Effects of salinity on antioxidant system in ten grape genotypes

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Abstract

Salinity is an important environmental factor that limits plant growth and production. Grape is classified as salt sensitive plants. The object of this study was to evaluate effects of salinity on membrane lipid peroxidation, antioxidant components, and antioxidative enzymes activity in ten grape genotypes native to the regions around Urmia Salt Lake. Malondialdehyde content and protective enzymes activities in roots and leaves of ten genotypes increased significantly ($p \leq 0.05$) under salinity. Salinity had an obvious effect on the accumulation of total phenolics content and induced phenylalanine ammonia-lyase enzyme activity in all genotypes. H6 showed low increases in Malondialdehyde content, also this genotype showed good enzymes activities and total phenolics content and PAL activity. There were significant positive correlations ($p \leq 0.01$, $r^2=0.7$) between antioxidative enzyme activities, total phenolics content, and phenylalanine ammonia-lyase activity in the leaves of all genotypes. It seems that H6 showed a higher capacity to tolerate salinity compared to the other genotypes.

Keywords: Anti-oxidative enzymes; salt stress; phenylalanine ammonia-lyase activity; lipid peroxidation

Mohammadkhani, N. and N. Abbaspour. 2017. 'Effects of salinity on antioxidant system in ten grape genotypes'. *Iranian Journal of Plant Physiology* 8 (1), 2247- 2255.

Introduction

Soil salinity is a major threat to global food security. Up to 20% of the world's irrigated land, which produces one third of the world's food, is salt affected (FAO, 2007). *Vitis vinifera* grapevines are classified as being moderately sensitive to salinity (Maas and Hoffman, 1977).

Plants produce reactive oxygen species (ROS) under normal conditions essentially from photosynthesis, photorespiration and respiration. The most common ROS generated under normal conditions are O^{-}_2 and H_2O_2 perhaps as a result of electron leakage from the photosynthetic and respiratory electron transport chains to oxygen. Another source of ROS (H_2O_2) is photorespiration resulting from the oxygenase activity of Rubisco (Lacuesta et al., 1997). However, it is evident that plants producing high levels of antioxidants have a greater resistance to salt stress than those with low levels of antioxidants. The balance between production and removal of ROS is controlled by the antioxidant systems (Ashraf, 2009).

Lipid peroxidation causes degradation and impairment of structural components. This leads to changes in selective permeability of membranes and enzyme activities that are bound to membranes. Therefore, the cell membrane stability has been used to discriminate stress

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Received: June, 2017
Accepted: September, 2017

tolerance in crops (Liang et al., 2003). Malondialdehyde (MDA) is a major product of lipid peroxidation and has been used as an indicator of ROS production under oxidative stress (Hong et al., 2000).

Enzymatic ROS-scavenging mechanisms in plants include production of superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), and glutathione reductase (GR). The extent of oxidative stress experienced in a cell is determined by the levels of superoxide, H_2O_2, and hydroxyl radicals. Additionally, a balance among APX, and CAT activities is crucial for suppressing toxic ROS levels within cells (Ozden et al., 2009).

CAT together with SOD form the most efficient antioxidant machinery in preventing cellular damage (Scandalios, 1993). Ascorbate peroxidase (APX) uses ascorbate as the electron donor for the reduction of H_2O_2 and is important in the detoxification of H_2O_2 along with catalase (Asada and Takahashi, 1987). Several researchers have observed variations in antioxidative defenses under saline conditions (Silveira et al., 2001).

Grape quality is a complex concept that mainly refers to berry chemical composition including sugars, acids, phenolics, and other aroma compounds (Lund and Bohlmann, 2006). The composition and concentration of these chemical compounds change during berry development and can be affected by many factors, either environmental, endogenous, or management practices (Dai et al., 2011).

