Effects of salinity on antioxidant system in four grape (Vitis vinifera L.) genotypes

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Summary

Salinity is a major environmental stress that restricts plants growth and production. Grapes are classified as moderately sensitive to salinity. The aim of this work was to investigate the salinity effects on lipid peroxidation level, antioxidant compounds and anti-oxidative enzymes activities and photosynthetic pigment contents in four grape genotypes that are commonly grown in the region around Urmia salt lake. Malondialdehyde content and protective enzymes activities in roots and leaves of four genotypes increased significantly (P < 0.05) under salinity. 'Chawga' showed lower and higher increases in malondialdehyde content and enzymes activities, respectively. Salinity had an obvious effect on the accumulation of total phenolics content and induced phenylalanine ammonia-lyase enzyme activity in all genotypes. There were significant positive correlations (P < 0.01, r² = 0.838) between anti-oxidative enzyme activities, total phenolics content and phenylalanine ammonia-lyase activity in the leaves of all genotypes. Chlorophyll a, b contents in leaves of all genotypes were reduced and carotenoid content increased significantly (P < 0.05) under salinity. 'Chawga' showed lower decrease in chlorophyll content and a higher increase in carotenoid content than others. It seems that 'Chawga' had a better antioxidant system compared to other genotypes and showed a higher capacity to tolerate salinity.

Key words: anti-oxidative enzymes, abiotic stress, phenylalanine ammonia-lyase activity, photosynthetic pigments.

Introduction

Salinity is a major environmental stress for plants growth and yield (ASHRAF and FOOLAND 2007). Abiotic stresses like salinity have restricted grape production and productivity. Recently, there have been several studies on the effects of abiotic stress on Vitis at the molecular level, but less focus has been put on the physiological aspects associated with the influences of abiotic stimuli on Vitis. Vitis vinifera grapevines are classified as being moderately sensitive to salinity (MAAS and HOFFMAN 1977).

Reactive oxygen species (ROS) are produced because of abiotic stresses such as salinity. Increased concentrations of ROS damage organelles and hence impair plant growth and yield (ASHRAF 2009). 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 change in selective permeability of membranes and enzyme activities bound to membranes. Therefore, the cell membrane stability has been used to discriminate stress 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).

Plants are equipped with oxygen radical detoxifying enzymes to survive under stress. Different plants have different protection mechanisms to eliminate ROS. Among them, non-enzymatic (phenolic compounds and carotenoids) and enzymatic antioxidants such as catalase (CAT; EC 1.11.1.6), guaiacol peroxidase (POD; EC 1.11.1.7) and ascorbate peroxidase (APX; EC 1.11.1.11) provide the first line of defense system against stress (YAHUBYAN et al. 2009).

H₂O₂ is an important ROS and can cause plasma membrane lipids and proteins impairment. H₂O₂ is mainly detoxified by CAT in glyoxysomes and peroxisomes and by APX in chloroplasts, mitochondria and peroxisomes (SHIGEOKA et al. 2002).

Phenolic compounds are important secondary metabolites in grape that play an essential role in determining grape quality. They are also involved in various antioxidant properties. Phenolic compounds are involved in plant resistance to biotic and abiotic stresses (SOLECKA and KACPERSKA 2003). Total polyphenol content increased in Mentha pulegium leaves (OUESLATI et al. 2010) under salinity.

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; it 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). So phenylalanine ammonia-lyase enzyme may induce stress resistance via regulating the biosynthesis of phenolic compounds.

Salt also affects photosynthetic pigments such as chlorophylls, and carotenoids. Changes in these parameters such as decrease in chlorophyll content depend on the severity and stress duration (DUBEY 1994). Carotenoids form a key part of the plant antioxidant defense system, but they are sensitive to oxidative stress (MATAMOROS et al. 2003).

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In this study four 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 look into salinity-induced alternations in four grape genotypes, lipid peroxidation level, antioxidant compounds and enzymes activities and photosynthetic pigments contents under salt stress were studied.

Material and Methods

Plant materials and growth conditions: Hardwood cuttings of four genotypes of grapevine ('LaaleSefid', 'Chawga', 'Khalili' and 'Shirazi') 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 with opened leaf bud were transferred to 2 L aerated pots containing modified Hoagland nutrient solution (hydroponics growth culture) with the following composition: 0.125 mM KNO3, 0.125 mM Ca(NO3)2, 0.05 mM MgSO4·7H2O, 0.0125 mM KH2PO4, 5.75 μM H3BO3, 1.34 μM MnCl2, 4H2O, 0.1 μM ZnSO4·7H2O, 0.038 μM CuSO4·5H2O, 0.025 μM Na2MoO4·2H2O and 8.88 μM Fe-EDTA. Solutions were replaced every 2 d and nutrient solution by incremental increases until the final concentrations were reached. Our experimental design was Complete Randomized Block Design (CRBD), three replicates per treatment and 2 plants per replicate were designed. The root and leaf tissues were stored at -80 °C until enzymatic assays.

