Biochemical and Physiological Changes in Response to Salinity in Two Durum Wheat (*Triticum turgidum* L.) Genotypes

Ezatollah ESFANDIARI, Vaghef ENAYATI, Amin ABBASI

University of Maragheh, Faculty of Agriculture, Department of Agronomy and Plant Breeding, Madar Square, Daneshgah Avenue, Maragheh, 55181-83111, Iran; esfand1977@yahoo.com (corresponding author)

Abstract

The effects of salt stress on the activity of antioxidative enzymes, some oxidative stress indices and Na\(^+\) and K\(^+\) content were studied in leaves of two durum wheat cultivars, 'Egypt 449' (salt-tolerant) and 'Syria 371' (salt-sensitive), grown under control (nutrient solution) or salt stress (nutrient solution containing 200 mM NaCl) conditions. Leaves of control and salt-stressed plants were harvested from 10 days old plants beyond salt treatment. The results showed significant increase for activities of antioxidant enzymes such as ascorbate peroxidase (APX) and guaiacol peroxidase (GPX), in 'Egypt 449' under salinity. At the same time, in cultivar 'Egypt 449', activity of SOD and CAT were not changed. Meanwhile, under salinity condition the activities of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and GPX in cultivar 'Syria 371' was lower than control. There was no significant difference between salinity situation and control ones regarding APX activity. Salt stress elevated the amounts of malondialdehyde (MDA) in both cultivars. However, the increasing rate in 'Syria 371' was more than (four times) that of 'Egypt 449'. Membrane stability index (MSI) of both cultivars negatively influenced by salinity. This negative impact on 'Syria 371' was highlighted than on 'Egypt 449'. Hydrogen peroxide (H\(_2\)O\(_2\)) content of salinity faced 'Syria 371' was higher than control. Both cultivars displayed increasing and decreasing trend for Na\(^+\) and K\(^+\) content, respectively. Moreover, K\(^+\)/Na\(^+\) ratio was decreased in both cultivars due to salinity. The studied parameters elucidated that salt resistance of 'Egypt 449' might be due to increased activity of antioxidant enzymes, low lipid peroxidation, assumingly lower changes in membrane stability index and avoidance of Na\(^+\) absorption.

**Keywords:** antioxidant enzymes, salinity, oxidative stress, wheat

Introduction

Soil salinity is a major abiotic stress adversely affects physiological and metabolic processes, leading to diminished growth and yield (Azizpour *et al*., 2010). Salinity affects the availability of nutrients and water. Moreover, it induces osmotic stress; the physiological drought, which typically reduces the growth and photosynthesis in plants (Munnes and Tester, 2008). Growth reduction due to salinity is also attributed to ion toxicity and nutrient imbalance, which 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). Salt stress in addition to the known functions of osmotic stress and ion toxicity, is also manifested as an oxidative stress (Esfandiari *et al*., 2007a).

One of the biochemical changes encountered in plants subjected to salinity is the production of reactive oxygen species (ROS). The main sites of ROS production in the plant cell during salinity are the organelles with highly oxidizing metabolic activities or with sustained electron flow; chloroplasts, mitochondria and peroxysome (Esfandiari *et al*., 2007b). ROS are highly reactive and when the plant capacity for scavenging of those molecules is less than ROS production rate they can seriously disrupt normal metabolism through oxidative damages on lipids, proteins and nucleic acids (Murillo-Amador *et al*., 2006). Plants posses a number of antioxidant enzymes such as SOD, CAT, peroxidase (POX) that protect plant cells them from these potential cytotoxic effects (Edreva, 2005). SOD is a major scavenger of O\(_2\)\(^.-\) and its enzymatic action results in the formation of H\(_2\)O\(_2\) (Sen Gupta *et al*., 1983). CAT and POD catalyze the breakdown of H\(_2\)O\(_2\). Therefore, these enzymatic systems eliminate the damaging effects of toxic oxygen species (Katsuhara *et al*., 2005).