Phenolic compounds include many secondary metabolites in plants that exhibit antioxidant properties. Precursors for the synthesis of phenolic compounds are made in the shikimic acid and chorismic acid pathways. As a result of the processes of methylation, hydration, and dehydration of cinnamic acid, phenolic acids are produced as part of the response of the plant to abiotic stresses (Dixon and Paiva, 1995). Some of the phenolic compounds such as phenolic acids or flavonoids are widely known and present in most of the plant species (Jwa et al., 2006). Phenolic compounds are considered as by-products of metabolic alteration. Results of investigations unequivocally suggest that phenols not only plays a major role in defensive reactions of plants but also influence humans and animals that consume products enriched with phenolic compounds (Amarowicz and Weidner, 2009).

The phenylpropanoid pathway is important in secondary plant metabolism and produces a variety of phenolics with defense-related functions. Phenylalanine ammonia-lyase (PAL; EC 4.3.1.5) is a crucial enzyme in that pathway which catalyzes the formation of trans-cinnamic acid. It is induced by abiotic stresses, which result in the accumulation of phenolic acids (Solecka and Kacperska, 2003).

In this study ten grape genotypes commonly grown in the region around Urmia salt lake were evaluated from the view point of antioxidant system response to salinity. In order to evaluate salinity-induced alternations in ten grape genotypes, lipid peroxidation level, antioxidant compounds, and enzymes activities were studied under salt stress.

Materials and Methods

Plant materials and growth conditions

Hardwood cuttings of ten genotypes of grapevine, namely, Ghazandayi, Gharaghandomeh, Zardkeh, Kajhave, H4: (*V. vinifera* cv. Jighjigha *× V. riparia cv. Gloire*), Anghootkeh, GhezelUzum, H6: (*V. vinifera* cv. GharaUzum *× V. riparia* cv. Kober 5BB), Aghshani, and Silvestris (*V. vinifera sub*. Silvestris were obtained from Kahriz vineyard (West Azarbaijan). The cuttings were soaked in IBA (indol-3-butyric acid) 0.1% (w/v) for 5-10 s and put in a mist house (relative humidity 80%) with a heat-bed temperature of 20-30 °C. The rooted cuttings were transferred in pots containing modified Hoagland solution with the following composition: 0.125 mM KNO_3, 0.125 mM $Ca(NO_3)_2$, 0.05 mM $MgSO_4 7H_2O$, 0.0125 mM KH_2PO_4, 5.75 µM H_3BO_3, 1.34 µM $MnCl_2.4H_2O$, 0.1 µM $ZnSO_4.7H_2O$, 0.038 µM $CuSO_4.5H_2O$, 0.025 µM $Na_2MoO_4.2H_2O$, and 8.88 µM Fe-EDTA. Two- month-old plants were treated with NaCl (0, 25, 50 and 100 mM) in Hoagland solution for 2 weeks. NaCl was added to the nutrient solution by incremental increases until the final desired concentrations were reached. The root and leaf tissues were stored at -80 °C until enzymatic assays.

