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EFFECTS OF SALT CONCENTRATIONS ON ANTIOXIDANT ENZYME ACTIVITY OF GRAIN SORGHUM

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Abstract
The present study was conducted to determine salt response of grain sorghum (Sureno) plants through antioxidant defense enzymes and to determine their salt resistanceat biochemical level. Sorghum plants were grown in climate chambers for 15 days in 3 replications in Hoagland growth medium under different salt concentrations (0, 50, 100, 150, 200 mM). At the end of growing period, roots and leaves were separated and the effects of salt stress were assessed spectrophotometrically through antioxidant enzymes, chlorophyll and carotenes. Root CAT increased until 100 mM, SOD, APX and GST activities increased with increasing salt concentrations until 150 mM and then they all decreased. Increasing salt concentrations elevated MDA accumulation in sorghum roots. Leaf SOD and APX activities and proline contents increased until 150 mM and CAT, GR and GST activities increased until 100 mM and then they all decreased. Leaf MDA contents also increased with higher salt concentrations. However, increasing salt concentrations decreased chlorophyll contents at 100 mM, carotene contents increased until 150 mM and then decreased. Although ascending antioxidant enzyme activity improved salt resistance of sorghum plants, increasing concentrations were not found to be sufficient. Thus, further studies with higher concentrations should be carried out to elucidate the case.

Keywords: Sorghum, Salt stress, Antioxidant system, Enzyme activity, ROS

1. INTRODUCTION

Salt stress is one of the most important environmental factors limiting both the growth and productivity of plants (Allakhverdiev et al., 2000). Sorghum varieties are known to resist salty soils (Boursier and Lauchli, 1990). Impacts of salt stress may vary on plants depending on; thier varieties, type of salt and exposure time, and dose (Munns, 2002).

Salt stress imposes oxidative stress by increasing the amounts of ROS species as super oxide (O^{2-}), hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH^-). Response of the plants in salty environments may vary depending on their genotypic differences. Antioxidative systems of plants are enough to detoxify the free radicals formed routinely. Enzymatic systems become activated under stress conditions (Smirnoff, 1993; Mullineaux and Creissen, 1997; Willekens et al., 1997; Apel and Hirt, 2004;).

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Positive correlation is known to exist in plants between the enzyme activities and tolerance levels to stress factors (El-Baz et al., 2003). Plants contain various amounts of antioxidants and antioxidative enzymes for preventing themselves from destructive effects of oxidative damage. This defence includes both enzymatic and non-enzymatic mechanisms. Non-enzymatic are those small molecules as triplet glutathione, cystine, hydroquinones, ascorbate (Vit C), lypohilic, vitamin E (α-tocopherol), flavanoids, carotenoid pigments, and alkaloids (Larson, 1988). Plants developed small molecular weight antioxidant defense systems as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), glutathione peroxidase (GPx), and glutathione reductase (GR) for decreasing the oxidative damage of ROS. SOD, CAT, APX and GR activities of salt tolerant genotypes were considerably higher compared to those of susceptible species (Scandalios, 1997; Yaşar et al., 2008). Proline is one of the osmotic protectants too (Djilianov et al., 2005). It accumulates at higher levels compared with other amino acids. MDA is also accepted as an indicator of lipid peroxidation (Turkan et al., 2005). Thus, it is used for differentiating culture plants with highly stable cell membranes from susceptible ones.
In the present study, effects of varying salt concentrations (0, 50, 100, 150 and 200 mM) on proline amount, lipid peroxidation, catalase, superoxide dismutase, ascorbate peroxidase, glutathione reductase, and glutathione S-transferase activities both in root and leaves, and carotenes, chlorophyll a and b, and total chlorophyll content in leaves of Sorghum was investigated.

2. MATERIALS AND METHODS

Sureno variety is used in the study. The trials were carried out in culture plates (15cm width/6cm depth) in climate chamber under 25± 1°C, 70±5 % humidity and 16/8 light/dark conditions. Plants were grown under normal circumstances for 10 days without any stress on them. At subsequent 15 days salt stress was exposed on plants at various concentrations of NaCI (0, 50, 100, 150, 200 mM). After that, plant were harvested at cold and stored at -20°C until use. All experiments were performed in three replications

2.1. Preparation of crude enzyme extracts and determination of protein concentrations

Crude enzyme extracts were prepared in a buffer containing 1% PVP and 0.1% EDTA, and 0.1 M potassium phosphate (pH 7.4) by using the method of Misra and Gupta (2006). Protein concentrations were determined by the method of Bradford (1976). BSA was used as the standard. Results were recorded as $mgml^{-1}$ protein.

