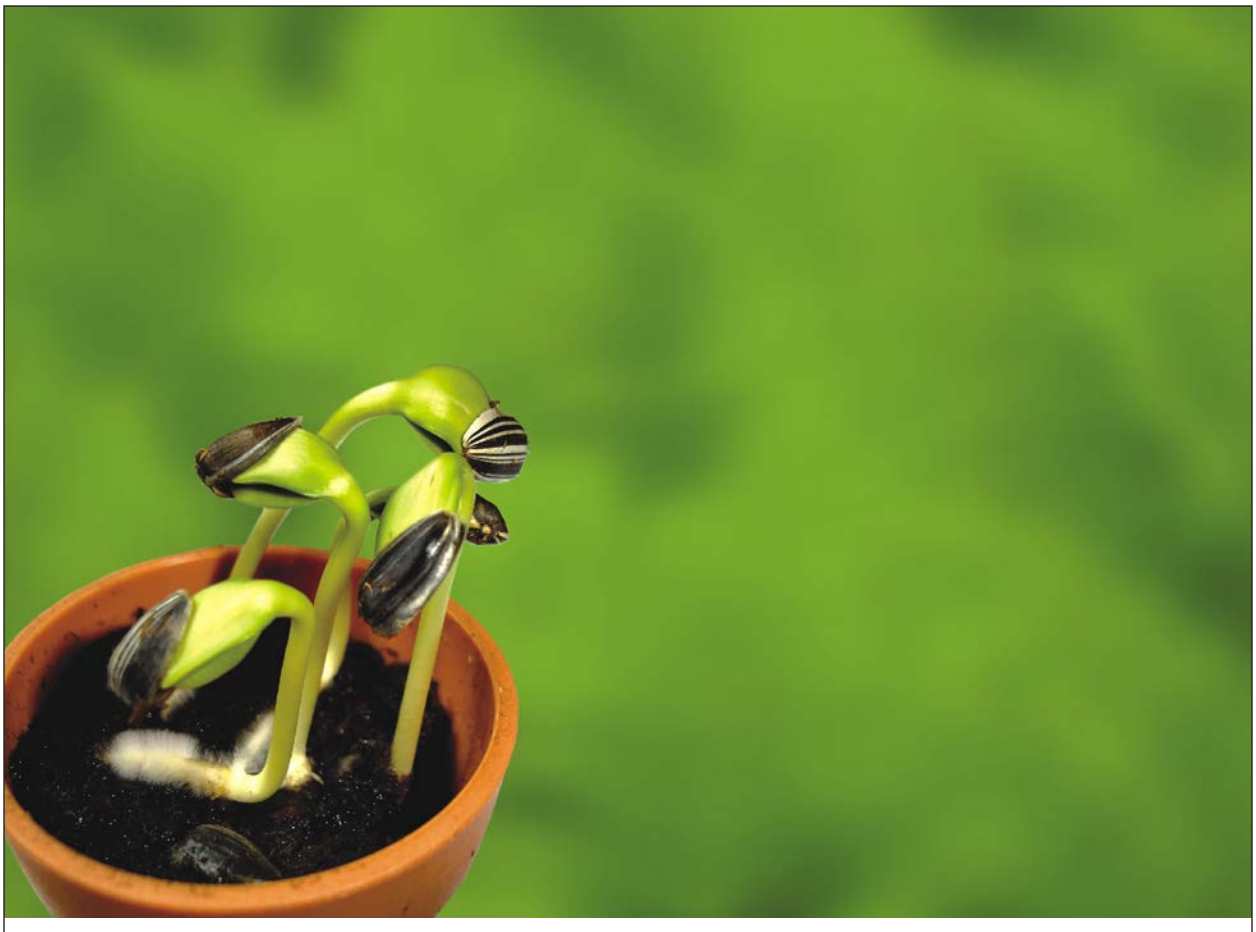

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Antioxidant-Enzyme System as Selection Criteria for Salt Tolerance in Forage Sorghum Genotypes (*Sorghum bicolor* L. Moench)

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Abstract: The involvement of antioxidant enzyme activities in mitigating the damage of NaCl stress was studied in 26 genotypes of forage sorghum exhibiting different responses to salinity, including a local hybrid with unknown performance under salinity stress. The 2 week old sorghum seedlings were subjected to 0, 50 and 100 mM NaCl for 4 weeks, which correspond to 0.7, 8.2 and 15.11 dS m^{-1} salinity levels. Plants were sampled for enzyme analyses and dry weight determinations. Salt stress resulted in significant reduction of dry weight of both tolerant and sensitive genotypes. The reduction was stronger in the later group compared with the former one at 8.2 dS m^{-1}. In contrast, at the highest salinity level, there was severe reduction in plant dry weights, meanwhile the highest value was recorded by the local genotype. Five out of the 21 salt tolerant genotypes and the local hybrid produced the highest dry weights at 50 and 100 mM NaCl. The effect of salinity levels on antioxidant enzymes and lipid peroxidation was examined. Both salinity levels induced significant increase in superoxide dismutase (SOD) activity, glutathione (GSH) levels and carotenoid concentrations in all tolerant genotypes and the local genotype compared to sensitive group. Moreover, the activities of peroxidase and Glutathione Reductase (GR) have increased at 8.2 dS m^{-1} NaCl for most of tolerant genotypes, then the activity was declined at 15.11 dS m^{-1} salinity level for the second enzyme and was somehow constant for the first enzyme. There was a common trend in increasing lipid peroxidation activity for sensitive genotypes at both levels of stresses and reducing the activity for some tolerant genotypes. Five salt tolerant genotypes and the local hybrid maintained beside high SOD, GSH, GR activities, reasonable lipid peroxidation and pigment contents. It could be concluded that the local genotype could be considered as salinity tolerant genotype as it exhibited the same trend of tolerant genotypes. Moreover, antioxidant system SOD, GSH, GR, ASPX and carotenoids could be considered as selection criteria for salt tolerance in sorghum species.