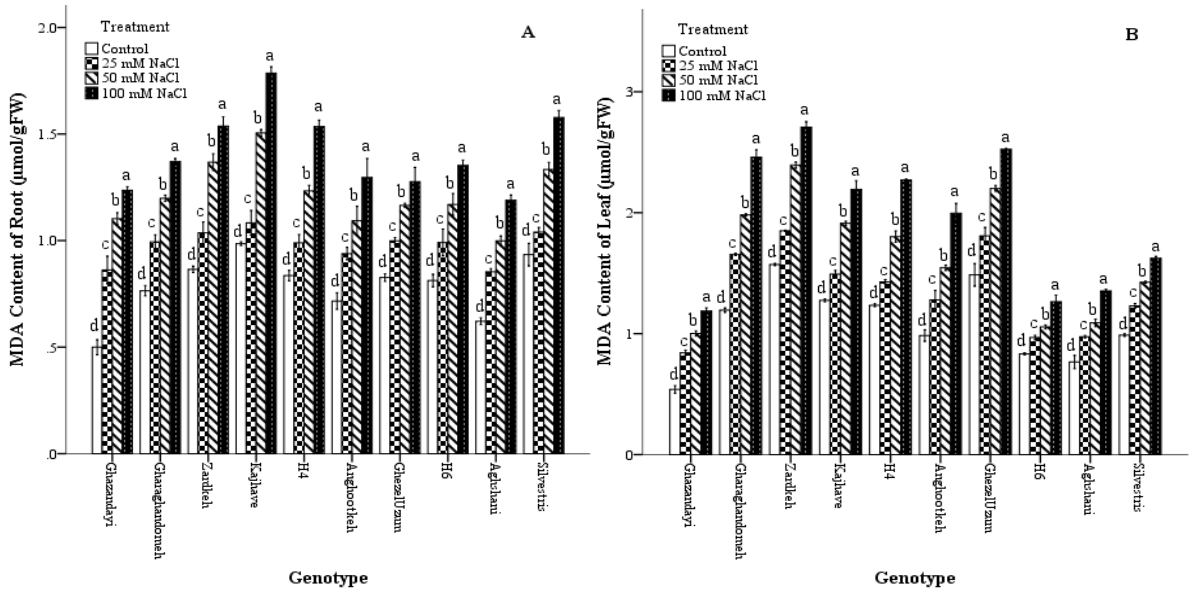

Fig. I. MDA content (µM. g FW^{-1}) in root (A) and leaf (B) of ten grape genotypes under different salinity treatments (control, 25, 50, and 100 mM NaCl); bars are the means ± standard Error. Different letters indicate significant difference (P<0.05) between treatments in each genotype according to Duncan test.

Determination of MDA content

Malondialdehyde (MDA) content was determined by TBA reaction as described by Heath and Packer (1968) using extinction coefficient of 155 mM^{-1} cm^{-1} and expressed as µM/g FW.

Anti-oxidative enzymes assay

Anti-oxidative enzymes extracts were prepared according to Garratt et al. (2002) method. APX (ascorbate peroxidase) activity was measured by the decrease in the absorbance of ascorbate (extinction coefficient 2.8 $mM^{-1}cm^{-1}$) within 1 min at 290 nm (Asada and Chen, 1989). CAT (Catalase) activity was determined by the decrease in the absorbance of H_2O_2 (extinction coefficient 0.036 $mM^{-1}cm^{-1}$) within 1 min at 240 nm (Maehly and Chance, 1959) using UV–visible spectrophotometer (WPA S2100).

Determination of total phenolics

Total phenolics were determined using Folin–Ciocalteu's reagent according to Bonilla et al. (2003) method. Total phenolic content was expressed as gallic acid equivalents (GAE) in mg/g of sample using a standard curve prepared with 100, 200, 300, 400, and 500 mg/l of gallic acid.

Assay for PAL activity

PAL activity was measured by Solecka and Kacperska (2003) method. One unit of enzyme activity was PAL amount that produced 1 µM of cinnamic acid in 1 h and expressed as µM cinnamic acid mg^{-1}protein h^{-1}.

Statistical Analysis

All statistical analyses were done using SPSS. The mean values of three replicates and SE were calculated. Duncan's multiple range tests (P≤0.05) and GLM (General Linear Model) was performed to determine the significance of the results. Correlations between different factors were determined for all genotypes.

Results

MDA content

Effects of salinity stress on MDA content of the grape genotypes are shown in Fig. (I). MDA