2.2. Determination of chlorophyll a, b, total chlorophyll and carotene quantity

Chlorophyll and carotene content of leaves were determined spectrophotometrically using DMSO by the method of Hiscox and Israelstam (1979). Densities of the samples read against DMSO at 647, 663 and 470 nm. Chlorophyll quantities were determined as $mgml^{-1}$ fresh weight. Chlorophyll contents were estimated using the following formula;

Chla= (12.25xA663) – (2.79xA647);
Chl b= (21.5xA647) - (5.1xA663);
Chl total= (7.15xA663) + (18.71xA647)
Carotene= [(1000xA470) – (1.82xChl-a) – (85.02xChl-b)]/198

2.3. Determination of membrane lipid peroxidation (MDA)

MDA of the samples were determined using TCA and TBA solutions by the method of Madhava and Sresty (2000). Results were given as TBARS ($nmolgr^{-1}$ fresh weight).

2.4. Determination of proline quantity

Proline quantities of the samples were determined by acid ninhydrin method (Karabal et al., 2003). Absorbances of the samples were read spectrophometrically against toluene at OD520 nm. Proline

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contents were determined using the graph obtained from proline standart. Proline amounts were calculated as nmol gr^{-1} fresh weight.

2.5. Determination of enzyme activities

2.5.1. Catalase (CAT)

After addition of enzyme extract to 20 mM sodium phosphate buffer (pH 7.5) reaction was started with 20 mM H_2O_2 together with 50 µl crude extract. Changes in activity were recorded during 3 minutes at 240 nm. Results were recorded as unit mg^{-1}.

2.5.2. Superoxide dismutase (SOD)

It was determined in 20 mM sodium phosphate buffer (pH 7.5) containing 0.1 mM EDTA, 10 mM methionine, 0.1 mM NBT, 0.005 mM riboflavin using the method of Granopolitis and Ries (1977). 50 µl of crude extract was added to the solution and kept at 20 cm away from light source (500 lumen) for 15 min before measuring the absorbance at 560 nm. SOD amounts (unit mg^{-1}) of the samples were estimated as percent inhibition by using the graph of standard SOD enzyme (10-500 ng).

2.5.3. Ascorbate peroxidase (APX)

After addition of 50 µl crude extract into a solution containing 50 mM potassium phosphate ($KHPO_4$, pH 7.0), 0.15 mM ascorbic acid, 20 mM hydrogen peroxide (H_2O_2), and decrease in absorbance values were recorded during 3 min at 290 nm in 2 ml cuvettes. Results were recorded as unit mg^{-1}.

2.5.4. Glutathione reductase (GR)

Decrease in absorbance values were recorded for 5 min at 25°C in 100 mM potassium phosphate ($KHPO_4$, pH 7.5) containing 0.1 mM Na_2EDTA, 0.1 mM nicotine amide adenine dinucleotide phosphate (NADPH) and 1mM oxidized glutathione (GSSG). Results were recorded as unit mg^{-1}.

2.5.5. Glutathione S-Transferase (GST)

Activity was recorded in 100 mM potassium phosphate ($KHPO_4$, pH 7.5) containing 0.1 mM EDTA, 0.1 mM NADPH, 1 mM GSH and 1 mM CDNB. Results were recorded as unit mg^{-1}.

2.6. Statistical analysis

Variance and correlation analyses were performed by using SAS software (SAS Inst., 1999).Differences between mean values were tested by Duncan's multiple range tests. In order to visualize the relationships among traits in the same chart, bi-plot analysis was performed by using Microsoft excel package program as suggested by Lipkovich and Smith (2002).

3. RESULT AND DISCUSSION

The amount of chlorophyll a, b, and total chlorophyll were expected to increase gradually in salt tolerant plants and to increase in susceptible plants parallel with increasing external salt concentrations (Khan et al., 2009; Akram and Ashraf, 2011). That is way, chlorophyll accumulation in different cereals as pea (Noreen et al., 2010) and sunflower (Khan et al., 2009) was considered as the potential biochemical indicator of the salt tolerance. In the current study, we indicated that stability of Sorghum plant in terms of chlorophyll biosysnthesis is an indication of its high resistance to salt stress till 100 mM. Also, it can be inferred that carotene is a stress related compound considering its cotinuous increase till 150 mM salt treatment (Fig. 1). It was clear that carotene and chlorophyll biosynthesis indicated an apparent decrease in 200 mM concentration.

