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## Antioxidant Activity and Osmolyte Concentration of Sorghum (*Sorghum bicolor*) and Wheat (*Triticum aestivum*) Genotypes under Salinity Stress

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**Abstract:** Seedling of two sorghum genotypes (Payam and Sistan) and four wheat genotypes (Bolani, Hirman, Star and Toss) were grown in Hoagland nutrient solution containing 0, 100 and 200 mM NaCl in controlled environment. Antioxidant activities like catalase (CAT), ascorbate peroxidase (APX) and guaiacol peroxidase (GPX) and osmolyte concentrations, proline and soluble carbohydrates were determined in the leaves 20 days after induction of salinity stress. Results showed that the activity of APX, GPX and CAT increased in both sorghum genotypes. Wheat genotypes showed significant differences during the experimental period. By increasing NaCl levels from 0 to 200 mM the activity of APX and GPX decreased, but the activity of CAT increased in all wheat genotypes. At the 100 mM NaCl, the CAT activity in wheat genotypes was higher compared with that in 200 mM NaCl. The increase in salinity stress increased total soluble carbohydrates and proline both in wheat and sorghum genotypes. Results in this study showed sorghum genotype displayed better osmotic adjustment and antioxidant compounds under salt stress and the efficiency of Sistan was better than Payam. Contrarily in wheat, osmotic adjustment (carbohydrate and proline accumulation) was much more effective than antioxidant enzyme activity.

**Key words:** Antioxidant activity, osmolyte concentration, salinity, wheat and sorghum genotypes

### INTRODUCTION

Plant salt tolerance has been generally studied in relation to regulatory mechanisms of ionic and osmotic homeostasis (Ashraf and Harris, 2004). In addition to ionic and osmotic components, salt stress, like other abiotic stress, also leads to oxidative stress through an increase in Reactive Oxygen Species (ROS), such as superoxide ( $O_2^-$ ), hydrogen peroxide ( $H_2O_2$ ) and hydroxyl radicals ( $OH^\bullet$ ) (Mittler, 2002).

Among the abiotic stress, salt stress causes major problems in cereals like wheat. It has been currently reported that most abiotic stress including NaCl salt stress impose injury in plants by osmotic stress, ionic stress and generating reactive oxygen species (Shalata and Tal, 1998).

During oxidative stress, the excess production of Reactive Oxygen Species (ROS) causes membrane damage that eventually leads to cell death. For protection against ROS, plants contain antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), guaiacol peroxidase (GPX) and Glutathione Reductase (GR) or as well as a wide array of non-enzymatic antioxidants (Blokhina *et al.*, 2003). SOD is the major  $O_2^-$  scavenger and its enzymatic action results in  $H_2O_2$  and  $O_2$  formation. The  $H_2O_2$  produced is then scavenged by CAT and several classes of

peroxidases. CAT, which is found in peroxisomes, cytosol and mitochondria, dismutates  $H_2O_2$  to  $H_2O$  and  $O_2$  (McKersie and Leshem, 1994).

Salt stress imposes osmotic stress, by limiting absorption of water from soil and ionic stress, resulting from high concentrations of potentially toxic salt ions within plant cells. A variety of protective mechanisms have evolved in plants to allow them to acclimatize to these unfavourable environmental conditions for survival and growth. Although, the effects of salinity are studied in variety of glycophytic and halophytic plants, the mechanisms of salt tolerance are not well understood (Hasegawa *et al.*, 2000).

The synthesis and accumulation of low molecular weight metabolites, known as compatible solutes, is a ubiquitous mechanism for osmotic adjustment in plants. Their main role is to increase the ability of cells to retain water without affecting the normal metabolism (Hamilton and Heckathorn, 2001). Amino acids, sugars, betaines and proline compounds may accumulate, as compatible solutes, in many plant species (Serrano, 1996).

