants were harvested 25 days after start treatment and immediately sorted into leaves and roots. Roots were quickly and gently washed with deionized water to remove residual vermiculite. All enzyme preparations were performed with freshly harvested plants. For free amino acids (AA) and total soluble sugars (TSS) estimation were determined in dried leaves or roots.

Growth Parameters: After 25 days of NaCl treatment, 10 plants from each group were divided into separate leaves and roots fractions. Fresh weights of leaves and roots were weighed and lengths were measured. The samples were then dried in oven at 70°C for 72 h and dry weights were determined. Relative water content (RWC) was calculated as follows [15]: RWC (%) = [(Fresh Weight – Dry Weight)/(Fresh Weight)] x100.

Preparation of Antioxidants Enzyme Extracts and Determination of Protein Content: For determination of antioxidant enzyme activities, leaves or roots were homogenized in 50 mM potassium phosphate buffer (pH 7) containing 0.1 mM ethylenediamine-tetraacetic acid (EDTA), 1mM phenylmethylsulphonyl fluoride (PMSF), 2% (w/v) polyvinyl-pyrrolidone (PVP) (for leaves) and sand at 4°C, The homogenate was centrifuged at 15 000 g for 15 min at 4°C. The resultant supernatant was collected for determination of antioxidant enzyme activities and protein content. Protein content was estimated by the method of Bradford [16] using bovine serum albumin (BSA) as standard.

Antioxidants Enzyme Assay: Catalase activity was determined by monitoring the decomposition of H_2O_2 (extinction coefficient 39.4 mM cm^{-1}) at 240 nm following the method of Aebi [17]. Peroxidase activity was determined by monitoring the formation of guaïacol dehydrogenation product (extinction coefficient 6.39 mM cm^{-1}) at 436 nm following the method of Pütter [18]. GR activity was determined by the oxidation of NADPH at 340 nm (extinction coefficient 6.2 mM cm^{-1}) as described by

Rao *et al*. [19].

SOD Gel Activity: SOD gel activity assay method of Beauchamp and Fridovich [20] was applied with some modifications. After completion of electrophoresis, the gel (12.5%) was incubated in a solution containing 2.45 mM/L NBT for 25 min, followed by incubation in 50 mM/L potassium phosphate buffer (pH 7.8) containing 28 µM/L riboflavin and 28 mM/L TEMED under dark conditions. expression of SOD was achieved by light exposure for 10 to 20 min at room temperature.

Organic Solute Analysis: The proline content was determined using the method of Bates *et al*. [21]. Free amino acids were measured in roots and leaves extracts with ninhydrin reagent according to Magné *et al*. [22]. A standard curve was prepared with glycine.

Total soluble sugars (TSS) were estimated by anthrone reagent [23]. Aliquot (0.05 ml) is taken in test tubes and the volume was made up to 1 ml. To this solution 2 ml of anthrone reagent was added and mixture was heated in boiling water bath for 8 min followed by cooling. Optical density of green to dark green color was read at 630 nm.

Estimation of Chlorophyll: The extraction of leaf chlorophyll was performed with 80% acetone. The chlorophyll a chlorophyll b and total chlorophyll quantities were calculated according to the method of Arnon [24].

Extraction and Essay of Nitrate Reductase: Leaves or roots were homogenized in chilled mortar and pestle with 100 mM potassium phosphate buffer (pH 7.4) containing 7.5 mM cysteine, 1 mM EDTA, 1 mM PMSF and 1.5% (w/v) casein. The homogenate was centrifuged at 30,000g for 15 min at 4°C. Nitrate reductase activity (NRA) was determined according to the method described by Robin [25].

Extraction and Assay of Glutamine Synthetase: Leaves or roots were extracted in a cold mortar and pestle with grinding medium containing 50 mM Tris–HCl pH 8.0, 5 mM $MgSO_2$, 12 mM Glutamate, 2 mM EDTA, 10% (v/v) glycerol, 0.1% (v/v) 2-mercaptoethanol. The homogenate was centrifuged at 13 000g for 30 min at 4°C. Glutamine synthetase activity was measured using the transferase assay as described by Shapiro *et al*. [26].