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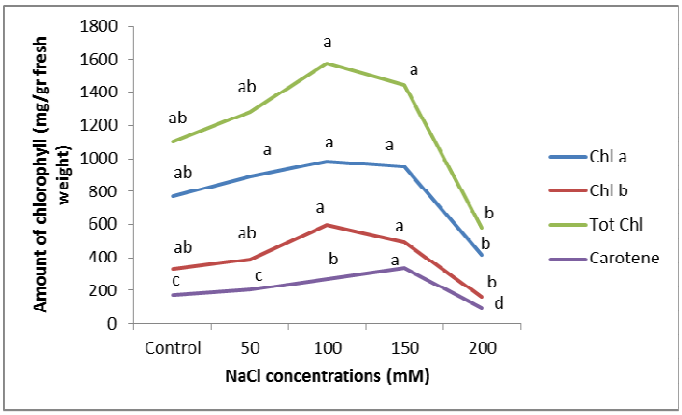

Figure 1. Amount of chlorophyll and carotene in the leaf tissues of the sorghum seedlings subjected to 50, 100, 150 and 200 mM NaCl

Therefore, 200 mM salt concentration was probably an indication that limiting value was exceeded for resistance mechanisms in Sorghum. Once the plant encounters salt stress, it firstly promotes the accumulation of phenolic compounds in roots. Increasing dose causes a radical shift from root to leaf in term of phenolics accumulation. Such a situation is an indication of direct salt transfer to the leaves without accumulating in roots. Stress caused after 100 mM treatment builds up antioxidative mechanisms and couples with decrease in phenolic accumulation (Fig. 2).

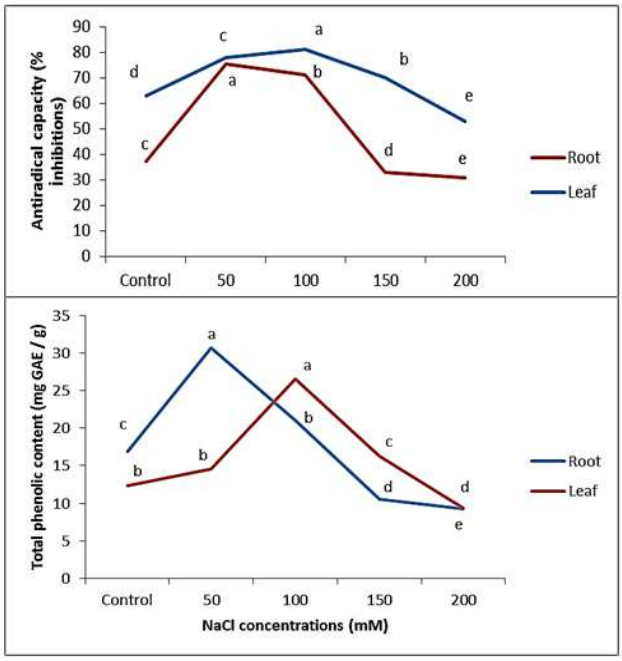

Figure 2. Antiradical capacity and total phenolic content of the sorghum root and leaf tissues subjected to 50, 100, 150 and 200 mM NaCl

Increase in phenolics together with antiradical capacity brings to mind that theytake part in mechanism of antoxidative capacity. This is because the changes in phenolic concentration coincide with that in antiradical capacity from the moment of first encounter with the stress. Antiradical capacity revealed considerable increase in leaves till 150 mM and deceased in later concentrations (Fig. 2).

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Antioxidant enzymes come into play immediately following the salt stress. Thus the damage to be caused by the stress is minimized. Such a situation can clearly be visualized by considering the MDA levels in tissues. It was clear that the salt concentrations over 150 mM caused an apparent damage in the plant (Fig. 3). Together with the antioxidant enzymes, proline is also released and used as a protective molecule in the plant. In a study carried out by Bavei et al., (2011) the proline amount reached 4-6 times higher levels in sorghum leaves at salt treatments ranging between 50-200 mM. Omari and Nhiri (2015) studied the adaptive response of sorghum under 50, 100 ve 150 mM salt concentrations and reported 2-3 times higher proline amount at especially 100 and 150 mM salt treatments. Similarly, El-haddad et al (1994) reported an increase in proline amount parallel with salt treatment. In the present study, significant increase was observed in proline amount both in root and leaves (Fig. 3). Proline accumulation as an adaptive response to salt stress may decrease the water potential and later can help to ensure continuity of water in leaves (Sabir et al., 2011).

