

Araştırma Makalesi/Research Article (Original Paper)

Evaluation of the Salinity Effects on Some Physiological and Biochemical Characteristics of Two Wheat Cultivars

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Abstract: Salinity is one of the most important abiotic stresses reducing wheat yield in most parts of the world. This study was conducted to evaluate the response of two wheat cultivars namely; 'Karkheh' (salt sensitive) and 'Golestan' (salt tolerant) to salinity stress grown in hydroponic system. The seedlings with 4-5 leaves were incubated under two conditions; lack of salinity (control) and salinity (200 mM NaCl) for 14 days. The results showed significant increase for activities of antioxidant enzymes such as catalase (CAT), ascorbate peroxidase (APX) and guaiacol peroxidase (GPX) in 'Golestan' under salinity. Furthermore significant increase in CAT activity was found in 'Karkheh' under saline situations compared the control. Salt stress elevated the amounts of malondialdehyde (MDA) in both cultivars. However, the increasing rate in 'Karkheh' was more than (two times) that of 'Golestan'. Moreover, unlike with 'Karkheh', membrane stability index was not affected by salinity in 'Golestan'. Both cultivars displayed increasing trend in Na⁺ content and Na⁺/K⁺ ratio. However, Na⁺/K⁺ ratio of 'Golestan' was greater than one due to salinity. Also, this cultivar obtained significantly higher amounts for soluble sugars content under salt stress compared to control. The studied parameters elucidated that salt resistance of 'Golestan' might be due to increased activity of antioxidant enzymes, low lipid peroxidation, assumingly minimum change in membrane stability index and more Na⁺/K⁺ ratio along with significant increase in soluble sugars and its probable ability to isolate Na⁺ in vacuoles.

Key words: Antioxidant enzymes, Oxidative stress, Salinity, Wheat

Tuzluluğun İki Buğday Çeşidinin Bazı Fizyolojik ve Biyokimyasal Özellikleri Üzerine Etkilerinin Değerlendirilmesi

Özet: Tuzluluk, dünyanın birçok bölgesinde buğday verimi azaltan en önemli abiyotik stres koşullarından birisidir. Bu çalışma, hidroponik sistem ortamında iki buğday çeşidinin [Karkheh (tuza duyarlı) ve Golestan (tuza tolerant)] tuz stresine karşı reaksiyonlarını değerlendirmek amacıyla yürütülmüştür. 4-5 yapraklı fideler 14 gün boyunca tuzsuz ve tuzlu (200 mM NaCl) koşullara tabii tutulmuştur. Sonuçlar, tuz stresi altında katalaz(CAT), askorbat peroksidaz(APX) ve GPX gibi antioksidan enzimlerin Golestan çeşidinde önemli bir artışa neden olduğunu göstermiştir. Ayrıca, Karkheh çeşidinde kontrolle karşılaştırıldığında CAT aktivitesinde önemli bir artış bulunmuştur. Tuz stresi her iki çeşitte de MDA(Malondialdehit) miktarını arttırmıştır. Bununla birlikte Karkheh çeşidindeki artış oranı Golestan çeşidine göre iki kattan daha fazla olmuştur. Ayrıca, Karkheh çeşidinin aksine Golestan çeşidinde membran stabilite indeksi tuzluluktan etkilenmemiştir. Her iki çeşit, Na⁺ içeriğinde ve Na⁺ / K⁺ oranında artış eğilimi göstermiştir. Bununla birlikte Golestandaki Na⁺ / K⁺ oranı Golestan çeşidinde Karkheh fazla olmuştur. Ayrıca, bu çeşit kontrolle karşılaştırıldığında önemli ölçüde çözünebilir şeker miktarına sahip olmuştur. Araştırma sonunda incelenen parametreler Golestan çeşidinde tuz toleransı nedeni olarak antioksidan enzim aktivitesinde artış, düşük yağ peroksidasyonu, membran stabilite indeksinde minimum değişiklik ve Na⁺/K⁺ oranının yüksek olmasının yanı sıra çözünür şeker miktarında önemli bir artış ve vakuollerdeki Na⁺ izolasyon yeteneğine bağlı olabileceğini göstermektedir.