Determination of MDA content: Malondialdehyde (MDA) was determined by TBA reaction as described by Heath and Packer (1968). The MDA content was calculated using extinction coefficient of 155 mM·1 cm-1.4.

Anti-oxidative enzymes assay: Anti-oxidative enzymes extracts were prepared according to the Garratt et al. method (2002). POD (guaiacol peroxidase) activity was calculated by the decrease in absorbance of tetraguaiacol (extinction coefficient 26.6 mM·1 cm-1) within 1 min at 420 nm (Upadhyay et al. 1985). APX (Ascorbate peroxidase) activity was measured by the decrease in 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 absorbance of H2O2 (extinction coefficient 0.036 mM·1 cm-1) within 1 min at 240 nm (MaeHy 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). Total phenolic content was expressed as gallic acid equivalents (GAE) in mg·g-1 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 as described by Solecka and Kacperska (2003). One unit of enzyme activity was the PAL amount that produced 1 μmol of cinnamic acid in 1 h and expressed as μmol cinnamic acid mg-1 protein h-1.

Measurement of photosynthetic pigment content: The chlorophylls and carotenoids (carotene and xanthophyll) content of leaves were measured with Lichtenthaler and Welbaum (1983) method.

Statistical analysis: All statistical analyses were done using SPSS (Version 14.0). The mean value of three replicates and standard errors were calculated. Tukey's multiple range tests (p < 0.05) and GLM (General Linear Model) were performed to determine the significance of the results. Correlations between different factors were calculated for all genotypes.

Results

MDA content: Effects of salinity stress on MDA content in the genotypes are shown in Fig. 1. MDA content increased significantly (P < 0.05) in roots and leaves of all genotypes with increasing salinity, but this increase in roots and leaves of 'Chawga' was lower than in other genotypes and in roots of all genotypes was higher than in leaves. Roots of 'Shirazi' and leaves of 'LaaleSefid' showed higher MDA content when compared to other genotypes (Fig. 1 A and B).

Antioxidative enzymes: Guaiacol peroxidase activity increased in roots and leaves of all genotypes under salinity, particularly in leaves of 'Chawga' (Fig. 2). However, POD activity decreased significantly (P < 0.05) in roots and leaves of 'LaaleSefid' and 'Khalili' at high salinity (100 mM NaCl).

Ascorbate peroxidase (APX) alterations in response to salinity treatments were similar to POD (Fig. 3). APX activity increased with increasing salinity; however, it decreased at high salinity in leaves of all genotypes, except for 'Chawga'. Roots of 'Khalili' and leaves of 'Chawga' showed a higher APX activity than that of others at high salinity.

Similar to antioxidant enzymes, catalase (CAT) activity increased in roots and leaves of all genotypes with increasing salinity (Fig. 4). Unlike other genotypes, CAT activity in roots and leaves of 'Chawga' significantly (P < 0.05) increased at high salinity (100 mM NaCl).

Total phenolics content and PAL activity of leaf: Salinity had an obvious effect on accumulation of phenolics in leaves of all genotypes (Fig. 5 A). Total phenolic contents increased under salinity; it means that the total phenolic contents in all treatments were higher than those of the control. LaaleSefid and Khalili had higher and lower (2.41 and 1.45 fold more than control, respectively) increases in total phenolics content.

Salinity induced PAL activity in all treatments and all genotypes (Fig. 5 B). A maximum activity was observed in 'LaaleSefid' (86 % more than control). Whereas 'Chawga' had a lower increase in PAL activity (12 % more than control) than others at 100 mM NaCl.
Effects of salinity on antioxidant system in four grape genotypes

Fig. 1: MDA content (μmol·g DW\(^{-1}\)) in root (A) and leaf (B) of four table grapes (Vitis vinifera L.; 'LaaleSefid', 'Chawga', 'Khalili' and 'Shirazi') at 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.

Fig. 2: Guaiacol peroxidase (POD) activity (μmol H\(_2\)O\(_2\)·g DW\(^{-1}\)·min\(^{-1}\)) in root (A) and leaf (B) of four table grapes (Vitis vinifera L.; 'LaaleSefid', 'Chawga', 'Khalili' and 'Shirazi') at 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.

Fig. 3: Ascorbate peroxidase (APX) activity (μmol H\(_2\)O\(_2\)·g DW\(^{-1}\)·min\(^{-1}\)) in root (A) and leaf (B) of four table grapes (Vitis vinifera L.; 'LaaleSefid', 'Chawga', 'Khalili' and 'Shirazi') at 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.
Chlorophyll and carotenoids contents: Chlorophylls a, b and total chlorophyll contents in leaves of all genotypes were reduced under salinity compared to control (Fig. 6). Leaves of 'Chawga' showed a lower decrease than others particularly for Chlorophyll b (non-significant at $P < 0.05$). Unlike chlorophylls, carotenoids content increased significantly ($P < 0.05$) under salinity and that increase in 'Chawga' was higher (3.78 fold more than control) when compared to other genotypes. However, 'LaaleSefid' had a higher decrease in chlorophyll content and a lower increase in carotenoid content than others.