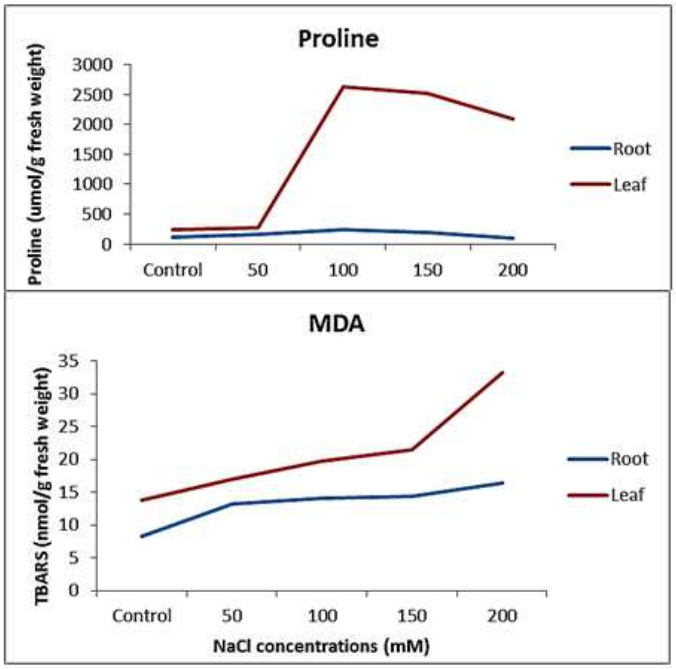

Figure 3. Prolin accumulations and TBARS levels of the root and leaf tissues in the sorghum seedlings subjected to 50, 100, 150 and 200 mM NaCl

As can be seen from the MDA figure, an apparent damage occurs after 150 mM concentration. Proline is also released together with antioxidant enzymes in the plant. Proline helps plant to protect from the damage to be caused by salt stress. The continuous increase in proline amount (at least in leaves) during the stress period can be considered as a proof that Sorghum plant is resistant to salt stress. Considering all the results from analysis, it can be inferred that Sureno varieties of sorghum is resistant to salt stress to some extent (till 150 mM concentration).

In Sureno variety, salt stress activates all antioxidative enzyme systems simultaneously and scavenging activities of CAT and GR, and SOD and APX were respectively retained till 150mM (Fig. 4). In addition, scavenging activity of GST enzyme continue even under 200 mM salt stress. These enzymes act together at the same time and exhibit nearly equal scavenging activity both in root and leaves. Increase in enzyme activity was only observed for GR in leaves compared to roots

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unde stress conditions. This situation brings in mind that the glutathione's mainly being GR arethe basic enzymes in leaves for protection against salt stress.
Omari and Nhiri (2015) reported that SOD activity in roots was higher that of leaves. Similarly, Gomez et al. (2004) observed an increase in SOD activity in pea chloroplasts upon long term salt treatment. The increase of SOD activity under salt stress is an indication that it is good oxidative stress tolerating enzyme (Panda and Khan, 2004). Bavei et al., (2011) found similar results in peroxidase activity in sorghum varieties they study. That is activity was considerably increased depending on the salt concentration. Bavei et al., (2011) found that peroxidase activity in roots was apparently higher than that of roots, but, the common trend was similar both in leaves and roots (Fig. 4).