Plant species adapt themselves to high salt concentrations in the soils with the compartmentation of inorganic molecules in the vacuole as well as accumulation of organic solutes in the cytoplasm and other organelles (Chinnusamy *et al*., 2005; Tejera *et al*., 2006). Cations such as K\(^+\) and Na\(^+\) are known to be the major inorganic elements, which provide necessary osmotic potential for water uptake by plant cells (Tejera *et al*., 2006). Regulation of K\(^+\) uptake beside prevention of Na\(^+\) entry and efflux of Na\(^+\) from the cell, and furthermore sequestration of Na\(^+\) in vacuole for osmotic adjustment are the common strategies for maintenance of desirable K\(^+\)/Na\(^+\) ratios in...
the cytosol. A high K+/Na+ ratio in the cytosol is essential for normal cellular functions of plants (Chinnusamy et al., 2005). The accumulation of compatible solutes such as proline, glycinebetaine and soluble sugars, which are non-toxic at higher concentrations to cytoplasmic functions, allows additional water uptake from the environment and subsequently turgor maintenance (Sairam et al., 2002). These compounds regulate cellular redox potential, preserve spatial conformation of proteins and integrity of membranes stabilize the function of some fundamental biochemical routes such as oxygen evolving PS-II complex (Chinnusamy et al., 2005) and finally enhance the activity of some related enzymes to scavenge the reactive oxygen species (Chinnusamy and Zhu, 2003).

Although durum wheat cultivars are more salt sensitive than bread wheat and their yield is lower under saline soils (Munns and James, 2003); the quality requirements of pasta are only satisfied by durum. For this reason breeding new cultivars of durum wheat capable of that can be grown on saline soils is of great interest. Understanding of salt tolerance mechanism and development of salt tolerant plants can be facilitated by the use of molecular, biochemical and physiological markers/traits.

The objective of the present study was to elucidate the differential physiological and biochemical responses of a salt-tolerant and a salt-sensitive variety of durum wheat to salinity under hydroculture conditions.

Material and methods

Plant material and induction of salt stress

Seeds of two durum wheat [Triticum turgidum L. subsp. durum (Desf.) Husn.] cultivars; ‘Egypt 449’ (salt tolerant) and ‘Syria 371’ (salt sensitive) were obtained from the International Center for Agricultural Research in the Dry Areas (ICARDA). Seeds were sterilized with 0.1 % SDS by stirring them for 20 minutes. Then, the seeds were washed out several times with deionized water and were germinated on filter paper at 25°C under dark conditions for three days. Seedlings were germinated on filter paper at 25°C under dark conditions, were taken in test tubes from each enzyme sample.

Enzyme extraction

Enzyme activity assay

SOD activity was estimated by recording the decrease in absorbance of superoxide-nitro blue tetrazolium complex by the enzyme (Sairam et al., 2002). About 3 ml of reaction mixture, containing 0.1 ml of 200 mM methionine, 0.01 ml of 2.25 mM nitro-blue tetrazolium (NBT), 0.1 ml of 3 mM EDTA, 1.5 ml of 100 mM potassium phosphate buffer, 1 ml distilled water and 0.05 ml of enzyme extraction, were taken in test tubes from each enzyme sample. Two tubes without enzyme extract were taken as control. The reaction was started by adding 0.1 ml riboflavin (60 μM) and placing the tubes below a light source of two 15 W florescent lamps for 15 min. Reaction was stopped by switching off the light and covering the tubes with black cloth. Tubes without enzyme developed maximal color. A non-irradiated complete reaction mixture which did not develop color served as blank. Absorbance was recorded at 560 nm and one unit of enzyme activity was taken as the
quantity of enzyme which reduced the absorbance reading of samples to 50% in comparison with tubes lacking enzymes.

CAT activity was measured according to Aebi (1984). Reaction mixture contained 100 mM potassium phosphate buffer (pH 7), 75 mM H₂O₂, enzyme extract and distilled water. Reaction started by adding H₂O₂ and the decrease in absorbance was recorded at 240 nm (ε= 36 mM⁻¹ cm⁻¹) for 1 min. Enzyme activity was computed by calculating the amount of H₂O₂ decomposed.

APX activity was measured according to Yoshimura et al. (2002) by monitoring the rate of ascorbate oxidation at 290 nm (ε=2.8 mM⁻¹ cm⁻¹). The reaction mixture contained 25 mM phosphate buffer (pH 7), 0.1 mM EDTA, 1 mM H₂O₂, 0.25 mM reduced ascorbate (AsA) and the enzyme sample. No change in absorption was found in the absence of AsA in the test medium.