Extraction and Assay of PEPC: Leaves or roots were extracted in a prechilled mortar and pestle with extraction medium containing 100 mM Hepes–KOH, pH 8, 10 mM MgCl2, 1 mM EDTA, 10% (v/v) glycerol, 14 mM b-mercaptoethanol, 1 mM phenylmethylsulphonyl fluoride (PMSF), 2% (w/v) polyvinylpyrrolidone (PVP) (for leaves) and sand. The homogenate was centrifuged at 15 000g for 15 min at 4°C. The resultant supernatant was collected for determination of phosphoenolpyruvate carboxylase activity. The activity of PEPC was assayed by coupling to NAD-malic dehydrogenase (MDH) and monitoring NADH oxidation at 340 nm spectrophotometrically in a 1 ml assay mixture containing 100 mM Hepes–KOH (pH 7.3), 5mM $MgCl_2$, 0.2 mM NADH, 5U of MDH (Malate dehydrogenase), 2.5 mM phosphoenol pyruvate (1 mM for root), 5 mM NaHCO3 and leaves or roots extract at 30°C.

Statistical Analysis: The data shown mean values ±S.E. Results were subjected to a one-way analysis of variance with a least significant difference (LSD) test between means using a Statgraphics 5.0. Levels of significance were represented by a at $P<0.05$, b at $P<0.01$ and c at $P<0.001$.

RESULTS AND DISCUSSION

Growth Parameters: Sorghum bicolor plants were grown for 25 days under salt stress conditions (Fig. 1). Many studies have shown that the fresh and dry weights of plants were affected, either negatively or positively, by changes in salinity concentrations, type of salt present, or type of plant species [27-29]. In the present study, salt treatments, have no significant effect on leaves and roots length, fresh weights, dry weights and RWC (Table 1). This may be due to the ability of the plant to increase the size of its sap vacuoles, which allows for the collection of a lot of water and this in turn dissolves salt ions that have accumulated [4].

Biochemistry Parameters: In leaves, protein content (Table 2) was significantly increased with increasing external applied salinity compared to control plants, however in roots it was increased significantly. Sibole *et al*. [30] reported that the treatment of clover plant (*Medicago citrna* L.) for 30 days with concentrations of NaCl increased soluble protein content in the seedlings, compared with control plants. Kapoor and Srivastava [31] on *Vigna mungo* (L.) supported the previous results. They observed an increase in protein

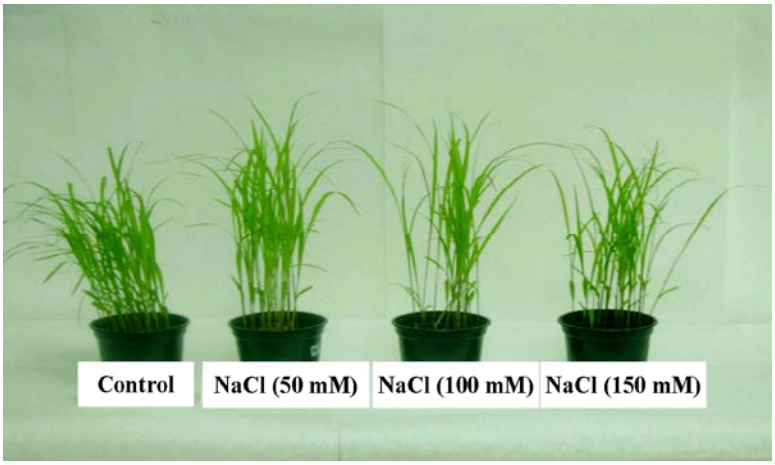

Fig. 1: Effect of salt treatments on growth of sorghum plants.

Table 1: Effect of salinity on length, fresh weight (FW), dry weight (DW) and water content in leaves and roots sorghum.

	Leaves			Roots			Water Content (%)	
	Length (cm)	FW (mg)	DW (mg)	Length (cm)	FW (mg)	DW (mg)	Leaves	Roots
Controal	32±1.63	217.2±17.7	42.3±2.51	29.7±5.8	44.1±5.2	10.8±1.5	80.5±1.9	75.5±3.7
50 mM	36.7±4	224.3±17.2	43±3.56	30.3±5	44.7±2.4	11.2±0.4	80.8±0.7	75±3.3
100 mM	34.5±2.4	221.5±20	43.4±6	30.15±4.4	43.9±1.7	11.4±1.7	80.4±1.92	74±3.6
150 mM	31.7±0.5	215.3±19	43±6	29.5±5.8	44.5±4	11.5±1.8	80±1.9	74.1±3.2

Each value represents the mean of ten leaves or roots with S.D. a, b and c indicate significance at P<0.05, P<0.01 and P<0.001 respectively when compared to control.