There are large differences in salt tolerance between crop species. One of the main reactions to salt or drought stress is closing of stomata. The  $C_4$  plants, in opposite to the  $C_3$ , are able to utilize very low concentration of carbon dioxide which enables them to assimilate  $CO_2$  even during considerable stomatal closure (El Bassam, 1998). This

might be one of the probable reason for the difference in resistance to stress between both plant groups. Photosynthesis is a complex process; therefore, it is possible that a number of elements in the C<sub>3</sub> and the C<sub>4</sub> may differ in resistance to salinity.

The objective of this study was to elucidate the role of some antioxidants and compatible osmolytes. On what basis was this tolerance dependent in four wheat and two sorghum genotypes.

## MATERIALS AND METHODS

This study was conducted in a greenhouse at the University of Zabol, Iran during April-June 2007. The experiment was laid-out a completely randomized factorial design with three replicates. Seeds of four wheat genotypes (Bolani, Hirman, Star and Toss) and two sorghum genotypes (Payam and Sistan) were grown in the containers with Hoagland nutrient solution. Surface-sterilized seeds were germinated in the dark on sand, moistened with distilled water. Seven days old seedling with uniform size were transferred to hydroponic culture in plastic container with 2 L of nutrient solution. Plants were grown under greenhouse conditions with a 12 h photoperiod of natural daylight, maximum and minimum temperatures were 26 and 18°C, respectively and relative humidity was 70% on average.

Three salinity treatments were imposed by adding S<sub>0</sub> : 0 (control), S<sub>1</sub> : 100 and S<sub>2</sub> : 200 mM NaCl to the nutrient solution after 10 day old plants. Twenty days after salt treatment, the plants were harvested. The extracts of mature leaf blades were used to determine soluble carbohydrates (Hendrix, 1993) and proline (Bates *et al.*, 1973) in both crop species.

For free proline content, leaf samples were homogenized in 5 mL of sulphosalicylic acid (3%) using mortar and pestle. About 2 mL of extract was taken in test tube and pestle. About 2 mL of extract was taken in test tube and to it 2 mL of glacial acetic acid and 2 mL of ninhydrin reagent were added. The reaction mixture was boiled in water bath at 100°C for 30 min. After cooling the reaction mixture, 6 mL of toluene was added and then transferred to a separating funnel. After thorough mixing, the chromophore containing toluene was separated and absorbance read at 520 nm in spectrophotometer against toluene blank.

### Enzyme assays

**Ascorbate peroxidase:** The enzyme was extracted in 50 mM phosphate buffer (pH = 7). The activity of ascorbate peroxidase (APX EC 1.11.1.11) was measured using method of Nakano and Asada (1981).

The APX activity was determined according to Nakano and Asada (1981). The reaction mixture consisted of 50 mM sodium phosphate buffer (pH = 7) containing 0.2 mM EDTA, 0.5 mM ascorbic acid (sigms), 50 mg of BSA (Sigma) and crude enzyme extract. The reaction was started by addition of H<sub>2</sub>O<sub>2</sub> at final concentration of 0.1 mM. Oxidation of ascorbic acid as a decrease in absorbance at 290 nm was followed 2 min after starting the reaction. The difference in absorbance was divided by the ascorbate molar extinction coefficient (2.8 mM<sup>-1</sup> cm<sup>-1</sup>) and the enzyme activity expressed as μmol of H<sub>2</sub>O<sub>2</sub> min<sup>-1</sup> mg<sup>-1</sup> protein, taking into consideration that 1.0 mol of ascorbate is required for the reduction of 1.0 mol of H<sub>2</sub>O<sub>2</sub> (McKersie and Leshem, 1994).