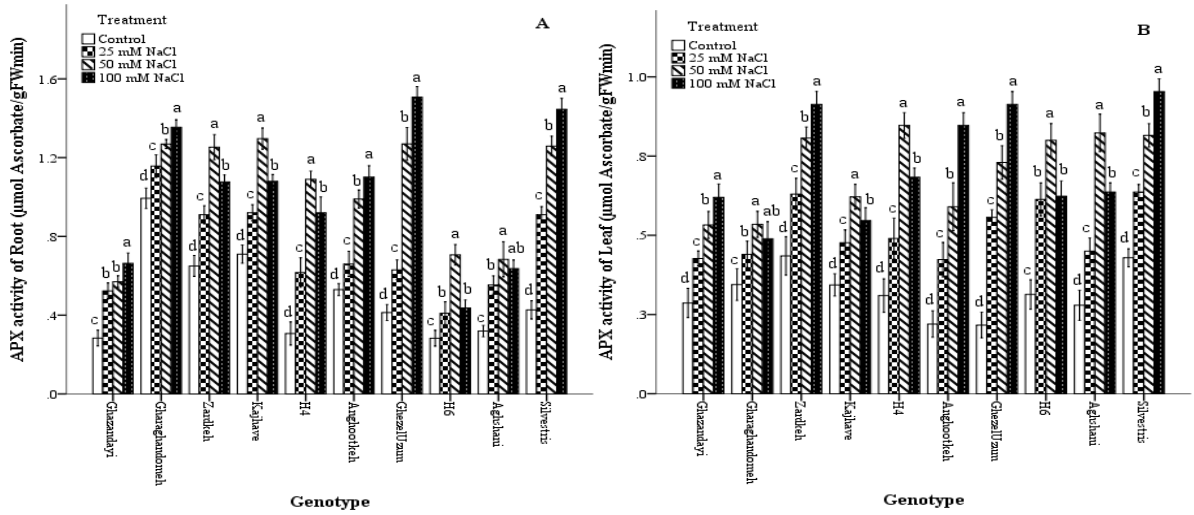

Fig. II. Ascorbate peroxidase (APX) activity (µmol H_2O_2. $gFW^{-1}.min^{-1}$) in root (A) and leaf (B) of ten grape genotypes under different salinity treatments (control, 25, 50, and 100 mM NaCl); bars are the means ± standard Error. Different letters indicate significant difference (P<0.05) between treatments in each genotype according to Duncan test.

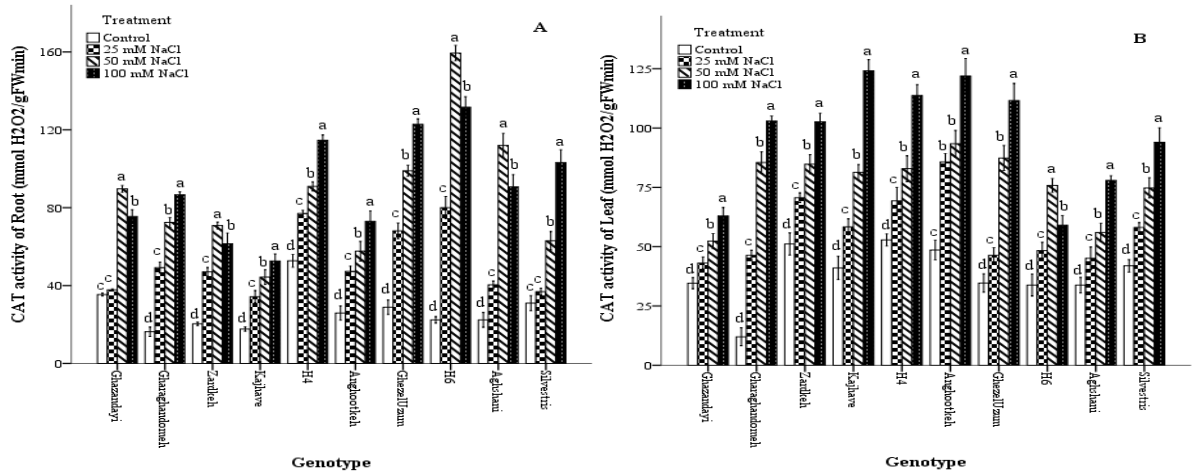

Fig. III. Catalase (CAT) activity (µM H_2O_2. g $FW^{-1}.min^{-1}$) in root (A) and leaf (B) of ten grape genotypes under different salinity treatments (control, 25, 50 and 100 mM NaCl); bars are the means ± standard Error. Different letters indicate significant difference (*P*<0.05) between treatments in each genotype according to Duncan test.

content increased significantly (P≤0.05) in roots and leaves of all genotypes with increasing salinity, but this increase in leaves of Ghazandayi and H6 was lower than the other genotypes. Roots of Kajhave and leaves of Zardkeh showed higher MDA content when compared to the other genotypes (Fi. I). Analysis of variance showed that the difference in MDA content of roots and leaves among genotypes, treatments, and genotype × treatment was significant (P≤0.05).