Key words: Antioxidant enzymes, oxidative stress, salinity, *Sorghum bicolor* L.

INTRODUCTION

Sorghum (*Sorghum bicolor* L.) is one of the stable crops grown in arid and semi-arid countries. It is the fifth most important cereal crops grown on 44 million ha in 99 countries in Africa, Asia and the Americans. The majority of sorghum plantings are concentrated in poor countries where it constitutes a valuable source of grains for human consumption. In addition, posses high nutritional source of animal feeding.

Sorghum exhibit excellent tolerance and yield potential to environmental stresses such as water shortage and salinity compared to millet (Boursier and Läuchli, 1990). In such regions, salinity is impose a limiting factor for crop production, where osmotic stress, ion toxicity and mineral deficiencies

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are all considered as consequence of the effect of salt stress on plant growth and performance. Abiotic stresses lead to oxidative stress through increase in the production of Reactive Oxygen Species (ROS). These species are toxic and cause damage to DNA, proteins, lipids, chlorophyll and almost every other organic constituent of the living cells (Imlay and Linn, 1988).

In this regard, there are many important adaptive mechanisms that plants use to cope with the adverse effects of salinity. Synthesis of compatible solutes such as: amino acid (proline), sugar alcohols (mannitol) and quaternary ammonium (glycinebetaine) that retain water within cells to combat from dehydration is one of these mechanisms (Nuccio *et al*., 1999). Lacerda *et al*. (2003, 2005) subjected the seedlings of two forage sorghum to 0 and 100 mM of NaCl and suggested that proline accumulation is an expression of the plant reaction to the stress damage and not a salt tolerance factors. On the other hand, Mickelbart *et al*. (1999) stated the role of glycinebetaine (GB) under a variety of unfavorable conditions. It has been shown that high concentration alleviate salt-induced destabilization of DNA helices and maintain the activity of enzymes when plants experiencing extremes of pH, high temperature and salt concentration.

Athor mechanism plants use to alleviate the effect of oxidative stress is evolving antioxidant systems such as: Superoxide Dismutase (SOD), Catalase (CAT), Ascorbate Peroxidase (ASPX), Glutathione Reductase (GR) (Alscher *et al*., 1997; Apel and Hirt, 2004). The response of field crops to salt stress through increasing activity of responsible enzymes were studied in cotton (Gosset *et al*., 1994), wheat (Sairam *et al*., 2002), rice (Vaidyanathan *et al*., 2003), sugar beet (Bor *et al*., 2003) and maize (Azevedo Neto *et al*., 2005). Most of the previous studies confirmed a correlation between salinity tolerance and the activity of the enzymes. On the other hand, results presented by Costa *et al*. (2005), on two forage genotypes of sorghum differ in their salt tolerance disagreed with previous data. Lipid peroxidation activity in leaves showed no difference between salt-sensitive and salt tolerant genotypes, this in turn may suggested that lipid peroxidation is not a useful marker for salt tolerance discrimination for all plant species.

Many valuable literatures are published on ion accumulation and compartmentation in salt-stressed grain and forage sorghum (Lacerda *et al*., 2005; Netondo *et al*., 2004; Sunseri *et al*., 1998).

Abundant information are reported on the capacity of antioxidant systems in conferring tolerance to salinity in many field crops, only one reference (Costa *et al*., 2005), presented a detailed study on the pattern of activity of most related antioxidant enzymes in this species.

Identification of salt-tolerant lines to design a breeding programs aim at tolerance for stress environments is a great challenge because of defining the suitable criterion associated with the stress and the complexity of the inheritance to stress environments as well. It is crucial to characterize and identify those criteria and determine their relative importance and contribution to the imposed stress. Then, characterization of the individuals under investigation in relation to these criteria is the next step to study the inheritance of these traits.

The present study was performed to understand the activity of antioxidant enzymes and their role in protecting against salt-induces oxidative damage in 26 forage sorghum genotypes during seedling stage. The experiment is a preliminary study to screen the genetic materials based on the activity of the anti-oxidant enzymes and select the promising genotypes for crossing and hybrid evaluation.

MATERIALS AND METHODS

Plant Materials, Salinity Treatments and Growth Conditions

The present study was conducted for 2 summer seasons, 2005 and 2006 under greenhouse conditions where no light or temperature supplements. Twenty five genotypes of forage sorghum were introduced from ICRISAT (International Crops Research Institute for the Semi-Arid Tropics, India) and used for this investigation, in addition to one local genotype (hybrid 101). The exotic materials

consist of 21 salt-tolerant and 4-salt susceptible control lines as certified by ICRISAT. No information is available on the performance of the local hybrid under salinity stress. Table 1 shows origin and classification of the studied germplasm based on ICRISAT notification.

Five seeds from each genotype were planted in plastic pot filled with a mixture of sand, peatmoss and vermiculite (1:1:1). Pots were watered regularly using the irrigation source for 14 days, then thinned to 3 plants per pot and salt treatments were applied. Salinization was induced by adding sodium chloride to the one-fourth strength commercial nutrient solution free from Na^+ and Cl^- salts in three concentrations; control (0), 50 mM and 100 mM NaCl L^{-1}. These corresponded to electrical conductivities of 0.7, 8.2 and 15.11 dS m^{-1}. The electric conductivity of the soil was estimated after the experiment termination in 1:1 soil extract and found to be: 0.86, 5.12 and 13.23 dS m^{-1} for the three NaCl levels, respectively. Salt treatments lasted for 4 weeks and then the experiment was ended.

Dry weight of plants was determined 4 weeks from the start of salt treatments, by uprooting fresh plants, drying at 70°C for 48 h and weighed.