Anahtar kelimeler: Antioksidan enzimler, Buğday, Oksidatif gerilim, Tuzluluk

Introduction

Salinity has been defined as one of the major abiotic stresses that adversely affect crop germination, growth, productivity and quality (Sairam et al. 2002; Chinnusamy and Zhu, 2003). Sodium chloride (NaCl) is the predominant form of salt in most saline soils (Zörb et al. 2004; Tejera et al. 2006).

Salinity decreases soil water potential and causes osmotic stress (Chinnusamy and Zhu, 2003; Karlberg et al. 2006). Osmotic un-adjustment imposes on the plants the subsequent secondary drought stress and reduces their ability to obtain nutrients from the root medium (Sairam et al. 2002). Furthermore, salinity directly affects nutrient uptake. It causes not only high sodium (Na^+) and chloride (Cl^-) accumulation in plants, but also antagonistically affects the uptake of essential nutrient elements such as potassium (K^+), calcium (Ca^{2+}) and magnesium (Mg^{2+}) in competition with Na^+ and also nitrate (NO_3^-) in contrast with Cl^- (Sairam et al. 2002; Zörb et al. 2004; El-Hendawy et al. 2005). Moreover, salt stress increases the saturation degree of membrane fatty acids and leads to conformational changes and loss of function of membrane proteins. Changes in the membrane structure/ composition in turn enhance plasma membrane permeability (Azizpour et al. 2010).

Another problems resulting from salinity lead to the reduced water absorption and gas exchanges in plant (Munns and Tester 2008). Furthermore, ionic and osmotic stress resulting from salinity goes to oxidative stress in plant cells due to the increased production of ROS(Reactive Oxygen Species)(Katsuhara et al. 2005; Hussain et al. 2008). ROS are partially reduced forms of molecular oxygen which are produced in plants common ongoing processes such as photorespiration, photosynthesis and respiration (Mittler et al. 2004). In order to water assembly in these processes, four electrons are required for complete reducing of oxygen. ROS typically result from the transference of one, two and three electrons, respectively, to O_2 to form O_2^- , H_2O_2 and $\text{HO}\cdot$ (Mittler 2002). These oxygen species are highly cytotoxic and can seriously react with vital biomolecules such as lipids, proteins, nucleic acids and etc., causing lipid peroxidation, protein denaturing and DNA mutation, respectively (Katsuhara et al. 2005; Esfandiari et al. 2007b). The resulting injuries lead to metabolic disorders which by itself highlights the crucial roles of biomolecules in cellular structure and metabolism.

Furthermore, the damages resulting from ROS as well as other bio-toxic compounds (such as 4-hydroxynonenthal) leads to oxidation of biological substances contribute to planned cellular death (Marrs 1996). These toxic compounds may be generated even when the environmental conditions are favorable (Edreva 2005). That is why plant cells must be equipped with specific defense mechanisms to combat the pernicious effects of these compounds whether in normal or stress conditions (Asada 2000).

Fortunately, plants have evolved various protective mechanisms to eliminate or reduce ROS, which are effective at different levels of stress-induced conditions. Enzymatic antioxidant system such as SOD(superoxid dismutase) is one of the protective mechanisms found in various cell compartments, catalyses the disproportionation of two O_2^- radicals to H_2O_2 and O_2 (Scandalios, 1993). Moreover, H_2O_2 is eliminated by various antioxidant enzymes such as CAT(Catalase) and POX(Peroxidase) which convert H_2O_2 to water. Other enzymes with functional role in ROS scavenging system are GR(Glutathione Reductase), MDHAR(Monodehydroascorbate Reductase) and DHAR(Dehydroascorbate Reductase) located at ascorbate-glutathione cycle.

Considering the wide distribution of salinity across agricultural lands, and important nutritional role of wheat in human diet, necessitate related works on salinity response evaluation. Salt stress, as illustrated above, negatively affects various stages of plant development, thereby threatening food security of human society. Furthermore, knowledge on physiological behavior of cells is necessary to overcome environmental restrictions and guarantee food security. In the present study the effects of NaCl salinity stress were investigated on antioxidant defense dynamics of two wheat cultivars.