Table 2: Effect of NaCl stress on biochemical parameters: Protein (mg g^{-1} FW), Proline (µmol g^{-1} FW), amino acid (AA) (µmol g^{-1} DW) and total soluble sugars (TSS) (µmol g^{-1} DW) in leaves and roots of sorghum.

	Leaves				Roots			
	NaCl (mM)				NaCl (mM)			
	Control	50	100	150	Control	50	100	150
Protein	8.45±0.22	11.33±0.3b	12.14±1.4c	15.3±1c	4.5±0.2	5.3±0.7	5.5±0.6	6.5±0.54b
Proline	2.2±0.17	2.06±0.02	3.16±0.07c	4.73±0.2c	1.9±0.18	1.97±0.02	4.46±0.3c	5.76±1.2c
AA	360±32	442±50	420±30	754±52c	470±30	523±24	720±24c	803±40c
TSS	36.3±4.4	40.63±7.3	41.3±1.8	53.2±6.67C	21.8±4	22.7±1.9	37.48±2.2b	41.8±3.1b

Each value represents the mean of three or four independent observations with S.D. a, b and c indicate significance at P<0.05, P<0.01 and P<0.001 respectively when compared to control.

content with increasing salt concentrations. In many cases, the synthesis of stress-induced proteins is a part of some stress tolerance mechanism [32]. Proteins are known to accumulate in plants grown under saline conditions and they may provide a storage form of nitrogen, which is re-utilized when stress is over [33] and may play a vital role in osmotic adjustment.

Proline content (Table 2) was increased significantly at 100 mM and 150 mM NaCl in both organs, the increase in roots was much greater than in leaves; at the high concentrations (150 mM NaCl); being approximately 2- and 3-fold in leaves and roots, respectively. Proline is one of the most important osmoprotectant in plants. Under salt stress most plant species exhibit a remarkable increase in their proline content [34]. In our experiments (Table 2) also it was observed a similar behaviour in the roots and leaves of sorghum El-haddad *et al* [35] and Khan *et al*. [36] reported an increase in proline content in sorghum leaf and rice roots respectively as the effect of salt stress. Accumulation of proline, as an adaptive response to salt stress, can decrease the water potential and then help maintain water content in leaves [37].

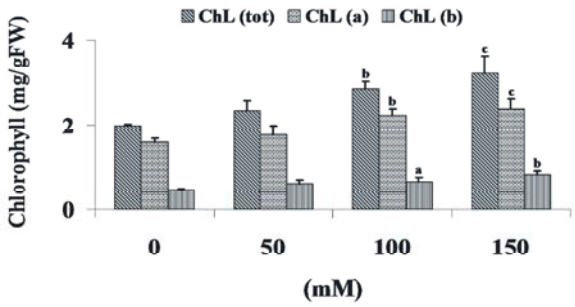

Fig. 2: Effect of NaCl treatments (0, 50, 100 and 150 mM) for 25 days on total chlorophyll, chlorophyll (a) and chlorophyll (b). Each value represents the mean of three or four independent observations with S.D. a, b and c indicate significance at $P<0.05$, $P<0.01$ and $P<0.001$ respectively when compared to control.

In both organs, total free amino acids content (Table 2) was increased with increasing salt concentration. In the leaves the significant increase was observed at 150 mM NaCl; approximately 2 fold. For roots tissues the significant increase was obtained at 100 mM (153%) and 150 mM (171%). These results are in agreement with those obtained by Lacerda *et al* and Abd-El Baki *et al*. [38, 39], who found the highest free amino acid contents in sorghum tolerant genotype and maize, respectively under salt stress. Free amino acids have been mentioned as important compound in osmoregulation in plants under water and salt stresses [40]. Accumulation of this compatible solute reduces osmotic potential in the cytoplasm and contributes to maintaining water homeostasis among several cellular compartments [41].

Salt led to an increase TSS% level in the leaves, but of the way significant only at 150 mM NaCl (Table 2). However in the roots, exogenous NaCl in culture medium induced an increase in TSS, this increase was significant at 100 and 150 mM NaCl; 1.72 fold and 1.92 fold, respectively (Table 2). The tolerant genotype is able to produce proportionally more carbohydrates for the osmotic adjustment and to maintain adequate conditions for growth under saline environment than the sensitive one [38]. El-haddad *et al*. [35] found that soluble sugars increased between 30% to 144% in stressed plants of sorghum as compared to control plants. In others studies, sorghum grown in highly saline soil [42] and maize grown under stress of 100 $molm^{-3}$ NaCl [43] showed 28% and 51%, respectively increased the content of soluble sugars as compared to control.