For enzyme assays, samples were taken 6 weeks after planting and 4 weeks from application of stress treatments, the following enzymes and pigments were determined.

Enzyme Extraction

Enzyme extracts were prepared by homogenizing plant tissue in a pre-chilled mortar in 20 mL chilled extraction buffer (pH 7.5). Extracts were then centrifuged at 6000 rpm for 20 min at 5°C. Enzyme assays were conducted immediately following extraction.

Super Oxide dismutase (SOD) was measured by the photochemical method described by Giannopolitis and Ries (1977). Assays were carried out under illumination. One unit of SOD activity was defined as the amount of enzyme required to cause 50% inhibition of the rate of ρ-nitro blue tetrazolium chloride reduction at 560 nm.

Table 1: Origin and classification of 26 forage sorghum genotypes according to their salt tolerance

Genotypes	Origin	Classification*
1	ICSV 93046	T
2	ICSV 745	T
3	SP 47513	T
4	SP 39262	T
5	SP 47529	T
6	S 35	T
7	ICSV 93048	T
8	ICSV 112	T
9	SP 47519	T
10	SP 39105	T
11	ICSR 93034	T
12	SP 39053	T
13	SP 40567	T
14	SP 47503	T
15	SP 39007	T
16	ICSR 170	T
17	A 2267-2	T
18	ICSB 707	T
19	ICSV 96020	T
20	NTJ 2	T
21	GD 65008	T
22	ICSB 406	S
23	ICSB 676	S
24	ICSV 21029	S
25	SP 36257	S
26	Hybrid 101	Local

*: T: Tolerant, S: Susceptible

Peroxidase activity (Per) was determined by following the dehydrogenation of guaicol at 436 nm (Malik and Singh, 1980).

Catalase (CAT) activity was assayed in a method following Aebi (1983). Activity was determined by following the decomposition of H_2O_2 at 240 nm.

Ascorbate peroxidase (ASPX) activity was determined using the method of Nakano and Asada (1987). Activity was determined by following the H_2O_2-dependent decomposition of ascorbate at 290 nm.

Glutathione Reductase (GR) activity was determined as the oxidation of NADPH at 340 nm (extinction coefficient 6.2 mM cm^{-1}) according to Donahue *et al*. (1997). Leaf samples (1 g) were homogenized in 5 mL phosphate buffer (pH 7.6), 2 mM EDTA. The homogenate was centrifuged at 15,000 g for 10 min and supernatant was used for analyses. The assay mixture contained 0.1 mM buffer (pH 7.6), 2 mM EDTA, 50 nicotinamioleaolenine-dinucleotide phosphate (NADPH), 0.5 mM glutathione oxidised (GSSG) and 500 μL of the extract. The reaction was initiated by addition of NADPH and followed for 5 min at 25°C.

Total glutathione content (GSH) was determined spectrophotometrically following the method described by Griffith (1980).

Lipid peroxidation (MDA) was assayed spectrophotometrically using TBA-MDA assay. Lipid peroxides were extracted with 5 mL of 5% (w/v) metaphosphoric acid and 100 μL of 2% (w/v in ethanol) butyle hydroxytoluene. An aliquot of the supernatant was reacted with thiobarbituric acid 95°C and cooled to room temperature. The resulting thiobarbituric acid malondialdehyde adduct was extracted with 1-butanol (Hodges *et al*., 1999).

Determination of Pigments

The pigments were extracted in 80% chilled acetone. The amount of total Chl a and b and carotenoids were estimated spectrophotometrically according to Lichtenthaler (1987).

Statistical Analysis

The experimental design was split-plot with four replicates per treatment and 3 plants/replicate. Main plots represented salt concentrations, whereas the 26 sorghum genotypes constituted the split-plots. Data were combined over the two seasons for statistical analyses.

For each enzyme assays, three replicate extracts were used per treatment. All statistical analyses and Least Significant Differences (LSD) were performed by GenStat Software statistical Program, release 4.24.

