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# Salinity Effects on Compatible Solutes, Antioxidants Enzymes and Ion Content in Three Wheat Cultivars

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**Abstract:** In order to study effects of different salinity levels on antioxidant enzyme activities, catalase (CAT), ascorbate peroxidase (APX) and guaiacol peroxidase (GPX) associated with compatible solutes, proline and carbohydrate and mineral nutrient content in shoots, sodium and potassium, in three wheat cultivars an experiment was conducted as completely randomized 3×4 factorial design with three replicates in a greenhouse. Three wheat cultivars (Pishtaz, Kavir and Hamon), that differ in their salt tolerances, were grown in four different salinity levels ($S_0$ = control, $S_1$ = 100, $S_2$ = 200 and $S_3$ = 300 mM NaCl). Twenty days after wheat cultivars subjected to salt stress, data showed salinity stress induced increase in the antioxidant enzyme activities. Among the cultivars, salinity stress decreased leaf-APX but increased the activities of leaf-GPX in Pishtaz cultivar. Our results showed a positive correlation between praline accumulation and Leaf-APX ($r^2$ = 0.56), Leaf-GPX ($r^2$ = 0.63) and Leaf-CAT ($r^2$ = 0.73). In these cultivars, in their shoots $Na^+$ showed an increase in concentration with salinity that approximately matches a decrease in $K^+$ concentration. It seems that $Na^+$ concentrations in the shoot may have had a more significant effect on plant antioxidant enzyme activities and compatible solutes such as proline and carbohydrates. These results indicated which in wheat under salinity stress antioxidant enzymes and compatible solutes help to plant adaptation. In this study we found a positive correlation between $Na^+$ concentration in the shoots and the antioxidant enzyme activities and compatible solutes in the leaves.

**Key words:** Antioxidants enzymes, proline, ion content, salinity, wheat

## INTRODUCTION

Soil salinity is one of the major factors of soil degradation. It has reached 19.5% of the irrigated land and 21% of the dry-land agriculture existing on the globe (FAO, 2000). Salinity inhibition of plant growth is the results of osmotic and ionic effects and the different plant species have developed different mechanisms to cope with these affects (Munns, 2002).

High salt concentrations, usually sodium chloride, cause osmotic stress by decreasing water potential within the cells and ionic stress due to specific inhibition of metabolic processes. Plants respond to salinity by sequestering toxic ions in the vacuoles and accumulation of compatible solutes in the cytoplasm to balance the decrease of water potential (Di Martino *et al*., 2003).

Biochemical studies have shown that plants under salinity stress accumulate number of metabolites, which are termed compatible solutes because they do not interfere with biochemical reactions. These metabolites include carbohydrates, such as manitol, sucrose and raffinose oligosaccharides and nitrogen-containing compounds, such as amino acids and polyamines (Bohnert *et al*., 1995).

In many researches, salinity tolerance has been studied in relation to regulatory mechanisms of osmotic and ionic homeostasis (Ashraf and Harris, 2004). Salt stress, like other abiotic stresses 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 (Apel and Hirt, 2004). To minimize the effects of oxidative stress, plant cells have evolved a complex antioxidant system, which is composed of low-molecular mass antioxidants (glutathione, ascorbate and carotenoids) as well as ROS-scavenging enzymes, such as: superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), guaiacol peroxidase (GPX) and glutathione reductase (GR) (Alscher *et al*., 1997; Apel and Hirt, 2004).

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The relationship between osmoregulation, salt tolerance and nutrient uptake is not clear. Studies about the effects of salt stress on wheat plant mostly concerned on the changes in growth parameter and osmoregulation for breeding purpose. In this study, we aimed to provide an insight view to salt stress and antioxidants enzymes system in wheat plant. In order to understand the mechamisms relevant in salt. tolerance. Therefore, the objective of this investigation was to evaluate the effects of salinity stress on three wheat cultivars and correlate these effects to changes in ionic, organic solute accumulation and antioxidants enzymes.

## MATERIALS AND METHODS

This study was conducted in the university of Zabol, Iran during April-June 2007. The experiment design was a completely randomized 3×4 factorial design with three replicates.

Seeds of three bread wheat cultivars (Kavir, Hamon and Pishtaz) were sown in trays containing vermiculite, water daily with distilled water and kept in a greenhouse.

Plant were grown under greenhouse conditions with a 13 h photoperiod of natural daylight, maximum and minimum temperatures were 26 and 18°C, respectively and relative humidity was 40% on average. Five days from seedling. emergence they were transferred to trays containing half-strength Hoagland's nutrient solution and 10 days later they were transferred to plastic plots containing 3 L of full strength nutrient solution.