**Catalase:** Catalase (CAT, EC 1.11.1.6) activity was assayed spectrophotometrically by monitoring the decrease in absorbance of H<sub>2</sub>O<sub>2</sub> at 240 nm. CAT was measured according to the method of Beers *et al.* (1952). The enzyme was extracted in 50 mM phosphate buffer (pH = 7). The assay solution contained 50 mM phosphate buffer and 10 mM H<sub>2</sub>O<sub>2</sub>. The reaction was started by addition of enzyme aliquote to the reaction mixture and the change in absorbance was followed 2 min after starting the reaction. Unite activity was taken as the amount of enzyme, which decomposes 1 M of H<sub>2</sub>O<sub>2</sub> in 1 min.

**Guaiacol peroxidase:** Total GPX (EC 1.11.1.7) activity was determined as described by Urbanek *et al.* (1991) in a reaction mixture (0.2 mL) containing 100 mM phosphate buffer (pH 7.0), 0.1 μM EDTA, 5.0 mM guaiacol, 15 mM H<sub>2</sub>O<sub>2</sub> 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 min. Enzyme activity was quantified by the amount of tetraguaiacol formed using its molar extinction coefficient (26.6 mM<sup>-1</sup> cm<sup>-1</sup>).

**Statistical analysis:** All data were analyzed with SAS Institute Inc., 6.12. All data were first analyzed by ANOVA to determine significant (p≥0.05) treatment effects. Significant differences between individual means were determined using Fisher's protected Least Significant Difference (LSD) test. Data points in the figures represent the means±SE of three independent experiments at least three replications per cultivar per treatment combination each.

## RESULTS

**Enzyme activity:** APX activity in leaves for control and salt stressed plants changed in genotypes of both wheat and sorghum crop species. The activity of APX increased

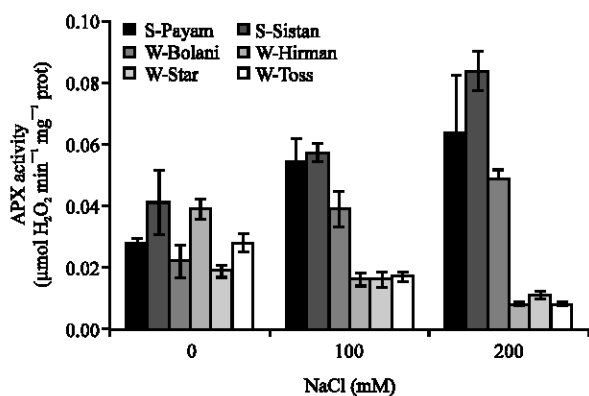


Fig. 1: Changes in the APX content in the leaves of sorghum (S) and wheat (W) genotypes subjected to increasing salinity

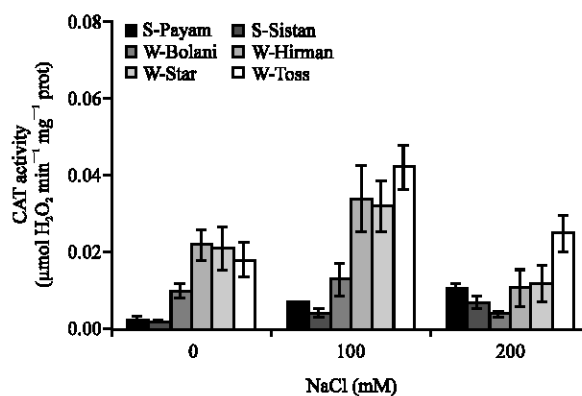


Fig. 3: Changes in the CAT contents in the leaves of sorghum (S) and wheat (W) genotypes subjected to increasing salinity

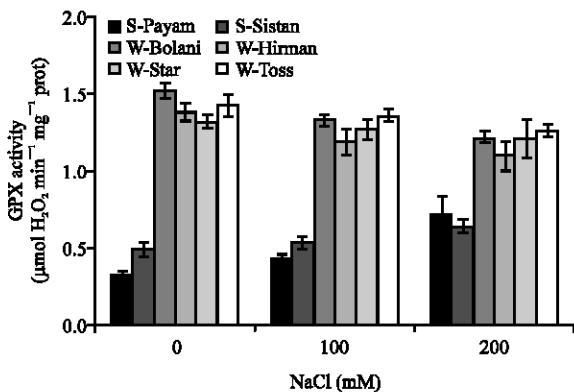


Fig. 2: Changes in the GPX contents in the leaves of sorghum (S) and wheat (W) genotypes subjected to increasing salinity