RESULTS

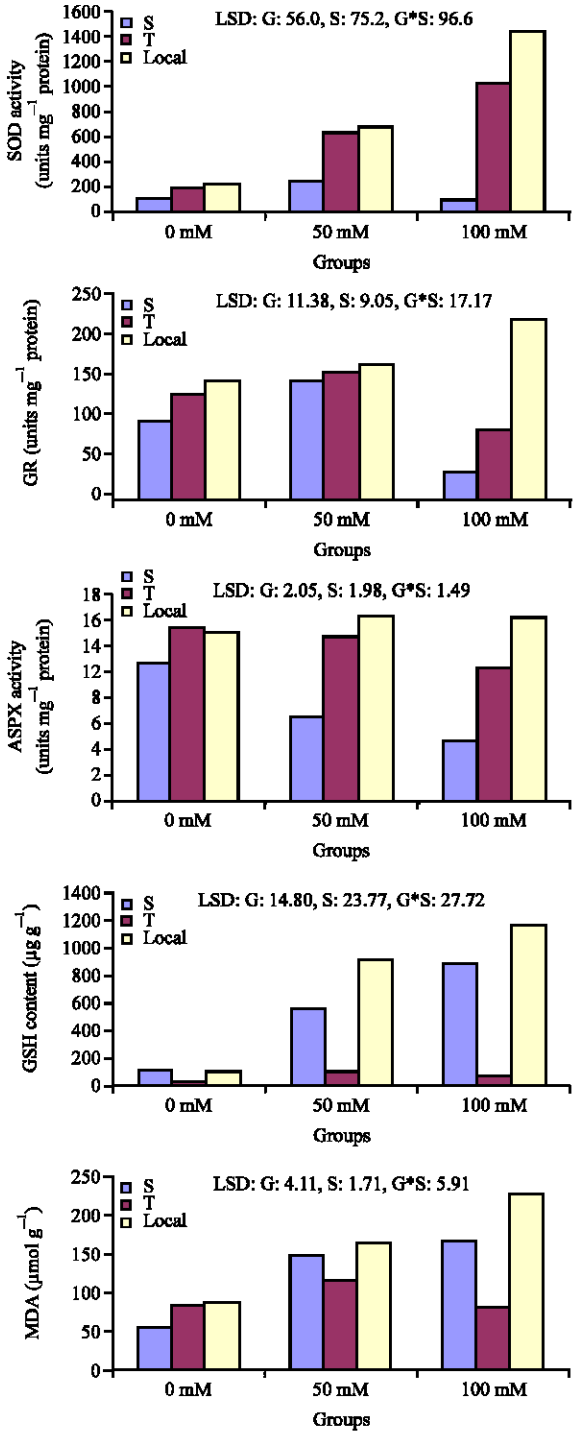
Analysis of variance revealed significant effect of genotypes, salinity levels and the interaction between both factors on plant dry weight. Growth of sorghum plants was sensitive to salinity, as indicated by the reduction of dry weight of all genotypes compared to control treatment. The reduction was severe at 100 mM NaCl since tolerant, sensitive and local genotypes were strongly affected, although the local genotype showed the highest dry weight $plant^{-1}$ (Table 2). The tolerant genotype, 1 and the sensitive genotypes, 23 and 25 had a dry weight record above 1.00 g and presented the least reduction at 100 mM salinity concentration. The reduction percent ranged from 29.4-59.0% for the abovementioned genotypes. At 50 mM, the reduction in dry weight was less compared to the highest salinity level, the majority of tolerant genotypes maintained high dry weight relative to sensitive group. The genotypes; 11, 16, 3, 12, 20 and 26 maintained high dry weight at 50 mM.

Table 2: Response of plant dry weight (g plant^{-1}) averaged over two seasons for 26 genotypes of forage sorghum to increasing NaCl concentration during seedling stage

	NaCl concentrations (mM)			
Genotypes	0	50	100	Mean
1	5.22	2.62	1.62	3.15
2	3.34	2.59	0.49	2.14
3	3.07	3.90	0.54	2.50
4	3.06	2.89	0.52	2.16
5	4.24	1.96	0.39	2.20
6	3.31	2.12	0.65	2.03
7	2.25	2.49	0.59	1.78
8	2.22	2.02	0.56	1.60
9	3.65	2.46	0.53	2.21
19	5.67	2.55	0.59	2.94
11	9.50	4.99	0.73	5.07
12	5.10	3.78	0.48	3.12
13	4.81	2.50	0.84	2.72
14	4.66	2.48	0.56	2.57
15	4.45	3.01	0.51	2.66
16	4.20	4.09	0.49	2.93
17	3.60	2.75	0.82	2.39
18	2.91	1.93	0.72	1.85
19	2.16	1.74	0.27	1.39
20	6.07	3.34	1.13	3.51
21	3.43	2.15	0.70	2.09
22	2.71	1.11	0.51	1.44
23	2.54	1.87	1.32	1.91
24	3.86	2.04	0.55	2.15
25	3.00	0.95	1.23	1.73
26	6.46	3.32	2.15	3.98
Mean	4.06	2.60	0.75	
LSD (0.05)				
Salt levels (S)	0.13			
Genotypes (G)	0.31			
S × G	0.53			

The activities of anti-oxidant enzymes were statistically analyzed for sorghum plants at different salinity levels and presented as group mean values (T, S and Local) and means of individual genotypes. The profile of enzymes activities has differed among the tested groups at control treatment, indicating genetic differences for the enzyme contents in this species. On the other hand, when the plants were subjected to NaCl, salinity induced oxidative stress in plant tissues and the groups were differentiated accordingly. The mean activities, as shown in Fig. 1, of the enzymes; SOD, GR, ASPX and GSH were increased markedly in Tolerant group (T). The activities of SOD and GSH levels were increased in both tolerant and sensitive groups at 50 mM, then decreased in S group at higher salinity, whereas increased in T group. On the other hand, GR and ASPX activities were increased in both groups up to 50 mM and then declined for both groups at 100 mM. The increasing in activity was 345.67, 120.10, 94.6 and 503.82% for the previous enzymes in tolerant group in response to 50 mM NaCl compared to control treatment. The sensitive group, in contrast, exhibited the lowest activities for the same enzymes at the same salinity level (229.69, 148.6, 61.0 and 286.78%, respectively). Interestingly, the local genotype surpassed the tolerant group in the mean activity of these enzymes at 100 mM, but showed close values for GR activity at 50 mM relative to control treatment (114.60%).

Lipid peroxidation, assessed through malondialdehyde (MDA) content, was higher in plants from sensitive group comparing with plants of tolerant group when salt-stressed and with elevating salinity