Chlorophyll content is fundamental to understand the plant response to the environment in which it resides [44]. To know if sorghum plants are damaged in the photosynthetic metabolism as a consequence of salt stress we decided to measure the leaves chlorophyll. Total chlorophyll, chlorophyll a and b were were gradually increased with increasing external NaCl application after 25 days of salt stress (Fig. 2). It is generally known that in salt tolerant species, chlorophyll content increases, whereas it decreases in salt-sensitive species under saline regimes [45, 46]. In view of this, an accumulation of Chl has been proposed as one of the potential biochemical indicators of salt tolerance in different crops, e.g., in pea [47] and sunflower [45]. The increased chlorophyll accumulation in response to abiotic stress could be due to chloroplast development or to increased thylakoid number [48].

Enzymes Antioxidants: In order to analyze the changes of SOD isoenzymes under salt stress, tissue extracts of leaves and roots were subjected to native PAGE. The native gel used, don't contain any denaturing agents and permit to make migrate the native proteins, it will allow surveying the raw state activity and the total molecular weight of the total protein. Six SOD activity bands were identified in leaves and roots tissue extracts sorghum (Fig. 3). In comparison to control of leaves, salt stress induced an increase of SOD isoenzymes 6 and 5, however, induced a significant increase of SOD isoenzymes 5, 4, 3 and 2 in sorghum roots. These results indicated that SOD isoenzymes had a differential sensitivity to NaCl in the leaves and roots and appear more effective in roots. Our results of increase in SOD are in accordance with Gómez *et al*. [49], who found an increase in all SOD isoenzymes of pea chloroplasts following a long-term NaCl treatment. The increase in SOD activity under NaCl-salinity stress suggests better oxidative stress tolerance [50].

H_2O_2, which is toxic and produced by the activity of SOD to prevent cellular damage, must be eliminated by conversion to H_2O in subsequent reactions. CAT and POD regulate H_2O_2 level in plants [51]. Our results showed that in leaves CAT activity was enhanced with increasing external salinity application, while POD activity did not change. Contrarily in roots, CAT activity was increased slowly at 50 mM and 100 mM, while POD activity enhanced significantly in presence of salt treatments (Table 3). It possibly means that the POD is the main H_2O_2-detoxifying enzyme in salt-treated sorghum roots, in accordance with the results obtained for common bean [52], marigold [53] and rice [54] roots exposed to salt.

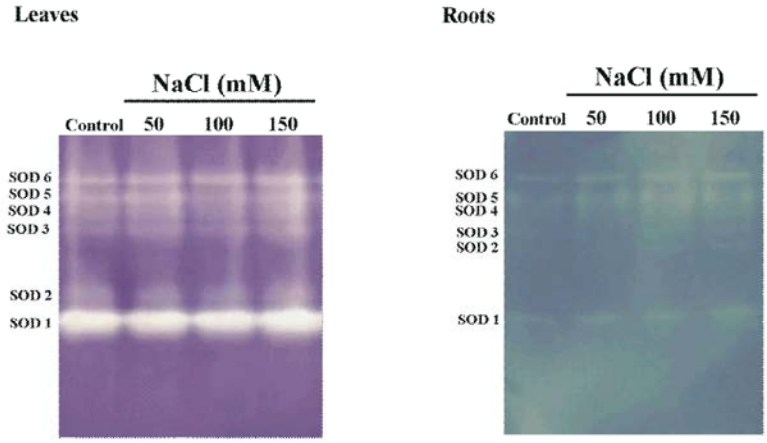

Fig. 3: SOD isoenzymes activities in leaves and roots of control and NaCl treated sorghum plants

Table 3: Activities of Catalase (CAT) (µmol H_2O_2 min^{-1} g^{-1} FW), Gaïacol peroxidase (POD) (µmol min^{-1} g^{-1} FW) and Glutathion reductase (GR) (µmol NADPH min^{-1} g^{-1} FW) in leaves and roots of sorghum subjected to 50 mM, 100 and 150 mM NaCl stress.