Four salinity treatments were imposed by adding $S_0$ = 0 (control), $S_1$ = 100, $S_2$ = 200 89 and $S_3$ = 300 mM NaCl to the nutrient solution. 20 days after beginning of salt additions the plants were harvested, organic and inorganic solutes were extracted from mature leaf blades. In this extract, soluble carbohydrates (Horwitz, 1975) and proline (Bates *et al*., 1973) were determined. The contents of $Na^+$ and $K^+$ were determined by using a Jemway PFP7 Flame photometer.

**Enzyme assays:** The APX activity was determined according to Nakano and Asada (1981). The assay mixture consisted of 50 μL of the enzyme extract, 50 mM phosphate buffer (pH = 6.0), 0.1 μM EDTA, 0.5 mM ascorbate and 1.0 mM $H_2O_2$ in a total volume of 1.5 mL. Ascorbate oxidation was monitored by reading the absorbance at 290 nm at the moment of $H_2O_2$ addition and 1 min later. The difference in absorbance was divided by the ascorbate molar extinction coefficient (2.8 $mM^{-1}$ $cm^{-1}$) and the enzyme activity expressed as μmol of $H_2O_2$ $min^{-1}$ $mg^{-1}$ protein, taking into consideration that 1.0 mol of ascorbate is required for the reduction of 1.0 mol of $H_2O_2$ (Mckersie and Leshem, 1994).

Specific GPX and CAT activity were measured according to Urbanek *et al*. (1991) and Beers and Sizer (1952), respectively.

**Statistical analyses:** 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.

## RESULTS

**Antioxidant enzyme activities:** Results of this study showed, the activity of antioxidant enzymes are elevated with increased salinity from $S_0$ = 0 (control) to $S_3$ = 300 mM NaCl. Figure 1 shows that leaf-CAT level is increased with increasing salinity meanly Pishtaz cultivar which had the highest CAT activity and 300 mM NaCl induced 85.1% increase in the activity of this enzyme in plants than control treatment.

As a result of salt stress, leaf-APX activity varied according the stressed variety: in fact, we observed three different behaviours: Pishtaz showed the same APX activity whatever the salt concentration and Kavir showed an APX increase with increasing salt concentration. Hamon cultivar had the highest APX activity and $S_2$ salinity induced 65.8% increase in the active of this enzyme than control treatment (Fig. 2).

In addition to APX, the activities of GPX in Hamon cultivar increased only until $S_1$ (100 mM NaCl) after that, the leaf-GPX in Hamon cultivar decreased. Leaf GPX-activity of other two cultivars (Kavir and Pishtaz) increased with increasing salinity levels. Between Kavir

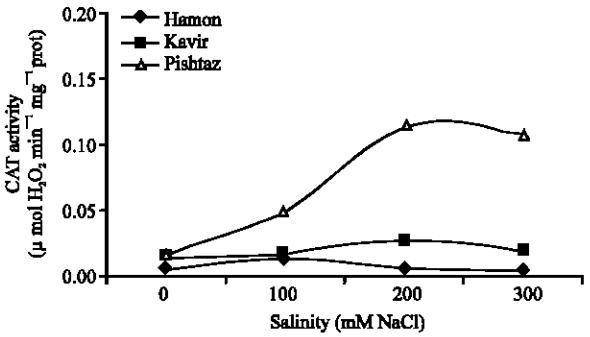


Fig. 1: Catalase (CAT) activity in leaves of three wheat cultivars at salinity levels

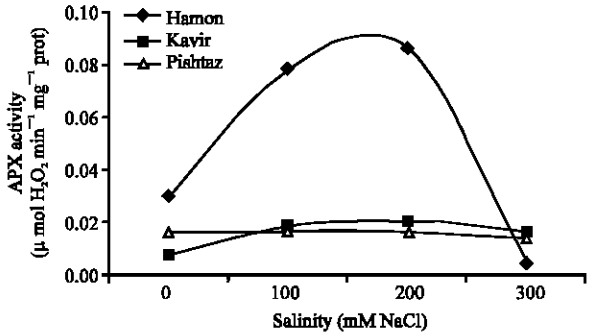


Fig. 2: Ascorbate peroxidase (APX) activity in leaves of three cultivars wheat at salinity levels

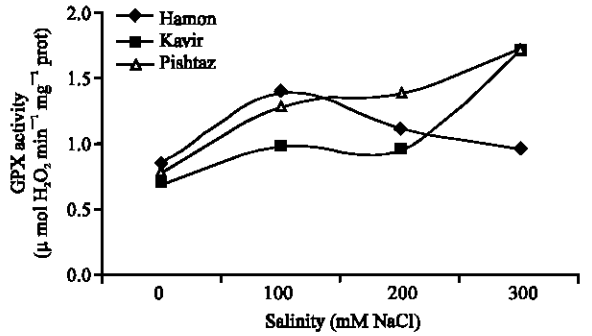


Fig. 3: Guaiacol peroxidase (GPX) activity in leaves of three wheat at salinity levels

and Pishtaz cultivars, Pishtaz had the maximum GPX activity and 300 mM NaCl induced 55.5% increase in the activity of this enzyme in the plants (Fig. 3).