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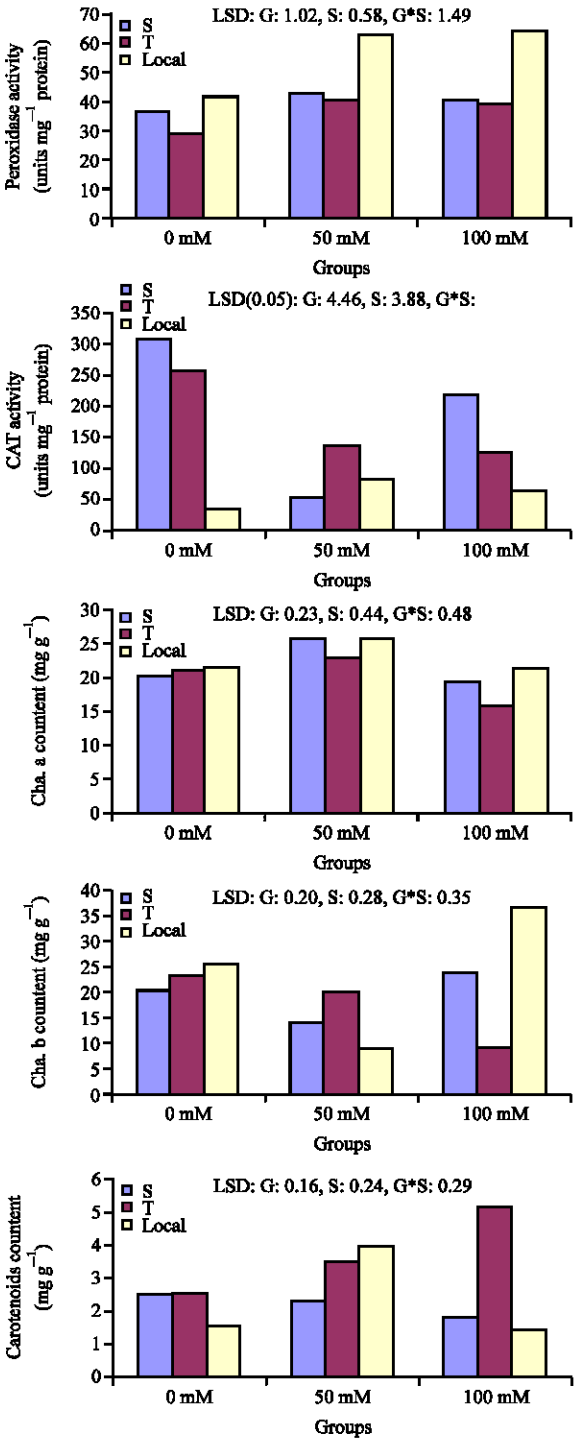

Fig. 1: Activity of antioxidant enzymes measured in seedlings of 26 forage sorghum genotypes, irrigated with 0, 50 and 100 mM NaCl. The assayed enzymes are: SOD, GR, ASPX, GSH level, MDA content, Peroxidase and CAT. The determined pigments are: chl. a, chl. b and carotenoid contents. T, S and local represents: tolerant, sensitive and local groups, respectively

level (Fig. 1). The MDA contents decreased for the tolerant group at 100 mM to values equal to control treatment. Increase in MDA content was 279.47, 139.4 and 188.24% for the sensitive, tolerant and local groups at 50 mM NaCl, respectively.

Chlorophyll a content (Fig. 1) was increased due to salinity effect for all the three groups at 50 mM and then decreased at 100 mM. Carotenoid contents, in contrast, increased with increasing salinity stress for T at both levels of stress (Fig. 1), in contrast, sensitive group showed some decline in contents toward stress intensity. The local genotype still has the highest content of pigments at 50 mM salinity concentration (Fig. 1).

The pattern of peroxidase and CAT activity values (Fig. 1) were different from other enzymes. Both T and S groups showed slight increase in the activity of Peroxidase when exposed to both levels of salinity relative to unstressed treatment. Whereas the local genotype, hybrid 101, showed the highest activity at all levels of salinity. The view of CAT activity was differed from other enzymes, it showed reduced activity at 50 mM for all groups compared to control treatment, then the activity was increased at 100 mM but still lower than unstressed condition. At 50 mM NaCl the plants of T group demonstrated the highest activity, followed by the local genotype. In contrast, the mean activity of the sensitive group has exceeded those of tolerant and local genotypes at the highest level of salinization.

Data on the activities of different anti-oxidant systems of individual genotypes treated with different concentrations of NaCl are presented in Fig. 2 and 3. The enzymes activities were affected significantly by the genotypes, salt application levels and the interaction of both factors. At both levels of NaCl (Fig. 2) the genotypes from the tolerant group: 20, 12, 17, 21, 18, 15 and 5 showed the highest SOD activity. The local genotype showed close value of SOD activity to genotypes from T group at 100 mM. All tested genotypes showed reduction in ASPX activity (Fig. 2), except, 19 and 4 which recorded the highest values at 50 mM. On the other hand, the genotypes 4, 17 and 18 maintained high and close values by increasing NaCl levels when compared with other genotypes. There was significant increase in GR activity for the genotypes: 19 and 25 at 50 mM NaCl, in contrast other genotypes showed weak activities at both levels of stress (Fig. 2). The genotypes: 15, 13, 18, 21, 20, 26 and 19 were apparently distinguished from other genotypes for its high GSH content at 50 mM (Fig. 2), however the highest salinity level caused clear increase in GSH production for the same genotypes but lower than 50 mM concentration. The activity of CAT was low for all genotypes under stress conditions, although its activity was somewhat high under control (Fig. 2). The only three genotypes: 1, 19 and 18 exhibited the highest activity at both levels of stress. Lipid peroxidation, measured in the form of its degradative product malondialdehyde, increased under salt stress. There was a general tendency in the direction of increasing MDA content under stress conditions for all tested genotypes (Fig. 3). There was a constant increase in MDA contents of S genotypes at both levels of NaCl, in contrast T genotypes fluctuated in these contents. The lowest content was observed in the genotypes: 11, 21, 13 and 14 at both NaCl concentrations. Exposing plants to NaCl decreased peroxidase activity in tested genotypes, furthermore, only three genotypes: 25 (sensitive group) and 8, 18 and 3 (tolerant group) and the local one demonstrated high peroxidase activity under salt conditions (Fig. 3). In general, carotenoids showed increased contents at 100 mM compared to control and the lowest level of stress (Fig. 3), in contrast were Chl. a and b which represented reduction with increasing NaCl level. There was much reduction in Chl. a pigment for the majority of genotypes as a result of stress except for: 16, 18, 17, 19, 20, 26, 22, 23 and 24 which gave high contents at 50 mM, whereas at the highest concentration the Chlorophyll content was adversely affected in all genotypes with no exception (Fig. 3).