Anti-oxidative enzymes

Ascorbate peroxidase (APX) activity increased with increasing salinity; however, it decreased at high salinity treatments in roots and leaves of some genotypes (Fig. II). But roots and leaves of GhezelUzum and Silvestris showed a higher APX activity than that of others. The difference in APX activity of roots and leaves among genotypes, treatments, and genotype × treatment was significant (P≤0.05).

Similar to APX, catalase (CAT) activity increased in roots and leaves of all genotypes with increasing salinity (Fig. III). CAT activity in roots of H6 and leaves of Anghootkeh was higher than the other genotypes. GLM analysis showed that the difference in CAT activity of roots and leaves among genotypes, treatments, and genotype × treatment was significant (P≤0.05).

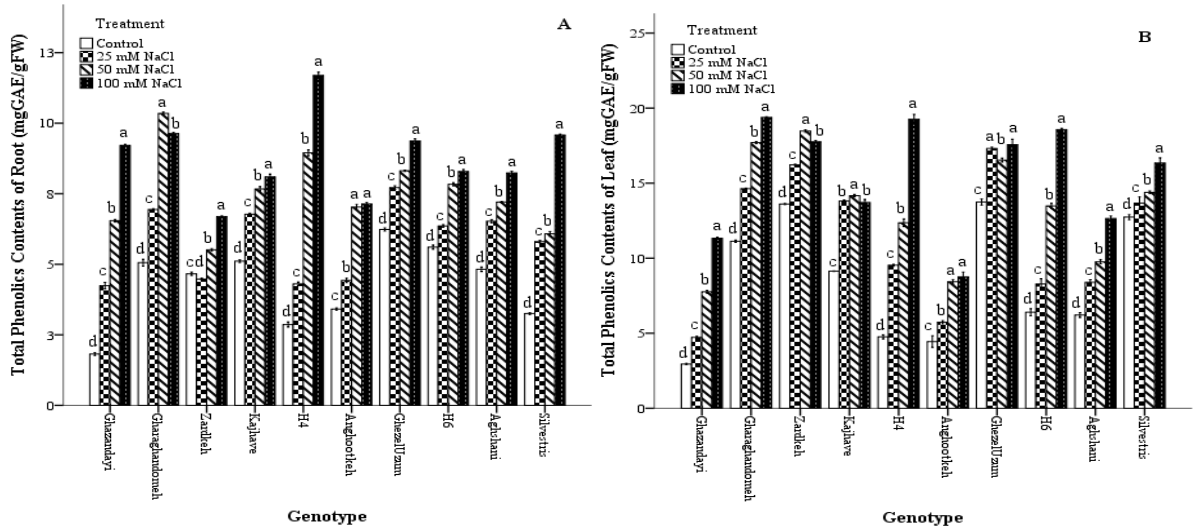

Fig. IV. Total phenolics content (mg GAE/g FW) in root (A) and leaf (B) of ten grape genotypes under different salinity treatments (control, 25, 50, and 100 mM NaCl); bars are means ± standard Error. Different letters indicate significant difference (P<0.05) between treatments in each genotype according to Duncan test.

