



PHYSIOLOGICAL AND BIOCHEMICAL RESPONSES OF WHEAT CULTIVARS UNDER SALINITY STRESS

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ABSTRACT

Effects of NaCl salinity was studied in eight wheat cultivars, Shirazi, Toss, Roshan, Hirman, Bolani, Falat, Kavir and Star, grown under salt stress (nutrient solution containing 0, 100, 200 and 300 mM NaCl) conditions. The results revealed that salinity caused significant decreases in the growth parameters such as root and shoot dry weight. Toss and Falat cultivars had the highest decreasing. The negative effect of salinity on plant was due increasing Na⁺ and decreasing K⁺ content in the leaves. Bolani and Toss cultivars had the highest Na⁺ and lowest K⁺ content. In leaves of plants, ascorbate peroxidase (APX) and catalase (CAT) activity increased and guaiacol peroxidase (GPX) decreased with salinity. Changes in proline and soluble carbohydrate content in wheat cultivars showed that at 300 mM NaCl, Toss and Star cultivars had the highest carbohydrate and proline content. The results suggest that cv. Roshan, exhibits a better protection mechanism against salinity.

Keywords: wheat cultivars, salinity stress, growth, ions, antioxidant enzymes, compatible solutes.

INTRODUCTION

A wide range of environmental stresses (such as high and low temperature, drought, alkalinity, salinity, UV stress and pathogen infection) are potentially harmful to the plants (Van Breusegem *et al.*, 2001). Salt stress in soil or water is one of the major stresses especially in arid and semi-arid regions and can severely limit plant growth and productivity (Koca *et al.*, 2007). Salt stress causes inhibition of growth and development, reduction in photosynthesis, respiration, and protein synthesis and disturbs nucleic acid metabolism. Decrease in uptake of K⁺, Mg²⁺, Ca²⁺ and thereby decrease in growth at higher sodium concentration has also been reported (Koca *et al.*, 2007).

Accumulation of proline has been widely advocated for use as parameter of selection for salt stress tolerance. However, proline accumulation cannot be regarded as marker for salt tolerance, as it accumulates under various conditions of stresses such as temperature, drought, and starvation (Naik and Joshi, 1983). Where as in many salt stressed plants its levels decreases (Siddiqui and Krishnamoorthy, 1987). Plant salt tolerance has generally been 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 stresses, also leads to oxidative stress through an increase in reactive oxygen species (ROS), such as superoxide (O²⁻), hydrogen peroxide (H₂O₂) and hydroxyl radicals (OH[•]) (Neill *et al.*, 2002). These ROS are highly reactive and can alter normal cellular metabolism through oxidative damage to lipids, proteins and nucleic acids (Imlay, 2003).

Therefore, the aim of this study was to evaluate the effects of salt stress on the activity of antioxidative enzymes in leaves of eight wheat cultivars differing in salt tolerance, in order to better understand the physiological and biochemical mechanisms of salt tolerance.

MATERIALS AND METHODS

This study was conducted in a greenhouse at the University of Zabol, Iran during February–April, 2011. The experiment was laid-out a completely randomized 8×4 factorial design with three replicates. Seeds of eight wheat cultivars (Shirazi, Toss, Roshan, Hirman, Bolani, Falat, Kavir and Star) were grown in the containers with Hoagland nutrient solution. Plant were grown under greenhouse conditions with a 13 h photoperiod of natural daylight, maximum and minimum temperatures were 26°C and 18°C, respectively and relative humidity was 50% on average.

Three salinity treatments were imposed by adding S₀ = 0 (control), S₁ = 100, S₂ = 200 and S₃ = 300 mM NaCl to the nutrient solution after germination of seeds. Twenty days after salt treatment, 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 Flam photometer. Ascorbate peroxidase (APX) was assayed as described by method of Nakno and Asada (1981). Catalase (CAT) activity was assayed spectrophotometrically by monitoring the decrease in absorbance of H₂O₂ at 240 nm. CAT was measured according to the method of Sizer (1952) and total Guaiacol peroxidase (GPX) activity was determined as described by Urbanek *et al.*, (1991).