Materials and Methods

Plant Material and Induction of Salt Stress

Seeds of two bread wheat [*Triticum aestivum* L.], cultivars, 'Karkheh' (salt sensitive) and 'Golestan' (salt tolerant) were obtained from the Seed and Plant Improvement Institute, Karaj, Iran. They were surface sterilized with 10% H₂O₂ for 20 minutes. Then, the seeds were washed out several times with deionized water. The sterilized seeds were germinated in 25 °C and dark conditions on filter paper for two days. Seedlings were hydro-cultured initially in aerated water and were grown inside the growth chamber under light conditions of 16:8 light and darkness, 25 °C, 65% relative humidity and light intensity of 6000 Lux. The source of light inside the growth chamber was a combination of yellow and white florescent lamps (Azizpour et al., 2010). Seven and 14 days after germination of the seeds, the tap water was replaced by half and full-strength Hoagland's nutrient solution (Hoagland and Arnon, 1950) respectively. The composition all amounts the nutrients in solution in mmol were 1 calcium nitrate [Ca(NO₃)₂] 4H₂O]; 0.1 mono-potassium phosphate (KH₂PO₄); 0.5 potassium sulfate (K₂SO₄); 0.5 magnesium sulfate (MgSO₄) and in μmol for boric acid, 10 (H₃BO₃); manganese chloride, 20 (MnCl₂ 4H₂O); zinc sulfate, 0.5 (ZnSO₄ 7H₂O); copper sulfate, 1 (CuSO₄ 5H₂O); molybdenum trioxide, 0.1 (MoO₃), and iron sulfate, 100 (FeSO₄ 7H₂O). These solutions were continuously aerated by electrical pumps (Resun, AC 9904, China) and renewed every three days.

The pH of the nutrient solution was measured by a pH meter (HANNA, HI9811, Hanna Instruments, Padova, Italy) and adjusted to 5.5 by adding 1 N sulfuric acid (H₂SO₄). Once the seedling grew up to 4 to 5-leaf stage, salt stress was applied by using 200 mM NaCl. Plants were kept under stress conditions for 14 days. Then full expanded leaf samples were transferred to liquid nitrogen and maintained at -20°C until measurement of biochemical parameters. At the same time, samples for membrane stability index assay collected from fully expanded leaves were brought into the laboratory in ice buckets. For measurement of physiological parameters the second group of fully expanded leaves was dried at 70°C during two days.

Measuring physiological and biochemical parameters

The MSI (Membrane Stability index), Na⁺, K⁺ and soluble sugars content were determined according to the methods presented by Azizpour et al. (2010), Bandehhag et al. (2004) and Sairam et al. (2002), respectively. Antioxidant enzymes were extracted according to the method developed by water stress, antioxidant enzyme activity and lipid peroxidation in wheat seedling (Esfandiari et al 2007b). SOD, CAT, GPX (Guaiacol Peroxidase), APX (Ascorbate Peroxidase) and the amount of lipid peroxidation were measured through the methods offered by Sen Gupta et al. (1993), Aebi (1984), Panda et al. (2003), Yoshimura et al. (2000) and Stewart and Bewley (1980), respectively.

Statistical analysis

The factorial experiment (cultivar and salinity as factors) based on completely randomized design was carried with five replications. All physiological and biochemical parameters were recorded. The data were analyzed with MSTATC software. Mean comparison were carried out by LSD method (Steel et al., 1996).

Results and Discussion

The amount of MDA (Malondialdehyde) significantly increased compared to control due to salinity in both studied cultivars. This increase in the case of 'Karkheh' was approximately twice as much as that in 'Golestan' (Fig 1A). Moreover, the MSI content was meaningfully decreased in the case of 'Karkheh' compared to control. The level of MSI did not vary in 'Golestan' (Fig 1B).

In both studied cultivars SOD activity was significantly decreased compared to the control under salinity conditions (Fig 2A). Furthermore, APX and GPX activities were statistically higher in 'Golestan' under salinity conditions. Meanwhile, in 'Karkheh', the activities of these enzymes were low compared to control (Fig 2B and C). Under salinity condition, the activity of CAT, another H₂O₂ scavenger, was predominantly increased in both cultivars compared to control (Fig 2D).