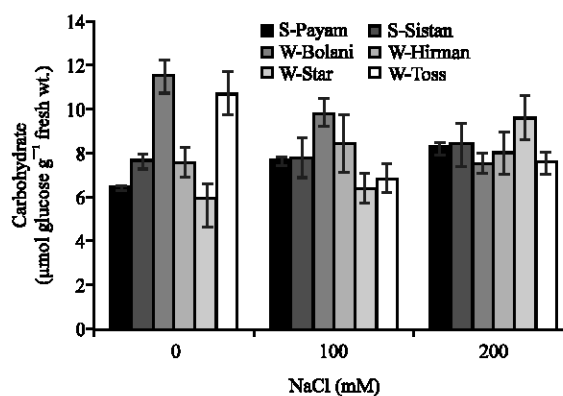


Fig. 4: Changes in the soluble carbohydrate content in the leaves of sorghum (S) and wheat (W) genotypes subjected to increasing salinity

remarkably in two sorghum genotypes as a result of salt stress. The activity of APX in Sistan was higher than Payam genotype. In all of wheat genotypes except Bolani, APX significantly reduced in leaves. The activity of APX was significantly lower in wheat than in the sorghum genotypes (Fig. 1).

The assay for GPX in different salinity levels showed that wheat genotypes represented significantly lower GPX activity than sorghum genotypes (Fig. 2). In wheat genotypes GPX activity decreased, but in sorghum it increased when under salt stress. Figure 2 shows that the GPX activity increased with increasing salinity in sorghum genotypes (Payam and Sistan). The activity of two enzymes, APX and GPX increased 56.2 and 23.4%, respectively at 200 mM NaCl compared to control in Sistan genotype. While, the increase of APX and GPX activity in payam genotype was 51.2 and 54.1%, respectively.

The activity of CAT was significantly lower in sorghum than wheat genotypes (Fig. 3). Increase in salinity levels significantly increased catalase activity in sorghum genotypes (79.1% in Sistan and 72.6% in Payam). The increasing CAT activity in wheat genotypes were observed only until 100 mM NaCl, after that by increasing salinity level to 200 mM NaCl, its activity decreased (Fig. 3). Wheat genotypes subjected to salinity treatment at the 100 mM NaCl, had the highest CAT activity (Hirman 35.2% and Toss 55.8%, Fig. 3).

**Osmolyte concentration:** Total soluble carbohydrate increased in all wheat (Bolani and Toss genotypes, at the 100 mM NaCl salinity treatment, had the highest carbohydrate) and sorghum genotypes with the increasing salinity (Fig. 4). Significant differences were observed between the genotypes of sorghum and wheat for total soluble carbohydrate. Sistan genotype in

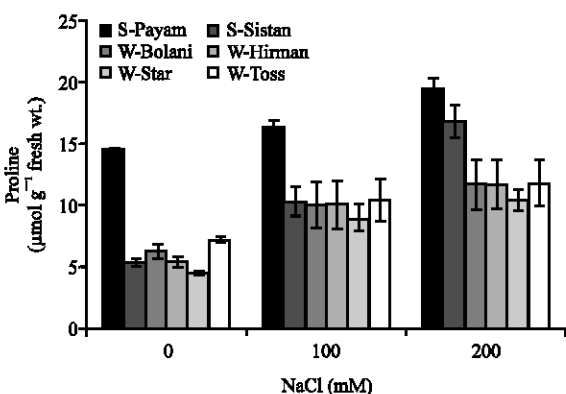


Fig. 5: Changes in the proline content in the leaves of sorghum (S) and wheat (W) genotypes subjected to increasing salinity

sorghum and Bolani and Toss genotypes in wheat had the highest carbohydrate concentration when under salt stress.