	Leaves				Roots			
		NaCl (mM)				NaCl (mM)		
	Control	50	100	150	Control	50	100	150
CAT	5.51±0.6	7.91±0.9b	9.15±0.6c	11.5±0.8c	12.3±0.46	16.2±1.4b	16±0.23b	13.72±1.1
POD	4.83±0.74	6.39±0.82	4.56±0.5	4.85±0.8	111±8	146±5b	125±3.2a	139±12.7b
GR	2.4±0.4	2.47±0.3	3.22±0.4b	4.58±0.4c	11.7±0.5	16.5±0.25b	13.5±0.7a	15.5±1.75b

Each value represents the mean of three or four independent observations with S.D. a, b and c indicate significance at P<0.05, P<0.01 and P<0.001 respectively when compared to control.

However we hypothesized that CAT is to be the most important H_2O_2-scavenging enzyme in leaves, these results were supported by Neto *et al*. [55].

The last enzyme of ascorbate-glutathione cycle, GR, catalyzes the NADPH-dependent reduction of oxidized glutathione. In our study it was observed a significant elevation in the GR activity in leaves and roots of sorghum under salt stress (Table 3). When compared with control, GR activity (Table 3) increased by 34% and 90% in leaves at 100 mM and 150 mM NaCl respectively, however it was significantly increases in roots at all salt treatment Meneguzzo *et al* [56] and Meloni *et al*. [57] also reported higher constitutive GR activity in roots of the tolerant cultivar wheat and pora, respectively. Gómez *et al*. [49] suggested that the induction of GR activity could increase the NADP/NADPH ratio, thereby ensuring the ability of NADP to accept electrons from the photosynthetic electron transport chain and allowing a moderate level of ROS to be maintained in the chloroplasts. GR is involved in maintaining high ratios of GSH/GSSG, which is required for the regeneration of ascorbate. Elevated levels of GSH would be associated with increased in oxidative stress tolerance [57].

Nitrate Reductase: In most plants, nitrate assimilation takes place predominantly in leaves. In accordance, our results show that NR activity (Fig. 4) was higher in leaves than in roots of sorghum. An elevated nitrate reduction in leaves compared to roots was related to the higher NR protein contents [38] and a sufficient availability of light and reducing power [58]. Reports concerning the effect of salinity on nitrate reductase activity are conflicting; the enzyme activity was inhibited by salinity in tomato [59], while increased in ryegrass [60]. However, results of this investigation showed that activities of nitrate reductase in roots and leaves no changed significantly en presence of salt stress (Fig. 4). These contrasting behaviours for the NR activity response to salinity stress may be linked to distinct approach, genetic variation and, mainly, changes in the intensity of NO_3- flux from roots to leaves [61]. Thus, in our study, we assume that the NO_3- flux from roots to leaves is predominantly no affected by NaCl stress.

Glutamine Synthetase: GS is the key enzyme involved in assimilation of inorganic nitrogen into organic forms. It catalyzes the ATP-dependent condensation of