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 AND DISCUSSIONS

Plant growth and ion concentration

Effects of NaCl treatments on the growth of wheat cultivars are shown in Figures 1 and 2. Root and shoot dry weights were affected after exposing the plants

to NaCl (Table-1). The dry weight of cultivar Roshan showed the lowest and cultivar Toss showed the highest significant reduction in root among the cultivars tested compared to the unstressed plants. Others cultivars also showed a significant reduction in dry weight of root following the same tend (Figure-1). Salinity significantly decreased dry weight of shoot (Table-1). Cultivars Shirazi and Hirman had the lowest and cultivar Toss had the highest decreasing in shoot among the cultivars (Figure-2).

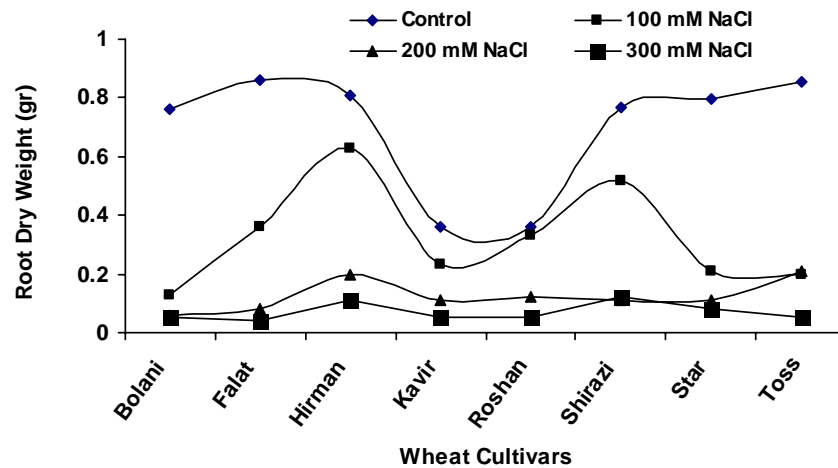


Figure-1. Root dry weight of wheat cultivars subjected to increasing salinity.

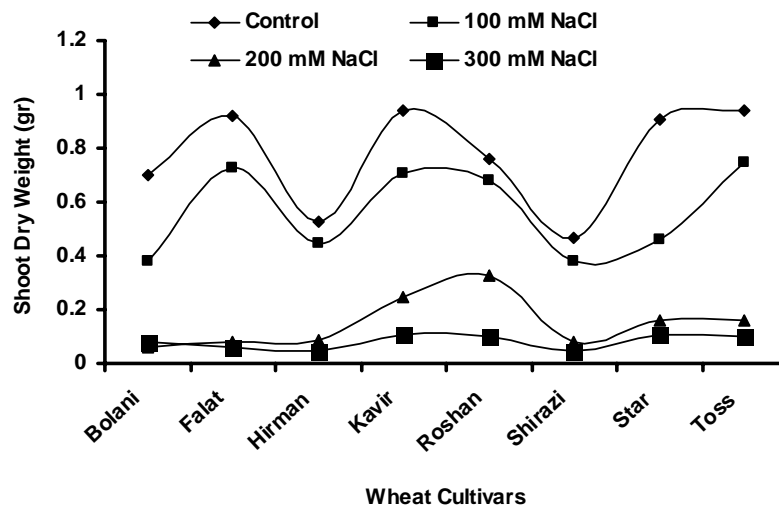


Figure-2. Shoot dry weight of wheat cultivars subjected to increasing salinity.