In all genotypes of sorghum and wheat, salinity stress stimulated proline accumulation (Fig. 5). Significant differences in proline accumulation were revealed under stress conditions ( $p < 0.01$ ). Payam genotype in sorghum and Toss genotype in wheat had the highest proline content. In this study, we did not find a close relationship between the accumulation of proline and carbohydrate in leaves ( $R^2 = 0.103$ ).

## DISCUSSION

In this study, among the antioxidative enzymes, activity of APX, GPX and CAT increased in both sorghum genotypes, but Sistan genotype had the higher enzyme activity than Payam genotype. The activity of these three enzymes increased in tolerant genotype (Sistan), which is similar to the results of Gossett *et al.* (1993). To tolerant high levels of salts, plants can adopt different strategies. Salinity stress induces Reactive Oxygen Species (ROS) production and lead to oxidative damages. The ROS may react with macromolecules, the proteins and lipid components of membranes causing damage through lipid peroxidation resulting in increased permeability of the membrane. The antioxidant defense system of the plant comprises a variety of antioxidant molecules and enzymes (Arora *et al.*, 2002). Wheat genotypes had different behavior during the experimental period. With increased salinity levels, the activity of APX and GPX decreased but that of CAT increased. At the 100 mM NaCl, the CAT activity in wheat genotypes was higher than that at 200 mM NaCl. Among the wheat genotypes, Toss and Hirman showed the highest CAT activity (Fig. 3). The higher CAT activity indicates that salt tolerant lines had better ability to scavenge  $H_2O_2$  (Gossett *et al.*, 1994).

Willekense *et al.* (1995) reported that the intracellular levels of  $H_2O_2$  is regulated by a wide range of enzymes. The CAT functions through an intermediate CAT- $H_2O_2$  complex and produces water and dioxygen. Ascorbate (APX) in addition to its role in the scavenging cycle, acts as areductant in the regeneration of  $\alpha$ -tocopherol and in the zeaxanthin cycle.

In this study, we found a positive relationship between antioxidant activity and osmolyte concentration, carbohydrates ( $R^2 = 0.33$ ) and praline ( $R^2 = 0.43$ ), in sorghum genotypes. Carbohydrate concentration, beside its role in decreasing water potential, contributes in preventing oxidative damage and maintaining the structure of proteins and membranes under moderate dehydration during drought period (Hoekstra *et al.*, 2001). Carbohydrate also serve as signaling molecules for sugar-responsive genes which leading to different physiological responses like defense responses and turgor-driven cell expansion (Sturm and Tang, 1999).

In this study, the activity antioxidant enzymes and osmolyte concentration increased in both sorghum genotypes. The general comparison of the examined antioxidants in wheat and sorghum genotypes revealed that in the salinity treatments, the salt tolerance is due, at least partially, to the higher constitutive antioxidant enzymes actives and increasing of osmolyte concentrations in the leaves. Between two kinds of plant, sorghum ( $C_4$ ) and wheat ( $C_3$ ), we found which, sorghum for salinity tolerance are use of two mechanisms (antioxidant activity and osmolyte concentration), but in wheat genotypes ( $C_3$ ) the mechanism of osmotic adjustment was acted better than antioxidant mechanism. In sorghum genotypes Sistan was better and had the highest antioxidant activity and osmolyte concentration.

## CONCLUSION

In addition to role of compatible solutes in osmotic adjustment, antioxidant enzymes also play a role in salt tolerance. Sorghum displayed better osmotic adjustment and antioxidant compounds under salt stress and the efficiency of Sistan was better than Payam. Contrarily in wheat, osmotic adjustment (carbohydrate and proline accumulation) was much more effective than antioxidant enzyme activity. Among wheat genotypes, Toss showed greater carbohydrate and proline content and CAT activity.

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