Salinity caused a notable increment in total soluble sugars content in 'Golestan'. But its variation was not significant in 'Karkheh' (Fig 3A). K^+ content of 'Golestan' decreased due to salinity ($P \leq 5\%$). But salt stress did not affect the K^+ level in 'Karkheh' (Fig 3B). Na^+ content of both cultivars increased compared to the control due to salinity ($P \leq 5\%$). It is worthy of note that Na^+ content of 'Karkheh' was about 5 and 2.5 folds higher than control and 'Golestan' respectively (Fig 3C). There was a declining pattern in K^+/Na^+ ratio in both cultivars under salinity stress conditions compared to control (Fig 3D). Moreover, K^+/Na^+ ratio in 'Golestan' was significantly higher than 'Karkheh' at salinity conditions. SOD activity was significantly dropped at salt stressed plants in both cultivars. The decrease in SOD activity level leads to the accumulation of O_2^- radical. This active oxygen radical is extremely cytotoxic, invading vital metabolisms in cells. The increase in the amount of this radical causes photosynthesis to diminish as a result of destroying protein D1 in PSII.

H_2O_2 is among the most deteriorate types of ROS which are produced in vital processes in the cell. The augmentation of H_2O_2 is liable to damage special positions in the cell, leading to metabolic disturbances including inactivity or lack of activity of bisphosphatase and ribulose monophosphate kinase, the two key enzymes involved in Calvin cycle (Yamazaki et al. 2003). Any interruption in the activity of these two enzymes may lower the CO_2 fixation. As a consequence, the $NADP^+/NADPH$, H^+ ratio will come down due to the imbalance between the output of photo-stage in photosynthesis and the consumption of them in Calvin cycle (Vaidyanathan et al. 2003; Esfandiari et al. 2007b). Reduced $NADP^+/NADPH$, H^+ ratio, leads to cease in electron transfer chain and subsequently higher and renewed ROS production within chloroplast.

Many researchers have been related the increased activity of antioxidant enzymes to a higher level of resistance against salt stress (Dalton et al. 1994; Milone et al. 2003; Srivalli et al. 2003; Costa et al. 2005; Koca et al. 2006; Esfandiari et al. 2007a; Gapinska et al. 2008; Mahmoud et al. 2009). The reason is that the amount of ROS in plant cells is restricted as a result of any increase in the activity of antioxidant enzymes which, in turn, mitigates the damages imposed on vital bio-molecules and metabolic disturbances explicated above. Hence, the cell is restored to a better conditions and oxidative stress is prevented. Srivalli et al. (2003), Costa et al. (2005), Koca et al. (2005), Gapinska et al. (2008) and Mahmoud et al. (2009) have asserted that increased activity of POX and CAT contributed to the plant resistance against salt stress. Similarly in this study, the activity of APX and GPX diminished significantly in the case of salinity-sensitive cultivar, 'Karkheh' (Fig 2B, C). Since, APX is active in both glutathione-ascorbate (Halliwell, 2006) and Mehler (Asada, 2000) cycles any decrease in its activity led to the lower efficiency at these two cycles in terms of 'Karkheh'.

Our result regarding in increased lipid peroxidation in wheat cultivars are in agreement with the findings of Morita et al. (1994); Gomez et al. (1999); Sairam and Srivastava (2002); Mahmoud et al. (2009) in rice, pea, wheat, potato, respectively. Membranes damage in 'Karkheh' is assumed to be due to diminished activity of O_2^- and $H_2O_2^-$ scavenging enzymes and the subsequent accumulation of these toxic molecules. In the case of 'Golestan', in spite of added lipid peroxidation (Fig1A), the evident increase in the activity of antioxidant enzymes exceptionally SOD (Fig2A-D), is indicative of the fact that toxic and damaging metabolites have overcome the defense mechanisms. Moreover, Esfandiari et al. (2007b) Reported that ascorbate had the potential to scavenge the O_2^- as well as this antioxidant is potent to prevent oxidative damage under low SOD activity situation.