After exposure of the wheat cultivars to salinity treatment, sodium and potassium content in the leaves significantly affected by salinity application (Table-1), with a NaCl concentration of 300 mM in the nutrient solution, the Na⁺ content in leaves increased three to four-fold values compared to the corresponding control (Figure-3). The lowest Na⁺ values were obtained in the Falat and Roshan cultivars and the highest of Na⁺ content in 300 mM NaCl were obtained in the Toss and Bolani wheat cultivars (Figure-3). The data in Figure-4 show that 300mM NaCl treatment induced a significant

decrease in K⁺ uptake by wheat plants. Among the cultivars, Bolani and Hirman had the highest and Roshan cultivar had the lowest decreasing in K⁺ content in leaves at 300 mM NaCl (Figure-4).

Deleterious effects of salinity are thought to result from low water potentials, ion toxicities, nutrient deficiencies or a combination of these factors. Nutrient deficiencies can occur in plants when high concentrations of Na⁺ in the soil reduce the amounts of available K⁺, Mg²⁺ and Ca²⁺ or when Na⁺ displaces membrane-bound Ca²⁺ (Cramer *et al.*, 1985).



Table-1. Results of variance analysis (ANOVA) of NaCl concentrations (S), wheat cultivars (G) and their interaction for plant growth, ion, proline, soluble carbohydrate and chlorophyll content.

Dependent variable	Independent variable		
	Salinity (S)	Genotype (G)	S*G
Root DW	1.971**	0.091**	0.047**
Shoot DW	2.77**	0.139**	0.029**
Sodium	1.23**	0.25**	0.035 ^{ns}
Potassium	12.02**	0.49**	0.224**
Proline	218.73**	11.38*	0.72 ^{ns}
Carbohydrate	8.66**	8.24**	5.72**
APX	0.0001**	0.0029**	0.0008**
GPX	0.336**	0.172**	0.0186**
CAT	0.00085**	0.0010**	0.0004**
Chlorophyll	0.391**	0.119*	0.033 ^{ns}

Number represents F-values at 5% level

^{ns} Non-Significant, * and ** significant at P<0.05 and P<0.01

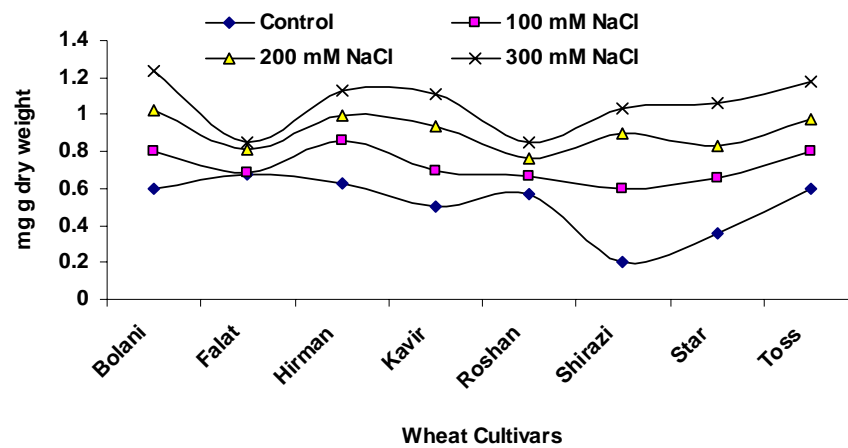


Figure-3. Na⁺ content of wheat cultivars subjected to increasing salinity.