GPX activity was measured according to Panda et al. (2003). Reaction mixture contained 100 mM potassium phosphate buffer (pH 7), 0.1 mM EDTA, 5mM guaiacol, 15 mM H₂O₂ and enzyme sample. The enzyme produced a colorful product by using H₂O₂ and guaiacol as substrates. The absorbance of the product was monitored at 470 nm (ε= 26.6 mM⁻¹ cm⁻¹), and peroxidase activity was expressed as units/mg protein.

MDA was measured by colorimetric method. 0.5 g of leaf samples were homogenized in 5 ml of distilled water. An equal volume of 0.5% thiobarbituric acid (TBA) in 20% trichloroacetic acid (TCA) solution was added and the sample incubated at 95°C for 30 min. The reaction stopped by putting the reaction tubes in an ice bath. The samples were then centrifuged at 10000×g for 30 min. The supernatant was removed, absorption read at 532 nm, and the amount of nonspecific absorption at 600 nm read and subtracted from this value. The amount of MDA present was calculated from the extinction coefficient (ε= 155 mM⁻¹ cm⁻¹) (Stewart and Bewley, 1980).

Hydrogen peroxide levels were determined according to Sersgine et al. (1997). Leaf tissues (0.5 g) were homogenized in ice bath with 5 ml 0.1% (w/v) TCA. The homogenate was centrifuged at 12000×g for 15 min and 0.5 ml of the supernatant was added to 0.5 ml 10 mM potassium phosphate buffer (pH 7.0) and 1 ml 1 M KI. The absorbancy of supernatant was read at 390 nm. The content of H₂O₂ was given on standard curve.

Protein content of samples was determined by method of Bradford (1976). Bovine serum albumin was used as a standard.

MSI was determined by recording the electrical conductivity of leaf ions leaching in double distilled water (Sairam and Srivastava, 2002). Leaf samples (0.1 g) were taken in test tubes containing 10 ml of double distilled water in two sets. One set was kept at 40°C for 30 min and another set at 100°C in boiling water bath for 15 min and their respective electrical conductivities, C1 and C2, were measured by a pH-EC meter (HANNA, HI9811, Hanna Instruments, Padova, Italy). Membrane stability index was calculated by the following formula:

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MSI = \left[1 - \frac{C1}{C2}\right] \times 100
\]

Potassium and sodium contents were measured by flame photometry method. Leaf samples were dried and pulverized. Powdered leaf materials (1 g) were kept at 560°C for 4 h for ash preparation. To these samples, 20 ml 1N HCl was added and the mixtures were heated at 90°C to drive off the hydrochloric acid. The digested ash was dissolved in 100 ml distilled water and then filtered. The filtrate was stored in a refrigerator until analysis. Concentrations of potassium and sodium ions were estimated by referring to 0, 5, 10, 20, and 30 ppm standard working solution. The test solution was diluted if its signal was above that of the highest standard. Content of the elements were calculated by using the AsA equation (Bandehhag et al., 2004):

\[
E = \frac{[(C \times V \times D)/(M \times 106)]}{100}
\]

Where E is the element (either potassium or sodium) content of the test sample, expressed in %. C is the element mass of the test solution, expressed in mg/l, read from the calibration graph. V is the volume, in ml, of the digested solution (V = 100). D is the dilution factor of the test solution carried out during the measurement step. M is the mass, in g, of the test sample used in the procedure.

Statistical analysis
All physiological and biochemical parameters were recorded with five replications. The data were analyzed with MSTAT-C software. Mean comparison were carried out by LSD method.

Results and discussion
The results showed that under salinity condition, the activity of antioxidant enzymes such as SOD, CAT and GPX in cultivar ‘Syria 371’ were lower than control (Fig. 1 A, B and C). Meanwhile, there was no significant difference between salinity situation and control ones regarding APX activity (Fig. 1D). At the same time, in ‘Egypt 449’, the activity of SOD and CAT were unchanged (Fig. 1A and B). Contrarily, APX and GPX showed significantly increased activity under salinity compared to control treatment (Fig. 1 C and D).

MDA amount significantly increased due to salinity in both studied cultivars. Although this increase in the case of ‘Syria 371’ was approximately four times higher than that of ‘Egypt 449’ (Fig. 2A). MSI of both cultivars were negatively influenced by salinity. This negative impact on ‘Syria 371’ was highlighted than on ‘Egypt 449’ (Fig. 2B).

H₂O₂ content of salinity faced ‘Syria 371’ was higher than control. However, its content was not affected in ‘Egypt 449’ (Fig. 2C).