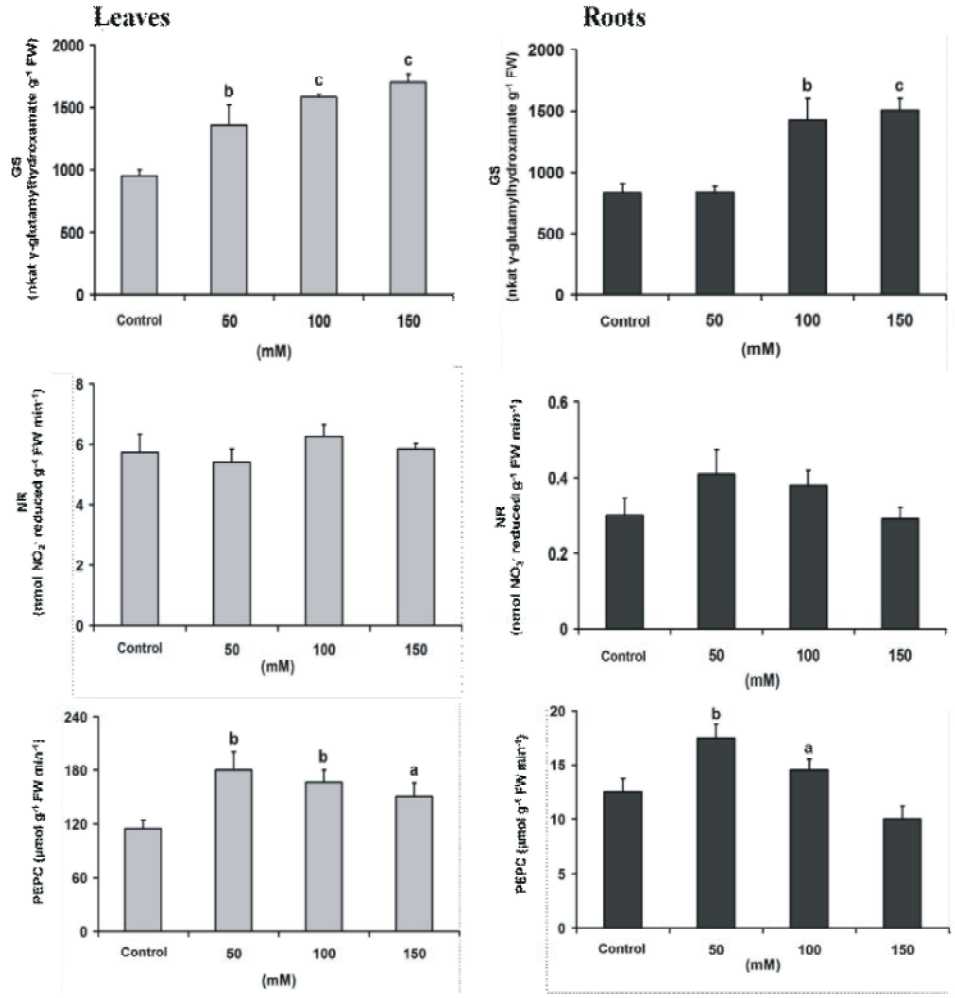

Fig. 4: Effect of NaCl treatments (0, 50, 100 and 150 mM) on Glutamine synthetase (GS) Nitrate reductase (NR) and Phosphoenolpyruvate carboxylase (PEPC) activities in leaves and roots sorghum. Each value represents the mean of three or four independent observations with S.D. a, b and c indicate significance at $P<0.05$, $P<0.01$ and $P<0.001$ respectively when compared to control.

ammonium with glutamate to yield glutamine, which then provides nitrogen groups for the biosynthesis of all nitrogenous compounds in the plant. Two different classes of GS have been identified in angiosperms: GS1 in the cytosol and GS2 in the chloroplasts [62]. GS1 is the predominant isoforme in roots and other non-photosynthetic tissues and is encoded by a small multigene family. In the present study we observed increased activity of GS in roots and leaves sorghum under salt treatments with a maximum value at 150 mM NaCl in both organs (Fig. 4). The salinity has been shown to stimulate GS activity, suggesting that the GS could play a key role in supplying glutamate for proline synthesis in plants growing under salt stress [63]. It was reported that proline accumulation was due, at least in part, to the increased GS activity under salt stress in the cashew [63]. Brugiere *et al*. [64] working with tobacco transgenic plants exposed to NaCl-stress suggested that GS plays a major role in regulating the proline production.

Phosphoenolpyruvate Carboxylase: In the present study it was observed that PEPC activity in leaves was significantly increased by all salt treatments with maximum at 50 mM (50%) (Fig. 4). Roots PEPC activity was not changed at 150 mM NaCl, but was significantly increased at 50 mM and 100 mM NaCl by 40% and 23%, respectively. The induction of PEPC under saline treatments was reported in leaves of CAM plants [65]. Sánchez *et al*. [66] have reported that the Atppc1 and Atppc3 transcripts of Arabidopsis phosphoenolpyruvate

carboxylase increased transiently in response to salt. [67] have been suggest that the activity important of the PEPC in presence of NaCl can be interpreted as a process permitting to counterbalance the excessive penetration of cations inorganic by organic acids essentially the malic acid required to control the pH-stat. In more we propose that PEPC plays a role in supplying glutamate for proline synthesis by provides the carbon skeletons via citric acide cycle intermediates under salt stress.

CONCLUSION

In conclusion, the results of the present study showed that the leaves and roots of the sorghum plants have developed physiological and biochemical strategies to tolerate NaCl stress. This plants exhibited higher adaptive potential under salinity stress by antioxidant defence, accumulation of osmoprotectants and efficient coordination between photosynthetic carbon and nitrogen assimilation.

More work is required for elucidating the roles of key enzymes that might be involved in the nitrogen and carbon assimilation pathways.

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