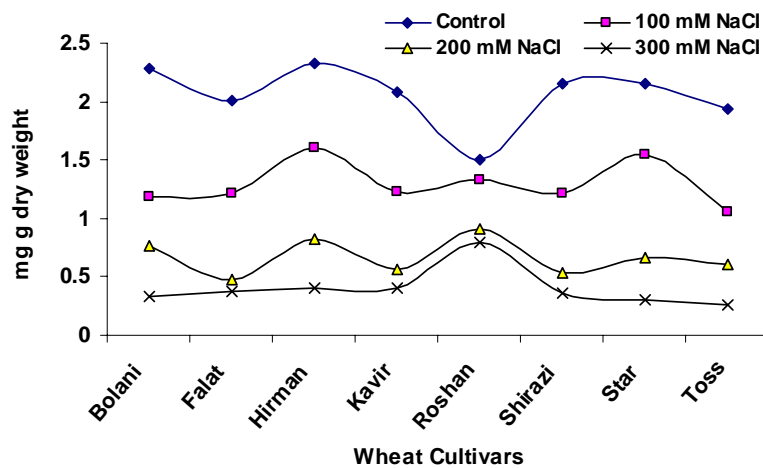


Figure-4. K⁺ content of wheat cultivars subjected to increasing salinity.



Activities of antioxidant enzymes

Results showed the activity of antioxidant enzymes are significantly changed with increased salinity treatment (Table-1). Figure-5 shows that CAT activity increased significantly in leaves of wheat cultivars in response to 100 mM NaCl, but except Roshan cultivar CAT activity increased until 200 mM NaCl. In S₂ (200 mM NaCl), salinity induced 86.9% increase in the active of this enzyme than control. Wheat cultivars grown at 200 mM NaCl (except Roshan cultivar), exhibited a slight decrease in CAT activity. Severe salt stress (300 mM NaCl), had significantly effect on CAT activity and decreased its activity (Figure-5).

As a result of salt stress, leaf-APX activity in eight wheat cultivars increased until S₃ (300 mM NaCl). Among the cultivars, Roshan cultivar had the highest APX activity and in S₃ salinity induced 76.4% increase in the active of this enzyme than control treatment. Among the cultivars, Shirazi cultivar had the lowest APX activity in this study (Figure-6). In addition to APX, the activities of GPX in total wheat cultivars

decreased by increasing salinity levels from 0 to 300 mM NaCl. Roshan cultivar had the lowest and Bolani had the highest decreasing leaf GPX-activity with increasing salinity levels (Figure-7). Salt stress accelerates the formation of active oxygen species. The lifetime of active oxygen species within the cellular environment is determined by the antioxidant system, which provides crucial protection against oxidative damage. The antioxidant system comprises numerous compounds of low molecular weights and enzymes (Noctor and Foyer, 1998). Our data show that APX, and CAT activities in leaves wheat cultivars play a central protective role in the O₂^{•-} and H₂O₂ scavenging process. Peroxidases (APX and GPX) are distributed throughout the cell and catalyze the reduction of H₂O₂ to H₂O. APX uses ascorbate as electron donor in the first step of the ascorbate - glutathione cycle and is considered the most important plant peroxidase in H₂O₂ detoxification (Noctor and Foyer, 1998).

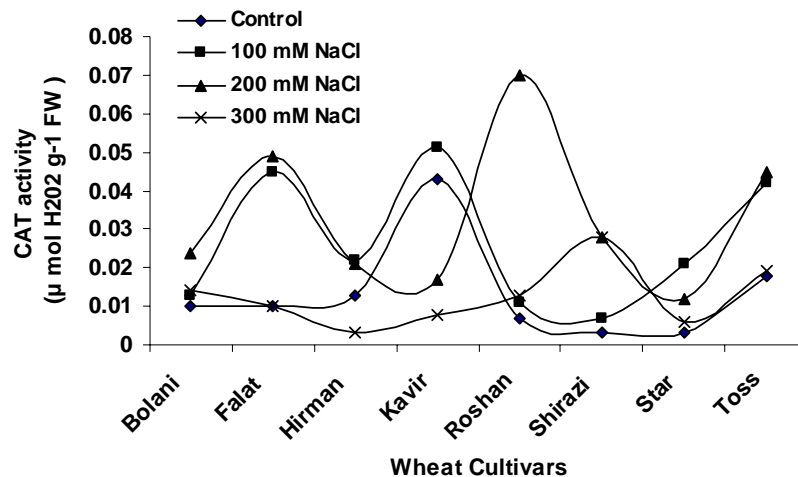


Figure-5. CAT activity of wheat cultivars subjected to increasing salinity.