**Biochemical components and nutrient uptake:** The results of this study indicate, that application of NaCl in the growing medium significantly ($p<0.001$) affected proline and carbohydrate concentration in the three wheat cultivars. By increasing salinity from $S_0$ = 0 to $S_3$ = 300 mM NaCl proline and carbohydrate contents increased meanly at Hamon and Kavir varieties.

Among the cultivars, Pishtaz cultivar had the maximum concentration of proline and carbohydrate. In the $S_3$ treatment Pishtaz had 76.1% proline content of $S_0$ in their green leaves tissue, but maximum carbohydrate content in Pishtaz saw in $S_2$ treatment after increasing salinity, carbohydrate content in green leaves tissue of this cultivar decreased (Fig. 4, 5).

Results of this study showed that considerable change in the plant's chemical composition. In general, potassium essential ion for plant growth and by increasing salinity levels from $S_0$ (control) to moderate salinity ($S_1$ = 100 mM NaCl) $K^+$ content of Kavir and Pishtaz cultivars decreased higher than Hamon cultivar. By increasing salinity levels from $S_1$ to $S_3$, Hamon had the

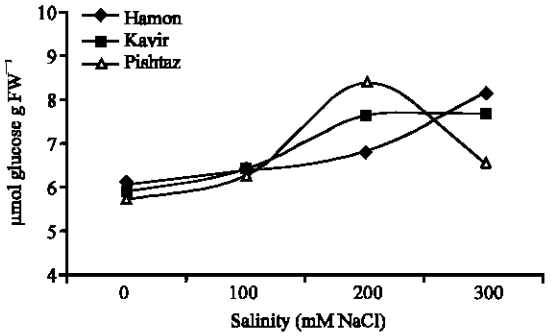


Fig. 4: Carbohydrate concentration in leaves of three wheat cultivars at salinity levels

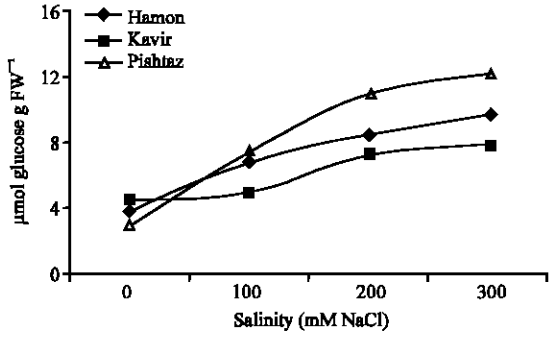


Fig. 5: Proline concentration in leaves of three wheat cultivars at salinity levels

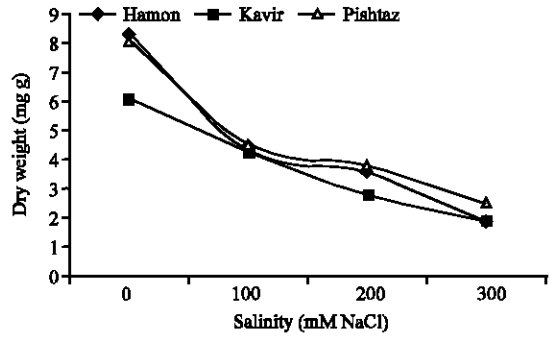


Fig. 6: $K^+$ content in leaves of three wheat cultivars at salinity levels

highest decreasing of $K^+$ content in its shoot. In $S_3$ treatment Hamon had the lowest and Pishtaz had the highest $K^+$ content among the cultivars (Fig. 6).

Compared to $K^+$, sodium content in the shoot was increased by each salinity level in the three cultivars. In the three cultivars and in the $S_3$ treatment, $Na^+$ content in shoot was 2.5 times than $S_1$. Among the cultivars, Hamon and Pishtaz had the highest and lowest $Na^+$ content in their shoots (Fig. 7).