Salinity caused depletion of potassium and concomitant increase in sodium content (Fig 3A-B). Sairam et al. (2002) and Azizpouer et al. (2010) have previously reported similar results in wheat. Meanwhile, leaf potassium concentration decreased due to salinity, potassium content in 'Karkheh' generally seemed to be unaffected. 'Golestan' had more potassium content compared to 'Karkheh' under salinity condition (Fig 3A). El-Hendawy et al. (2005) speculated that more potassium content observed in the salt-resistant genotypes might explain their higher tolerance to salinity. In both cultivars of wheat, salinity increased sodium content. Earlier studies have demonstrated that salt tolerance is not necessarily correlated with the content of leaf sodium in several plant species, including rice (Yeo and Flowers, 1983), maize (Cramer et al. 1994), and cotton (Leidi and Saiz, 1997). Similarly, Munns and James (2003) reported that several salt-tolerant tetraploid wheat genotypes do indeed demonstrate very high leaf sodium levels under saline sodic conditions. These genotypes may have a special ability to tolerate high internal levels of sodium. The higher concentration of sodium may result from greater capabilities for compartmentation of this ion within the vacuoles. It seems, therefore, that 'Golestan' had the ability to sequester sodium into the

vacuole more efficiently than ‘Karkheh’, and thus avoided sodium toxicity of the cytoplasm. According to Wyn Jones et al. (1979) potassium/sodium ratio for non-halophytes should be higher than one for normal functioning of all metabolic processes. In the present study, above ratio increased in both varieties by salt treatment, although value was higher than 1 in the tolerant cultivar, ‘Golestan’ (Fig 3C). Potential for maintenance of high potassium content may act as the major cationic osmoticum in the presence of external salt (Reggiani et al. 1995; Baalbaki et al. 2000; Chartzoulakis and Klapaki, 2000). Furthermore, vacuolar sequestration of sodium is another important strategy for osmotic adjustment (Niu et al. 1995; Blumwald et al. 2000). Although the use of ions for osmotic adjustment may be energetically more favorable than biosynthesis of organic osmolyte under salt stress, many plants accumulate organic osmolytes (Chinnusamy et al. 2005) to protect their cells by balancing the osmotic pressure of cytosol with that of vacuole and external environment (Gadallah, 1999). In the present study, in agreement with Misra and Dwivedi (2004) an important effect of higher osmolyte concentration was reflected by maintenance of higher MSI under salinity stress in ‘Golestan’ (Fig 1B). Decrease in MSI under salinity conditions has been reported by Bhattacharjee and Mukherjee (1996) and Azizpour et al. (2010) in *Amaranthus lividus* and wheat, respectively. Similar to our results, other studies have indicated that salt tolerant cultivars always show lower decrease in the MSI as compared to salt sensitive cultivars in the saline environments (Mansour and Stadelmann, 1994; Mansour and Salama, 1996; Mansour, 1997).

Conclusions

In conclusion, at the present study the main reasons for salt tolerance in ‘Golestan’ may be due to the increase in contents of some of antioxidant enzymes especially H_2O_2 scavenging ones as well as higher K^+/Na^+ ratio under saline situation. Furthermore, great potential of ‘Golestan’ for soluble sugars accumulation and also its high sodium blockage in vacuole are other aspects of salinity tolerance. The final role of the above mentioned physiological parameters would be the appropriate control of cell damaging factors.

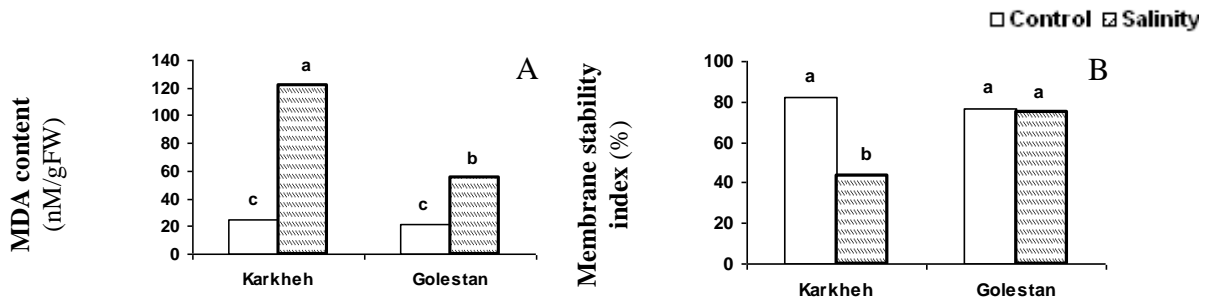


Fig 1: The effect of salinity on malondialdehyde content (A) and membrane stability index (B) in two wheat cultivars.