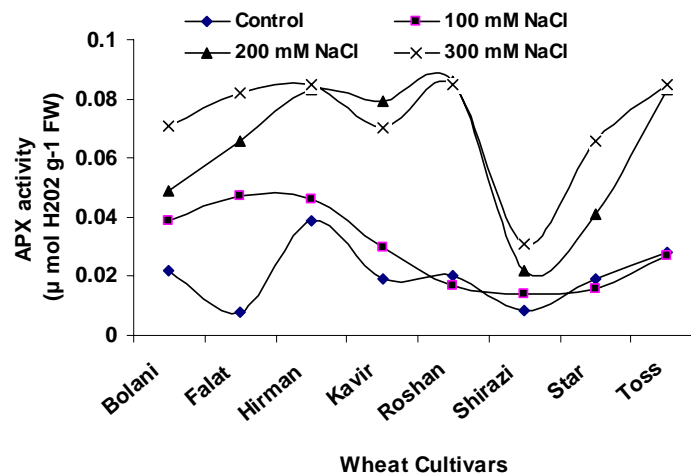


Figure-6. APX activity of wheat cultivars subjected to increasing salinity.



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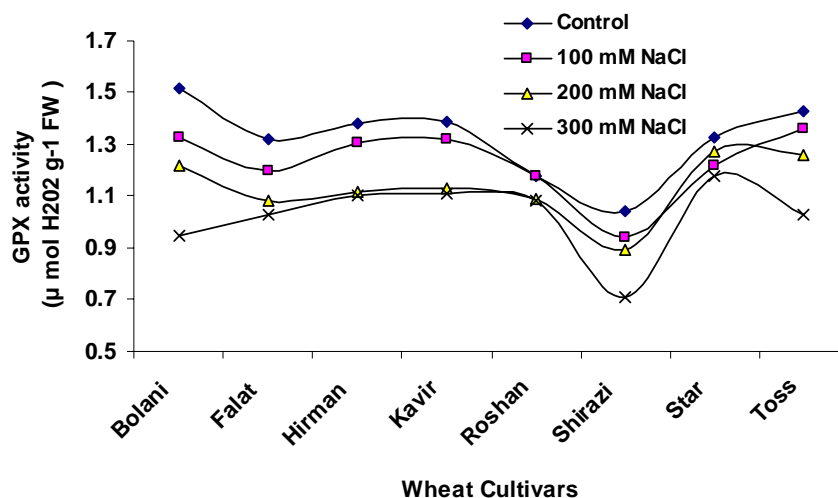


Figure-7. GPX activity of wheat cultivars subjected to increasing salinity.

Proline and soluble carbohydrate

By increasing salinity levels from 0 to 300 mM NaCl, proline content strongly increased in leaves of wheat cultivars. Although proline accumulated in all wheat plants were subjected to salinity stress, the highest content in 300 mM NaCl compared to control level, was found in Shirazi (45.6 %) and Hirman (60.1 %) cultivars (Figure-8).

The data herein obtained revealed that salinity significantly affected on soluble carbohydrates in leaves of wheat cultivars and stimulated its accumulation (Table-1). Soluble carbohydrates increased in all cultivars of wheat due to the addition of NaCl from 0 to 300 mM to the nutrient solution. The addition of 100 mM NaCl to the nutrient solution did not affect sugar content in all the genotypes (Figure-9). Soluble carbohydrates increased 34.4 % in Star and Toss, two of wheat cultivars in this study which had the highest soluble carbohydrates in their leaves in 300 mM NaCl compared to control level. Among the cultivars, Roshan

cultivar had the lowest (13.2 %) soluble carbohydrates in its leaves at 300 mM NaCl (Figure-9).