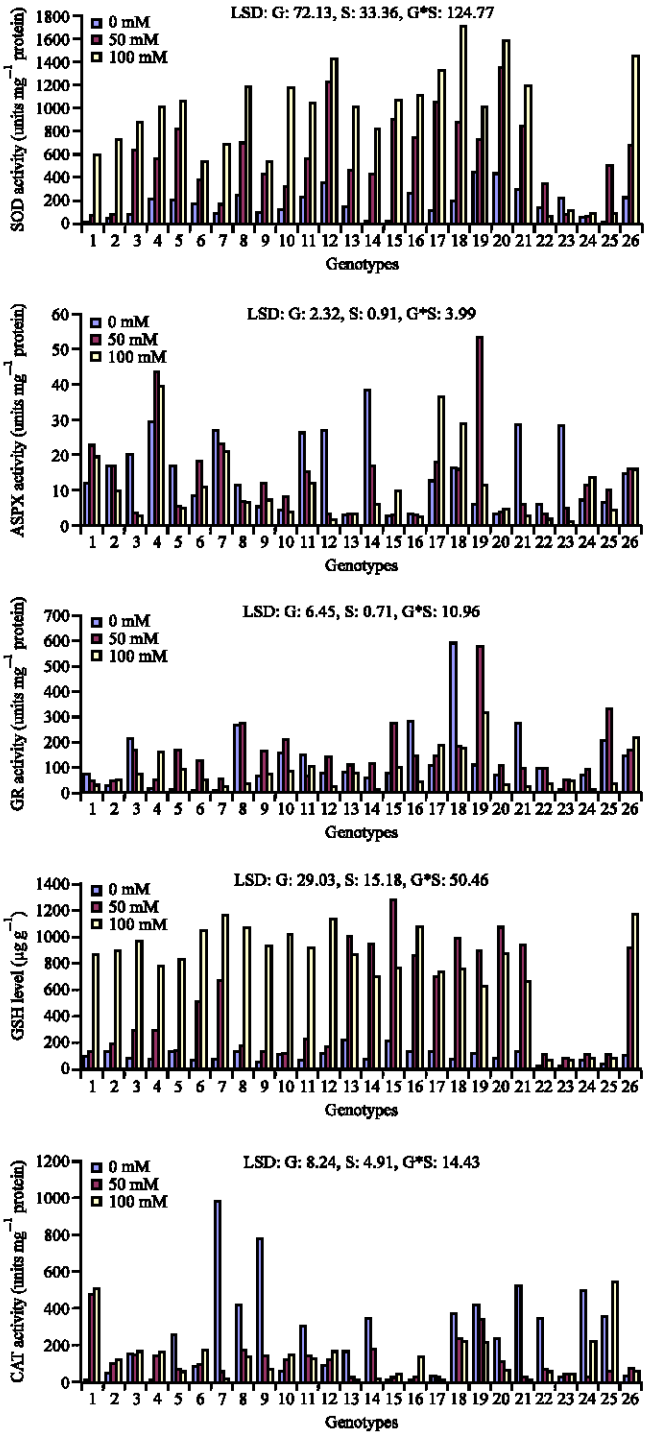

Fig. 2: Activity of antioxidant enzymes measured in seedlings of 26 forage sorghum genotypes, irrigated with 0, 50 and 100 mM NaCl. The assayed enzymes are: SOD, ASPX, GR, CAT and GSH level