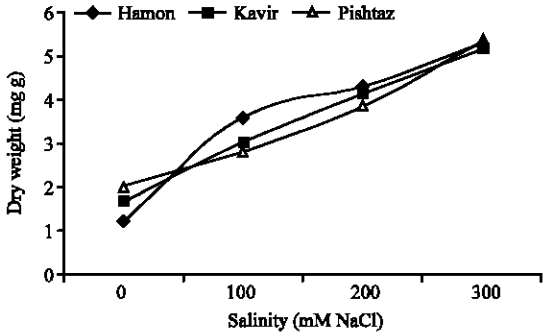


Fig. 7: $Na^+$ content in leaves of three wheat cultivars at salinity levels

## DISCUSSION

Even under optimal condition, reactive oxygen species including superoxide, hydrogen peroxide, hydroxyl radicals and single oxygen are metabolic by products of plant cell. These reactive oxygen species affect lipid peroxidation, protein denaturation and DNA mutation (Bowler *et al*., 1992). To remove reactive oxygen species, plant cells possess an antioxidant system consisting in low molecular weight antioxidants such as ascorbate, α-tocopherol, glutathione and carotenoids, as well as antioxidant enzymes. These include superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT) and glutathione reductase (GPX) (Nakano and Asada, 1981). In this study, present results showed the different antioxidant enzymes in three wheat cultivars in the control treatment which could be the result of genetic differences.

When the plants were subjected to salt stress, by increasing salinity level from control to 200 mM NaCl the increases in CAT activity was higher in the Pishtaz cultivar. After that in 300 mM NaCl, CAT activity was not affected by the salinity stress (Figure 1). This result is similar to reports of Sairam *et al.* (2002) in wheat.

In addition to CAT, the activities of the APX (Fig. 2) and GPX (Fig. 3) from three wheat cultivars were also affected by salinity levels. By application NaCl in the growing medium from 0 too 300 mM, the activities of leaf-APX and GPX in three wheat cultivars increased but among the cultivars Pishtaz cultivar had the lowest and highest APX and GPX activities, respectively.

Badawi *et al.* (2004) found a strong correlation between salt tolerance and APX activities in tobacco. However in this study Pishtaz had the lowest CAT in its leaf, but this cultivar had the highest APX and GPX activities in its leaf when subjected to salt stress.

A better understanding of physiological responses under drought and/or salinity may help in programs in which the objective is to improve the drought and/or salt tolerance of crop varieties. During the course of these stresses, active solute accumulation of compatible solutes such as amino acids, polyamines and carbohydrates is claimed to be an effective stress tolerance mechanism (Rosa-Ibarra and Maiti, 1995).

In this study present results showed that salinity affected compatible solutes and by increasing salinity levels from 0 to 300 mM NaCl proline and carbohydrate concentration in leaf green tissue of three wheat cultivars increased. Among the cultivars, Pishtaz cultivar had the highest carbohydrate (until 200 mM NaCl level) and proline concentration (Fig. 4, 5).

we found a positive correlation between proline accumulation and Leaf-APX ($r^2$ = 0.56), Leaf-GPX ($r^2$ = 0.63) and Leaf-CAT ($r^2$ = 0.73). These results indicated which antioxidant enzymes and compatible solutes help to plant adaptation when subjected to salt stress. Proline plays a protective role of subcellular structures and scavenging free radicals. Sucrose is accumulated in many plant tissues in response to environmental stress, including salinity for playing an osmoregulation role and cryoprotection (Balibrea *et al*., 1997). Under salinity stress osmotic adjustment by accumulation of compatible solutes such as amino acids and carbohydrate, can provide condition to continue water and nutrient uptake by plant.

In general, the content of potassium, essential ion for plant growth. In this study the $K^+$ contents shoot showed a marked decreased with the increase of salinity. Highly significant differences were absorbed among treatments and cultivars. The Pishtaz showed the maximum accumulative mean of four treatments and Kavir had the lowest $K^+$ contents in its shoot.

In the shoots of these cultivars, $Na^+$ showed an increase in concentration with salinity that approximately matches a decrease in $K^+$ concentration. It is well documented that NaCl depresses the accumulation of $K^+$ in whole plants (Al-Rawahy *et al*., 1992). It seems that $Na^+$ concentrations in the shoot may have had a more significant effect on plant antioxidant enzyme activities and compatible solutes such as proline and carbohydrates.

In this study, we found a positive correlation between $Na^+$ concentration in the shoots and the antioxidant enzyme activities and compatible solutes in the leaves. The findings match those of Schachtman *et al.* (1989) how showed that sensitivity to salt is correlated to $Na^+$ concentration in wheat shoots. The lower $Na^+$ concentration in shoot of Pishtaz cultivar compared to other cultivars indicated that Pishtaz cultivar can tolerant salinity stress better than other cultivars (Kavir and Hamon).

## CONCLUSION

Among the cultivars, Pishtaz cultivar had the best antioxidant enzyme activities and accumulate compatible solutes such as proline and carbohydrate in its shoot in all levels of salinity. This cultivar although had the highest $K^+$ and lowest $Na^+$ concentration in its shoot and it had the best salinity tolerance in this study.

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