Ashraf and Harris (2004) reported that effects of salt stress on proline content, D1-pyrroline-5-carboxylate (P5C) reductase activity and water relations in plants were dependent on the leaf position. The proline level and P5C reductase activity were highest in young leaves and decreased linearly with increasing leaf age. It may be argued that the decrease in the proline content of wheat leaves cultivars observed, was due to the increasing age of the plants which induced by salinity stress.

Although our experiments did not discriminate between osmotic and ionic (nutritional imbalance and toxicity) effects of NaCl salinity, the obtained results showed that the differences in the antioxidative enzyme activities of leaves may, at least in part, explain the greater tolerance of Roshan cultivar to salt stress when compared to others cultivars, and that the APX and CAT activity level in leaves may be an important biochemical trait for salt stress tolerance in wheat plants.

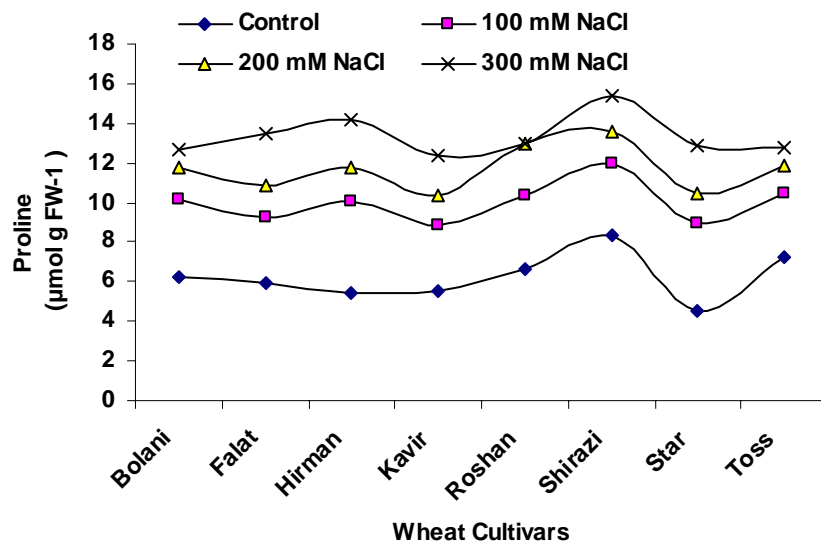


Figure-8. Proline content of wheat cultivars subjected to increasing salinity.

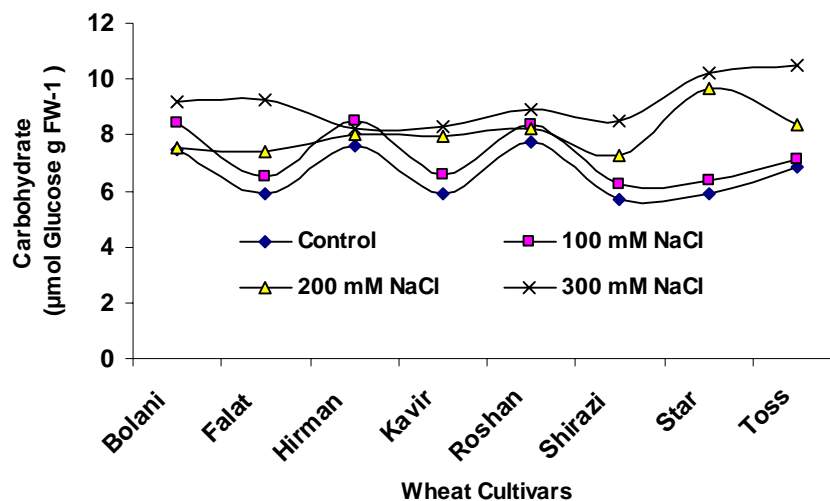


Figure-9. Carbohydrate content of wheat cultivars subjected to increasing salinity.

ACKNOWLEDGEMENTS

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