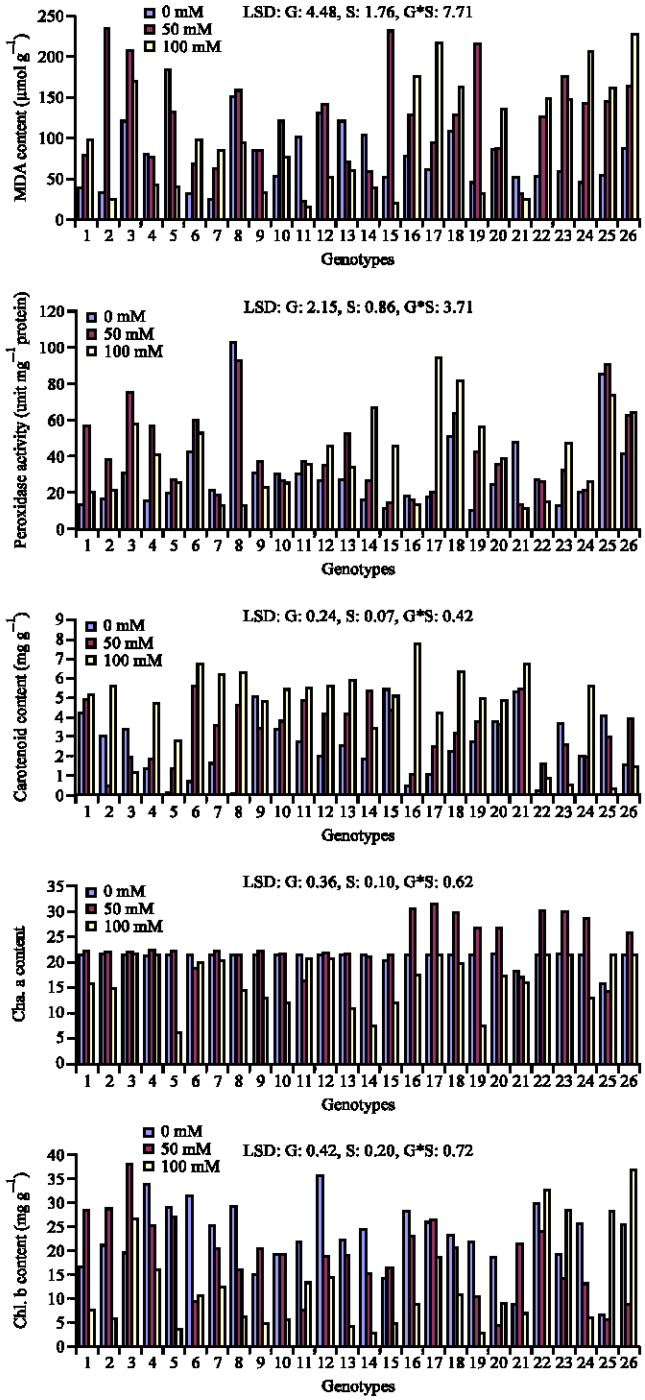

Fig. 3: Activity of antioxidant enzymes and pigment contents measured in seedlings of 26 forage sorghum genotypes, irrigated with 0, 50 and 100 mM NaCl. The determined enzymes are: MDA, Peroxidase, in addition to carotenoids, chl. a and chl. b pigments

DISCUSSION

The sensitivity of crop growth to environmental stresses is well-recognized and investigated by many researchers. In the present study growth of sorghum genotypes was severely affected by increasing salinization, as indicated by the reduction in dry weight of plants. The reduction percent reached a maximum values of 68 and 90% at 50 (8.2 dS m^{-1}) and 100 mM (15.11 dS m^{-1}), respectively. According to Maas (1990) sorghum is classified as moderately tolerant with threshold of 6.8 dS m^{-1} and slope percentage of 16 dS m^{-1}. Similar data were obtained by Netondo *et al*. (2004) who recorded a reduction of 75 and 53% in stem dry weight for two sorghum genotypes exposed to 15.01 dS m^{-1} salinity level. They commented that, salinity increases energy cost and carbon gain and reduce photosynthetic rates/unit leaf area.

The effects of various environmental stresses on plants are known to be mediated, at least partially, by an enhanced generation of ROS (Able *et al*., 2003; Herna`ndez *et al*., 2001). Salinity causes oxidative stress by inhibition the CO_2 assimilation, exposing chloroplasts to excessive excitation energy, which in turn increases the generation of ROS from triplet chlorophyll (Asada, 1994; Gosset *et al*., 1994). As soon as the carbon fixation inside chloroplasts decreases, there is also a lower NADP availability to accept electrons from PSI, thus initiating O_2 reduction resulting in the ROS generation (Sudhakar *et al*., 2001). In addition, considering the fact that Cl^- is involved in electron flux during the H_2O oxidation, the Cl^- toxicity is likely to disrupt the normal electron flow to PSII, which in turn leads to excess electron leakage and increased production of ROS (Gosset *et al*., 1994). Plants with higher levels of antioxidants, whether constitutive or induced, have been reported to posses greater resistance to different types of environmental stress conditions (Dionisio-Sese and Tobita, 1998; Young and Jung, 1999).

There are different arrays of mechanisms that plant breeders use to study the adaptive response of different genotypes and as criteria for selection under unfavorable conditions. Among those, is generation of reactive oxygen intermediate scavenging systems (ROS) (Able *et al*., 2003). The significant increase in the anti-oxidative enzymes: SOD, GR, ASPX and GSH at 50 mM for the three groups indicate the role of those enzymes in protection and tolerance against salinity damage at this level and genetic differences for enzyme production. Further increase in stress resulted in increasing activity of SOD and GSH level for the tolerant group and the local one.

The general comparison of the examined antioxidants in sorghum genotypes revealed that SOD activity and GSH levels were significantly higher under the two salinity levels when compared to control plants. The increase in these two parameters was highly pronounced in tolerant genotypes and local genotypes than in sensitive genotypes.

The diverse responses of the SOD enzyme activities in the plants subjected to saline conditions suggest that oxidative stress is an important component of salt stress. SOD is reported to play an important role in cellular defense against oxidative stress, as its activity directly modulates the amount of O_2^- and H_2O_2, the two substrates of the metal-catalyzed site-specific Haber-Weiss reaction resulting in generation of the high-reactive OH (Sudhakar *et al*., 2001). The higher SOD level observed in tolerant and local sorghum genotypes could be considered as an advantage that allows the plants to resist the potential oxidative damages. These results are in good agreement with that obtained by Acar *et al*. (2001) and Bor *et al*. (2003), who found a higher constitutive and induced level of SOD activity in more tolerant barley and sugar beet cultivars under drought and salt stresses.

Glutathione is widely used as a marker of oxidative stress to plants, although its part in plant metabolism is a multifaceted one (Grill *et al*., 2001). As it is a nonprotein sulphur-containing tripeptide, glutathione acts as a storage and transport form of reduced sulphur. Glutathione is related to the sequestration of xenobiotics and heavy metals and is also an essential component of the cellular antioxidative defence system, which keeps Reactive Oxygen Species (ROS) under control (Noctor and

Foyer, 1998). Antioxidative defense and redox reactions play a central role in the acclimation of plants to their environment, which made glutathione a suitable candidate as a stress marker. In view of the stress-response concept of the glutathione system, higher concentrations of glutathione would confer better antioxidative protection and would be considered as an acclimation. An increase in the GSH/GSSG ratio (more reduced would indicate an overcompensation by intensified recycling of glutathione to keep it in its active, reduced form.

The present study revealed also that GR activity increased significantly in most tolerant and local genotypes at 50 mM NaCl. The highest NaCl concentration, however, induced a fluctuating response of the two enzymes activity in all genotypes under investigation. The role of GR and glutathione in the H_2O_2 scavenging in plant cells has been well established in Halliwell-Asada pathway (Bray *et al*., 2000). GR catalyses the last rate-limiting step of the ascorbate-glutathione cycle. This enzyme maintained high ratio of GSH/GSSG which is required for the regeneration of ascorbate and for the activation of several chloroplastic CO_2-fixing enzymes. The GSH and GR action suggests that the more active ascorbate-glutathione cycle may be related to the development of relatively higher salt tolerance in sorghum.