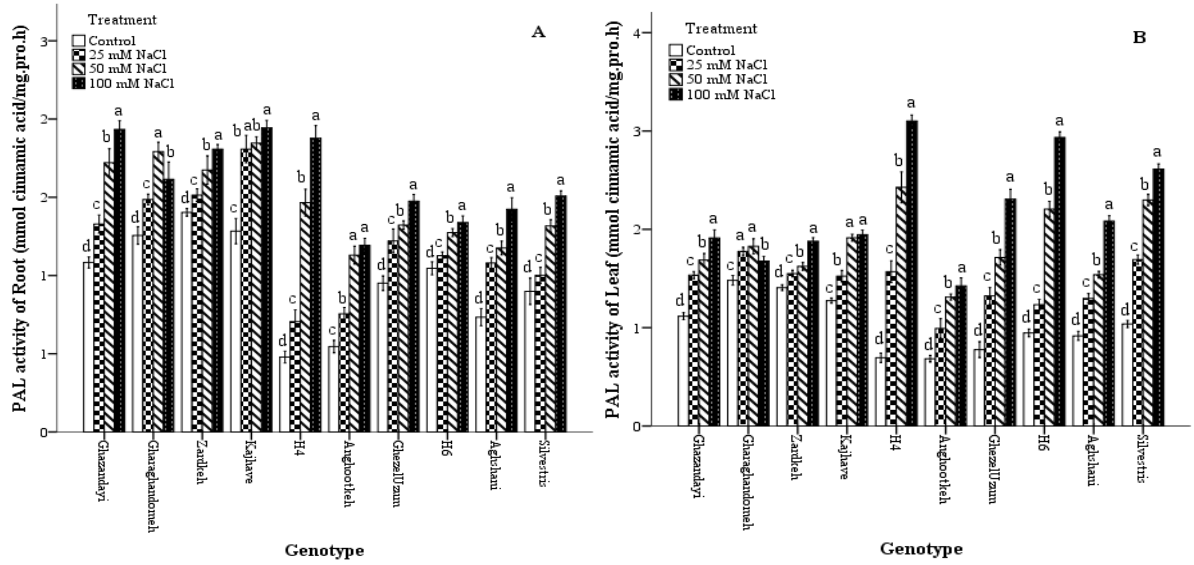

Fig. V. PAL activity (mM cinnamic acid/mg pro.h) in root (A) and leaf (B) of ten grape genotypes under different salinity treatments (control, 25, 50, and 100 mM NaCl). Bars are means ± standard Error. Different letters indicate significant difference (P<0.05) between treatments in each genotype according to Duncan test.

Total phenolics content and PAL activity of leaf

Salinity had an obvious effect on accumulation of phenolics in roots and leaves of all genotypes (Fig. IV). Total phenolic contents increased under salinity; it means that the total phenolic contents in all treatments were higher than those of the control. H4 genotype showed a higher increase (4.05 and 4.06 fold more than the control in roots and leaves, respectively) in total phenolics content. Zardkeh and Anghootkeh had a lower content in roots and leaves, respectively. The difference in phenolics content of roots and leaves among genotypes, treatments, and genotype × treatment was significant (P≤0.05).

Salinity induced PAL activity in all treatments and all genotypes (Fig. V). Similar to phenolics content a maximum increase in PAL activity was observed in H4 (3.90 and 4.57 fold more than control in roots and leaves, respectively). Also, Anghootkeh had a lower

increase in leaves. Analysis of variance showed that the difference in PAL activity of roots and leaves among genotypes, treatments and genotype × treatment was significant (P≤0.05).

Discussion

It is widely accepted that plant response to salinity depends on several factors such as plant organ, stress intensity, and physiological stage (Munns and Tester, 2008). In addition, the effective role of each antioxidative enzyme's activities (measured *in vitro*) in salt tolerance is still contradictory (Cavalcanti et al., 2004).

Salt-induced oxidative stress may disrupt membrane structure because ROS overproduction triggers lipid and protein peroxidation (Mandhania et al., 2006). Electrolyte leakage is markedly increased by salt, suggesting high membrane damage. This is an unexpected response, as high electrolyte leakage is generally accompanied by enhanced lipid peroxidation as an evidence of oxidative damage (Mandhania et al., 2006). It is possible that changes in the fatty acid profile of membrane lipids masked the estimation of membrane damage through the TBARS assay (Amor et al., 2006). In addition, oxidative injuries on the membrane proteins may also contribute to high electrolyte leakage (Yang et al., 2004). MDA can be used as a parameter for evaluating a plant's tolerance to environmental stresses. Variations in MDA content have been reported for different plant species under different conditions (Bor et al., 2003). Our results showed a significant (P≤0.05) increase in MDA content in roots and leaves of salt-treated plants. Roots of Ghazandayi and Aghshani and leaves of Ghazandayi and H6 showed a lower MDA content. Previous studies also reported that lipid peroxidation under salt stress was lower in salt-tolerant plants such as Beta maritima (Bor et al., 2003).