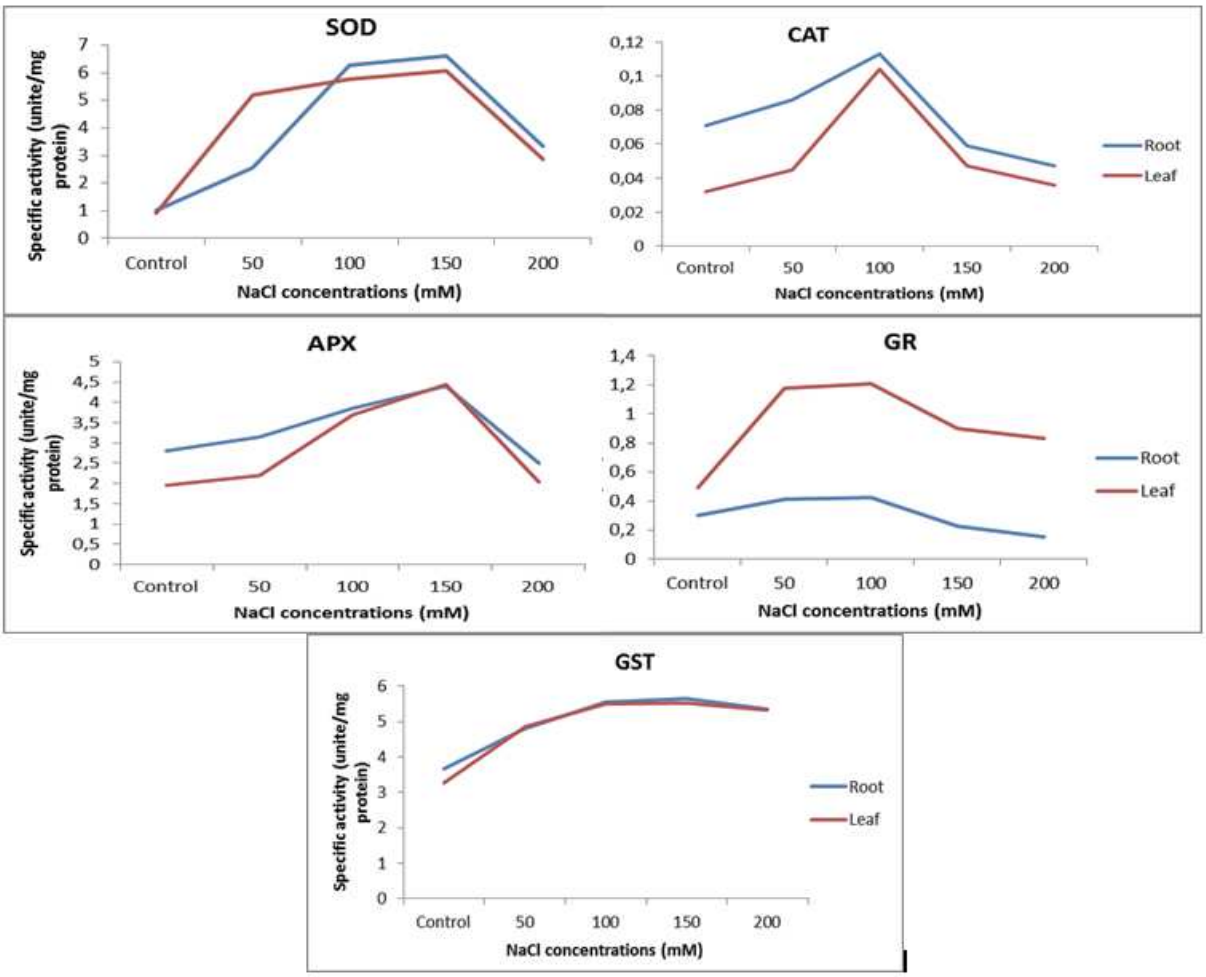

Figure 4. Antioxidant enzyme (SOD, CAT, APX, GR and GST) activities in the leaf and root tissues of the sorghum seedlings subjected to 50, 100, 150 and 200 mM NaCl

In a study conducted by Omari and Nhiri (2015), depending on the external salt applications, while an increase was seen in CAT activity, no change was observed in POD activity in leaves. In contrast, while CAT activity in roots indicated a gradual increased especially at 50 and 100 mM, a rapid risewas observed in POD activity. Such a case suggests that POD is the basic enzyme for detoxification of H2O2 in sorghum plant under salt stress. In the present study, SOD, CAT, APX and GR revealed a similar trend in Sureno variety and suggest that these enzymes take part jontly in detoxification processes.

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In addition, GST activity revealed a steady increase in parallel with the increase in stress factors, and thus considered that this was the basic detoxification enzyme. Our findings were compatible with the enzyme activities in roots of bean (Jebara et al., 2005), margold (Chaparzadeh et al., 2004), Sorghum (Omari and Nhiri, 2015), and rice (Khan and Panda, 2008) subjected to salt stress. However, Omari and Shiri (2015) stated that the most important H2O2 scavenging enzyme in the leaves is CAT as also proposed by Neto et al (2006). Moneguzzo et al (1999) in salt resistant wheat, and Meloni et al (2003) in salt resistant cotton (pora cultivar) observed significant rise in root GR activity at all salt treatments. In our study, enzyme activities both in roots and leaves demonstrated a parallelism. Gomez et al. (2004) suggest that the increase in GR activity raise the rate of NADP/NADPH and consequently elevates the electron acceptance capacity of NADP in photosynthetic electron transport chain, and thus helps regulate the level of ROS formed in chloroplasts.

4. CONCLUSIONS

For to be sure about the stress resistance of Sureno varieties, it is supposed to study the phenolic content and its variation under stress conditions. Besides all these, new detailed studies should be carried out for determining the possible relationships between phenolics and antioxidant enzyme activities under such conditions. Also proteomics level analysis of the enzyme/proteins has great importance for clarifying the mechanisms related with resistance.

5. ACKNOWLEDGEMENT

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