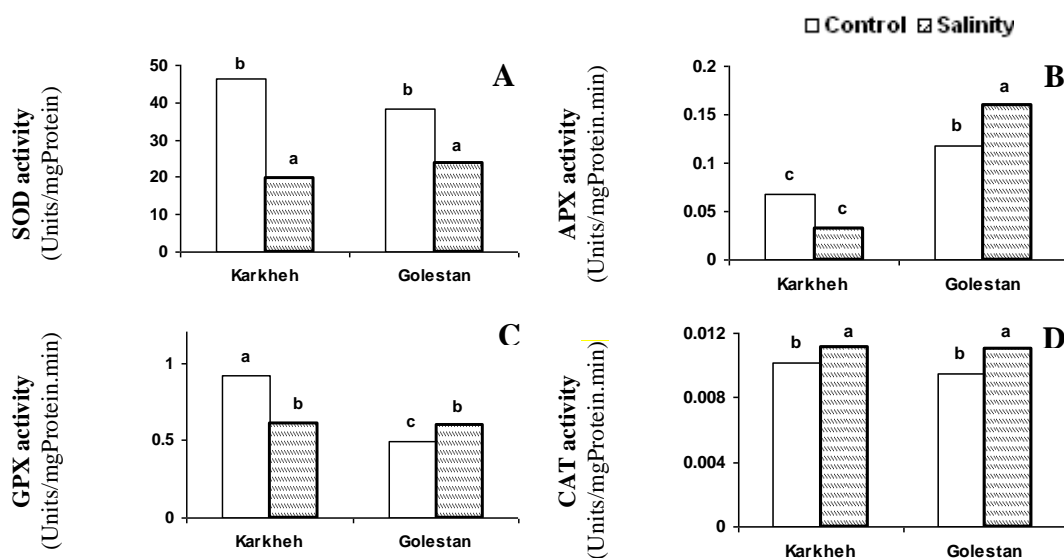


Fig 2: The effect of salinity on antioxidant enzymes activity in two wheat cultivars.

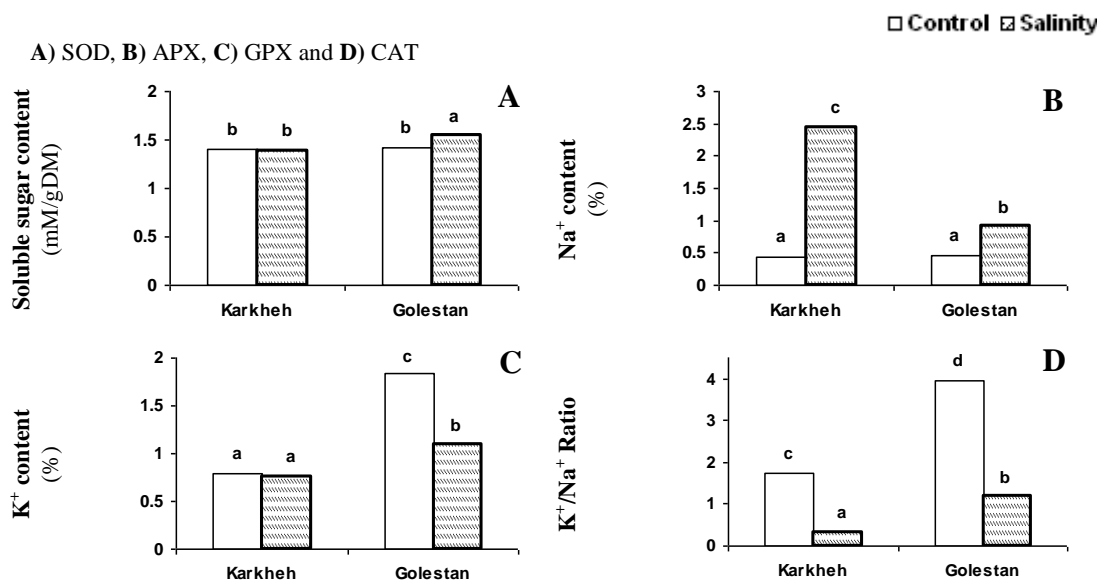


Fig 3: The effect of salinity on Sugar (A), Na⁺ (B), K⁺ (C) and K⁺/Na⁺ (D) in two wheat cultivars.

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