Salt tolerance is related to antioxidant enzymes activity in plants (Shalata et al., 2001). High antioxidant activities could be interpreted as symptoms of oxidative stress or damage (the plant upregulates the antioxidant enzymes because it is producing more ROS). Conversely, high antioxidant activity could be interpreted as higher tolerance to the oxidative stress (the plant suffers less oxidative stress because it has higher antioxidant activity). Also efficient antioxidative defense has often been viewed as upregulation of a full set of antioxidant enzymes (SOD, CAT, APX, etc.) although each of these enzymes performs a specific function and its activity should be assigned to a specific role in ROS detoxification i.e., efficient antioxidative activity does not necessarily mean the strong upregulation of the full set of antioxidant enzymes and vice versa (Abogadallah, 2010). The anti-oxidative enzymes activities were higher in salinity-stressed plants in comparison to the control ones in all genotypes studied in the present study.

Higher levels of APX activity have been reported in wild salt tolerant tomato and radish plants (Lopez et al., 1996). Overexpression of APX gene in plants has been reported to increase protection against oxidative stress (Wang et al., 1999). In the present study APX activity in leaves of all genotypes increased in response to high salinity up to 50 mM NaCl. For some genotypes increasing of APX activity in leaves was continued with increasing salinity up to 100 mM. Some genotypes like roots of Silvestris and leaves of GhezelUzum and Zardkeh showed high APX activity and also had high MDA content. This is consistent with the theory by Abogadallah (2010) suggesting that high antioxidant activities could be interpreted as symptoms of damage.

Catalase function in plants tissues is detoxification of hydrogen peroxide to water and oxygen. CAT activity is important in salinity tolerance and in accordance with the intensity of salt stress (Streb and Feierabend, 1996). The changes in CAT activity depend on the species, the development and metabolic state of the plant, as well as on the duration and intensity of the stress (Chaparzadeh et al., 2004). In the present study roots of H6 genotype showed high CAT activity and therefore low increase in MDA content under salinity. There were significant positive correlations (P≤0.05, r^2≥0.7) between roots and leaves anti-oxidative enzymes (APX and CAT) and MDA content.

Many authors demonstrated that the production of phenols in plant tissues rises under abiotic stress conditions (Dixon and Paiva, 1995; Weidner et al., 2009). Such large discrepancies in experimental results can be attributed to differences in abiotic stresses, e.g. type of stress,

its intensity, its duration, the stages of plant development (in general, the early stages of germination and plant development are the least tolerant to stress), and the biological material, e.g. whole seedlings or different parts of plants, such as roots or leaves, which are characterized by a great diversity of secondary metabolites (Weidner et al., 2009). In response to changing environmental conditions, plants evolved the capacity to biosynthesize different phenolic acids (Caldwell et al., 2007).

Phenylalanine ammonia-lyase (PAL, EC 4.3.1.5) is a key enzyme between primary and secondary metabolism (Dixon and Paiva, 1995), which regulates the biosynthesis of phenolic compounds from phenylalanine. Phenolic acids are accumulated under stress as a consequence of the increased PAL activity and protect plants against abiotic stresses (Dixon and Paiva, 1995).

In the present study total phenolic acids and PAL activity increased under salinity, so that a regular ascendant process from control to high salinity was observed in all genotypes. However, this process was higher in roots of H4 genotype and leaves of H4 and H6. These genotype had good enzymes activity. Also, H6 genotype showed low MDA content. It seems that these genotypes with higher antioxidant enzymes activity, showed higher increase in total phenolics content and PAL activity compared to the others. There were significant positive correlations ($P \leq 0.01$, $r^2 \geq 0.7$) between anti-oxidative enzymes (APX and CAT), total phenolics content and PAL activity in the leaves of all genotypes.

In conclusion, numerous studies have revealed that environmental stress often raises the accumulation of phenolic compounds and phenolic acids. It should be noted that most of the studies are concentrated on short-term stress. However, the mechanism of plant response to long and continuous stress is different. There were significant positive correlations ($P \leq 0.01$) between enzyme activities and total phenolics content. Considering the results obtained in this study, H6 showed low increases in Malondialdehyde content, also this genotype showed good enzymes activities and total phenolics content and PAL activity. It seems that genotypes possessed higher efficiency in its anti-oxidative system and can tolerate salinity better than others.

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