In both cultivars, salinity treatment led to the increased Na⁺ content. However, this increase in the case of ‘Syria 371’ was nearly three-times higher than ‘Egypt 449’ (Fig.
Moreover, salinity adversely affected K⁺ content in both cultivars (Fig. 3B). K⁺/Na⁺ ratio of both cultivars reduced via salinity (Fig. 3C). However, there was no statistical difference between cultivars considering this ratio.

As previously understood, salinity intensity goes to reduced water availability and/or absorption and hence lowered leaf turgor and finally leads to stomata closure (Azizpour et al., 2010). Stomata closure influences CO₂ acquisition and cause fluctuations and im-balances in ongoing light reactions and CO₂ fixation stage (Esfandiari et al., 2007b). The final outcome of these abnormal conditions would be reduced NADP⁺/NADPH,H⁺ ratio and increased ROS production (Esfandiari et al., 2011). Mehler cycle is the most efficient intrinsic mechanism for diminished production and coping with ROS molecules (Asada, 2000). SOD activity showed declining pattern in 'Syria 371' (Fig. 1A). It is likely that the lowest SOD activity under salinity might be the main factor for intense membrane damage and increased MDA content and oxidative stress (Fig. 2A and C). Reduced SOD activity follows by the accumulation of O₂⋅− in leaf cells and consequently results is the blockage of CAT and peroxidases activity (Fridivich, 1989). Any increase in above mentioned cycles activity under salinity goes to stability in cell mechanisms and reduced occurrence of oxidative stress (Edreva, 2005, Esfandiari et al., 2011). In 'Syria 371', in spite of oxidative stress, SOD activity was not increased (Fig. 1A). Mehler cycle operation under stress conditions help the plant to activate the xanthophyl cycle by acidification of lumen space (Ort, 2002). Furthermore, in 'Syria 371'; CAT and GPX activities were significantly decreased (Fig. 1B and C). These enzymes have the potential to neu-
neutralize $\text{H}_2\text{O}_2$ via its conversion to $\text{H}_2\text{O}$ molecules (Edreva, 2005, Ahmed et al., 2009). Any reduction in $\text{H}_2\text{O}_2$ scavenging enzymes activity causes the accumulation of these oxidants in leaf cells. Ionic absorption induced by salinity conditions i.e. intensified absorption of Na$^+$ and Cl$^-$ antagonistically affect K$^+$, Ca$^{2+}$, and Mg$^{2+}$ absorption and metabolism (El-Hendawy et al., 2005; Mansour et al., 2005; Murillo-Amador et al., 2006). There is strong evidence that plants have potential to cope with toxic levels of saline sodic ions via accumulation of those ions in vacuole or apoplast and furthermore, employ them as osmoticum molecules for cell turgor maintenance (Yeo and Flowers, 1983, Cramer et al., 1994, Leidi and Saiz, 1997). Similarly, Munns and James (2003) reported that several salt-tolerant tetraploid wheat genotypes do indeed accumulate very high leaf sodium levels. These genotypes may have a special ability to tolerate high internal levels of sodium. The higher concentration of sodium may result from the greater capability for compartmentation of this ion in the vacuoles.

Other scientists claim that tolerant plants prevent Na$^+$ and Cl$^-$ absorption and/or translocation. Na$^+$ content of ‘Syria 371’ and ‘Egypt 449’ meanfully increased due to salinity (Fig. 3A). However, intensified oxidative damage in ‘Syria 371’ might be in consequence of Na$^+$ and Cl$^-$ toxic levels. This means that ‘Syria 371’ was not able to combat toxic levels of Na$^+$ accumulation in cytosol. Meanwhile, ‘Egypt 449’ was capable to prevent toxic accumulation of Na$^+$ ion in its aerial parts and Na$^+$ content of plant was like normal condition (Fig. 3A). At the same time, in both cultivars, K$^+$/Na$^+$ ratio was low mainly due to lowered K$^+$ content (Fig. 3C).

In conclusion, the data obtained from the present experiment revealed that ‘Syria 371’ showed higher oxidative stress demonstrations compared to ‘Egypt 449’ largely due to the low activity of antioxidant enzymes and high Na$^+$ accumulation. Furthermore, the results showed that those plants were able to escape the ionic toxicity under saline sodic conditions via scavenging of ROS molecules and concomitantly controlled Na$^+$ absorption and translocation. Those capabilities potentiate the plants survival and productivity under stressful conditions.

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References