Discussion

Grapevine plants are moderately sensitive to salinity (Walker et al. 1981). Lipid peroxidation is correlated with oxidative damage under abiotic stresses (Bor et al. 2003). The MDA content is used as an index of oxidative stress such as salinity (Hernández and Almansa 2002). Our results showed a significant ($P < 0.05$) increase in MDA content in roots and leaves of salt-treated plants, that increase in Chawga was lower than others. The improved protection in membranes of 'Chawga' as compared to others may reflect efficient anti-oxidative enzymes as evidenced by the activity of CAT, POD and APX enzymes. 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 enzyme activity in plants (Shalata et al. 2001). Activity of antioxidant enzymes such as CAT, POD, and APX is increased under stress conditions, correlated to stress tolerance. The anti-oxidative enzyme activities were higher in salinity stressed plants when compared to control in all genotypes studied in this experiment. It has been shown that POD activity in salt-tolerant plants was more than salt-sensitive under salinity (Rahnama and Ebrahimzadeh 2005). Also Gao et al. (2008) showed high POD enzyme activity in salt tolerant cultivars compared to salt sensitive ones.
Effects of salinity on antioxidant system in four grape genotypes of *Jatropha curcas* seedlings. Our results showed that salinity caused increase in POD activities of all genotypes. However, the higher POD activity in 'Chawga' (4.93 and 2.79 fold increase in leaves and roots compared to control, respectively), suggested that a tolerant genotype shows higher protection against the oxidative stress than others.

It is well known that APX detoxifies H$_2$O$_2$ to water and oxygen during stresses. Because of higher activity in salt tolerant species, APX plays a key role in plants to tolerate salinity (Sudha and Ravishankar 2002). In the present study APX activity in leaves of all genotypes increased in response to high salinity up to 50 mM NaCl. For 'Chawga', increase of APX activity in leaves was continued with increasing salinity up to 100 mM.

Catalase function in plant 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 (Streif and Feierabend 1996). CAT activity in response to increased salinity was the same as APX. Decreased CAT activity under high salinity may be associated with the induction of H$_2$O$_2$ signaling pathways (De-Pinto et al. 2002). Only Chawga' showed an ascendant increase in CAT activity from control to high salinity.

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There were significant positive correlations ($P < 0.05$, $r^2 \geq 0.75$) between roots and leaves anti-oxidative enzymes (POD, APX and CAT) and MDA content, except for CAT in 'LaaleSefid' that showed negative correlation. It means that with increasing salinity, MDA content increased and subsequently anti-oxidative enzyme activities also increased.

Phenylalanine ammonia-lyase (PAL, EC 4.3.1.5) enzyme converts l-phenylalanine to trans-cinnamic acid, a precursor of phenylpropanoids such as phenolic acids (Schuster and Retey 1995). PAL is a key enzyme between primary and secondary metabolism (Dixon and Paiva 1995) and 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 an ascendant process from control to high salinity was observed in all genotypes. However, this process was higher in sensitive genotypes than in tolerant ones. It seems that sensitive genotypes ('LaaleSefid' and 'Shirazi') with higher membrane lipid peroxidation and lower antioxidant enzyme activity, showed higher increase in total phenolics content and PAL activity compared to tolerant genotypes ('Chawga' and 'Khalili'). There were significant positive correlations ($P < 0.01$, $r^2 \geq 0.83$) between anti-oxidative enzymes (POD, APX and CAT), total phenolics content and PAL activity in the leaves of all genotypes.

Salinity caused leaf injury and reduction in chlorophyll content of pea plant species (Hernandez et al. 2000). Chlorophyll content was decreased (chl a > chl b) in four grapevine genotypes under salt stress. Carotenoids have antioxidant properties and play an important role in scavenging ROS in addition to acting as accessory light-harvesting pigments (De Pascale et al. 2001). Carotenoids...
content increased under salinity and among the genotypes studied here, 'Chawga' showed a lower decrease in chlorophyll content and a higher increase in carotenoids content compared to others.

There were significant positive correlations ($P < 0.05$, $r^2 \geq 0.80$) between leaves anti-oxidative enzymes and carotenoids content, whereas a negative correlation ($P < 0.05$, $r^2 \geq 0.75$) was observed between leaves anti-oxidative enzymes and chlorophyll contents, except for CAT in 'LaaleSefid'.

**Conclusions**

In conclusion, genotype 'Chawga' had lower membrane damages than others because of a more efficient anti-oxidative system as evidenced by a higher activity of CAT, POD and APX enzymes. There were significant positive correlations ($P < 0.01$) between enzyme activities and total phenolic content. 'Chawga' showed a lower decrease in chlorophyll content and a higher increase in carotenoids compared to the others. Considering above mentioned results obtained in this study, 'Chawga' possessed higher efficiency in its anti-oxidative system and can tolerate salinity better than others.

**References**