The results for CAT and peroxidase activities were varied and did not follow the other enzymes pattern, the mean of CAT activity decreased at 50 mM NaCl for all groups, then increased for sensitive group at 100 mM concentration. Peroxidase did not discriminate both groups from each other as the values were nearly unchanged, but the local genotype was distinguished by possessing the highest activity. Both CAT and peroxidase are not considered a distinguished marker for selection for salinity in the present materials of sorghum. Its activity was generally low and only three genotypes namely: 1, 19 and 18 recorded the highest activity at both salinity levels, although the values are low. Costa *et al*. (2005), found increase in CAT activity when sorghum plants subjected to 75 mM NaCl and the increase was more conspicuous in tolerant than in sensitive genotype. Sairam and Srivastava (2001) stated that scavenging of H_2O_2 as represented by GR and CAT is limited and less efficient in susceptible wheat genotypes leading to higher H_2O_2 accumulation and increasing in lipid peroxidation under water limited environments. ASPX uses ascorbate as the electron donor for the H_2O_2 reduction and is known to be a major enzyme in the detoxification of H_2O_2 (Asada, 1992; Sairam and Saxena, 2000). The increase in ASPX activity (at 50 mM NaCl) observed in the present study was in agreement with gradual application of salinity. The increase in enzyme activity may be due to increasing the synthesis of the enzyme or an increased activation of constitutive enzyme pool. An increase in the transcription of genes involved in the synthesis of various stress metabolites, including antioxidant enzymes, has been reported (Scandalios, 1994). Malondialdehyde as the main decomposition product of polyunsaturated fatty acids in biomembranes is known to show greater accumulation under salt stress (Gosset *et al*., 1994; Meloni *et al*., 2003; Sudhakar *et al*., 2001). Such results are consistent with those in present investigation. A significant increase in the malondialdehyde level (used as an indicator of the extent of membrane damage) was observed in all sensitive genotypes and the local genotype. In contrast there was a progressive increase in MDA content in tolerant group. The study of Sairam and Srivastava (2001) conducted on wheat cultivars revealed a lower MDA content in tolerant genotypes compared to susceptible one under water stress conditions. As a result of the greater antioxidant defense in tolerant sorghum genotypes, the malondialdehyde content did not raise the high level that of sensitive genotypes. It is presumably due to the high constitutive level of the antioxidant enzymes activities in tolerant genotypes which is sufficient to avoid a substantial elevation in the lipid peroxidation. Moreover, the increase in antioxidants activities was negatively associated with the level of lipid peroxidation. Cell membrane stability has been widely used to differentiate stress-tolerant and susceptible cultivars of many crops and it could be correlated with better field performance (Premachandra *et al*., 1991). The Chls compared to carotenoids have markedly

decreased in most sorghum genotypes under investigation; this gives the appearance of senescing plant. The reduction in Chls was reduced with elevating stress while the opposite was true for carotenoids. The decrease in Chl. a and b contents could be attributed to the increase their degradation (Abdel-Kader, 2000). Moreover, the damage caused by ROS may also affect macromolecules as mentioned by Pastori and Foyer (2002) and Costa *et al*. (2005). On the other hand, carotenoids concentration was significantly increased in most tolerant and local sorghum genotype under salinity treatments. The most important role of carotenoids is preventing the formation of singlet oxygen and protecting Chls by quenching their triplet states via thermal dissipation of energy. Additionally, carotenoids play a central structural role for chl-binding proteins of both the antenna system and the reaction center (Paulsen, 1997). Recent reports have shown that b-Car is essential for the assembly of D1 protein during its turnover in the formation of functional PS2 complexes in *Chlamydomonas reinhardtii* under high light conditions (Trebst and Depka, 1997; Depka *et al*., 1998). The increase in carotenoid concentration in most tolerant sorghum genotypes may be due to a shift in the synthesis of carotenoids to protect chloroplast from oxidative damage. Carotenoids could be considered as salinity tolerant marker or criteria.

CONCLUSIONS

Although the exotic genotypes are classified as tolerant and sensitive (based on ICRISAT confirmation), there were significant differences in genotypic responses within each group in the pattern of anti-oxidative mechanisms represented by the activity of anti-oxidant enzymes. The H_2O_2 scavenging system: SOD, ASPX, GR and GSH in addition to carotenoid contents are more important in imparting salt stress in the present materials. MDA content, showed decreased level in tolerant compared to sensitive group, however some genotypes have shown some high values. Although most of the previous enzymes involved in the amelioration of oxidative stress, they do not show uniform increase/decrease in activity in a given genotype. The tolerance of the following genotypes: 11, 16, 12, 20, 15 and 26 seems to be related to the production of high dry weight plant^{-1}, efficiency of the anti-oxidant enzymatic systems: SOD, GSH and MDA content. Other genotypes which represented high enzymes activity although did not produce reasonable dry weight are: 18 and 21. The local genotype, hybrid 101, has some degree of salt tolerance as it showed similar enzyme activities as some tolerant genotypes and reasonable dry weigh. Sorghum plants can tolerate a maximum level of 8.0 dS m^{-1}, then obvious reduction in plant performance and productivity occur. Genotypes which produce high dry weight do not necessarily exhibit high enzyme activities, as there may be no correlation between high production and intensity of oxidation-protecting enzymes. The antioxidative systems, SOD, ASPX, GSH and the MDA and carotenoids content are considered good physiological markers to distinguish between tolerant and sensitive genotypes; consequently they becomes a selection criteria for breeding for saline environments.

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