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Salinity effects on expression of some important genes in sensitive and tolerant grape genotypes

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Abstract: The effects of NaCl on expression of some genes related to salinity tolerance in grape (*Vitis* L.) were investigated. According to our screening study on eighteen grape genotypes, H6 and Gharashani (tolerant) and Shirazi and GhezelUzum (sensitive) were selected for molecular analysis. Our tolerant genotypes showed a higher water potential and a lower MDA content compared to other genotypes. Plants were treated with 50 mM NaCl as a critical concentration that is not lethal for grape. The expression profile of *VvNHL1* in leaves of all genotypes and in roots of tolerant genotypes was similar to that of *VvEDS1*; these genes probably associated in defense responses. *VvP450* and *VvCPN21* transcripts were expected only in leaves, but they were also detected in roots. *VvChS* and *VvPAL* transcripts accumulated significantly ($P < 0.05$) in leaves of tolerant genotypes under salinity. Our results showed a significant difference between tolerant and sensitive genotypes and highlighted a strong relationship between the accumulation of specific transcripts and the degree of salinity tolerance.

Key words: Salt, *Vitis*, cell death, photosynthesis, phenols

1. Introduction

1.1. Salinity effects on water potential and MDA content

Salinity is a major environmental stress for plant growth and yield. Abiotic stresses like salinity have restricted grape production. Difference in water potential (Ψw) caused by salinity or water availability limits water flux from soil to leaves. Under salinity and drought stress, the leaf osmotic potential (Ψπ) should always be lower than the soil water potential (Ψw) in order to maintain water flux and leaf cell turgor pressure (Munns, 2002).

Many authors have proposed the pressure chamber method (Scholander et al., 1965) as an excellent tool to measure vine water status under irrigated and nonirrigated conditions. Vine water status can be assessed using different pressure chamber approaches, such as leaf water potential and stem water potential (Sibille et al., 2007). These methods are widely used from low to very high levels of water restriction on vine (Acevedo-Opazo et al., 2008).

Reactive oxygen species (ROS) are produced because of abiotic stresses such as salinity. Increasing concentration of ROS damages organelles and impairs plant growth and yield (Ashraf, 2009). Lipid peroxidation causes degradation and impairment of structural components. This leads to a change in selective permeability of membranes, and 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).

1.2. Salinity effects on gene expression in grape

Due to a worldwide increase in soil salinity, the identification of genes conferring tolerance to abiotic stresses has been the subject of intensive studies. Genes related to salt stress cover extensive aspects, including salt-tolerant genes, energy metabolism, ionic transmembrane, transport, photosynthesis, signal transduction, and many other pathways. Some genes were expressed exclusively under salt-stress conditions. Therefore, salt stress could induce changes in modulating gene expression to adapt to the environment (Lu et al., 2015).

Recent studies have shown that application of salt stress induced massive changes in grapevine gene expression (Vincent et al., 2007; Jellouli et al., 2008). Comparative gene expression analysis could be a useful approach for understanding the mechanisms of tolerance and susceptibility (Kozian and Kirschbaum, 1999).

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Grapevine (*Vitis vinifera* L.) is the most widely cultivated perennial plant in the world. Due to the recent unraveling of the grapevine genome, this plant may become a model for fruit tree genetics and abiotic stress tolerance through biotechnological approaches (Troggio et al., 2008).

Grapevine has a high content of interfering substances, which prevent the application of standard RNA isolation protocols. During salt stress, secondary metabolites accumulate significantly. It is a major challenge to obtain sufficient amounts of high-quality RNA from grapevine, especially under abiotic stresses (Daldoul et al., 2009).

Grape roots accumulate some defense compounds like stilbene and other phenolic compounds (Cushman and Bohnert, 2000). Despite the importance of roots, the expression of genes in roots has rarely been studied.

Molecular information is needed to determine the gene expression profile. Some important genes encoding proteins for ion channels, signaling factors, and salt-responsive enzymes have been recognized in previous molecular studies. That information is useful for improvement of grape quality (Deluc et al., 2007).

1.3. Salinity effects on expression of cell death related genes

Both abiotic and biotic stress conditions cause ROS to increase in plant cells. At lower concentrations, ROS may be perceived as signals that influence the expression of genes and help the plant alleviate the adverse effects of the stress (Apel and Hirt, 2004).

Singlet oxygen produced under stress conditions is very harmful for plants. *EDS1* is involved in the processing of hydrogen peroxide-/superoxide-derived signals and plays an important role during oxidative stress caused by singlet oxygen (Mateo et al., 2004). Chini et al. (2004) studied the expression of *EDS1* under drought conditions.

NDR1 encodes a small, highly basic, and plasma-membrane–localized protein. Transcription of *NDR1* is induced by pathogen infection. Overexpression of *NDR1* in *Arabidopsis* results in enhanced resistance to biotic stress. *NDR1* belongs to the large family of *NHL*, and functions of some *NHL*s are important in plant responses to stresses (Varet et al., 2002). Kamei et al. (2005) studied overexpression of *NHL* gene under salinity in *Arabidopsis*.

1.4. Salinity effects on expression of photosynthesis-related genes

VvP450 gene belongs to cytochrome P450 which is a part of the photosynthesis electron transport chain. Cytochrome P450s (P450s or CYPs) are key enzymes involved in hydroxylation/oxidation reactions (Nomura and Bishop, 2006).

A gene coding for chloroplast chaperonin 21 (ch-Cpn21) is a strong candidate for the control of seed development and seedlessness in grapes (Costenaro-da-Silva et al., 2010).

Chloroplast chaperonin 21 also facilitated refolding of rubisco protein. Cpn21 binds both α and β subunits of cpn60. L-subunit of chloroplast polypeptides assembles with imported S-subunits into a holoenzyme by chaperonin 60 (Baneyx et al., 1995).

1.5. Salinity effects on expression of phenylpropanoid pathway genes

Chalcone synthases provide the starting materials for some metabolites (like flavonoids) that have important roles in flowering plants such as providing floral pigments, UV protectants, and insect repellents (Hahlbrock and Scheel, 1989).

The phenylpropanoid is an important pathway in secondary plant metabolism and produces a variety of phenolics with structural and defense-related functions including phenolic acids and flavonoids. Phenylalanine ammonia-lyase is a crucial enzyme in phenylpropanoid metabolism. It is induced by various biotic and abiotic stresses (Solecka and Kacperska, 2003).

In the present study, the expression of genes associated with salinity tolerance was compared in four grape genotypes. In previous experiments we screened 18 grape genotypes from the viewpoint of salt-tolerance parameters (Mohammadkhani et al., 2013, 2014). Genotypes with lower (GhezelUzum and Shirazi) and higher (H6 and Gharashani) capacity for salinity tolerance were selected for molecular analysis. The aim of our molecular study was to compare expression of genes related to cell death, photosynthesis, and phenols in roots and leaves of tolerant and sensitive grape genotypes under salinity. In addition, water potential and MDA content of genotypes were reported to show that our genotypes were under osmotic stress.

2. Materials and methods

2.1. Plant materials and growth conditions

First we performed screening experiments on 18 grape genotypes containing growth factors (length, dry weight, leaf area, relative water content, water potential, and leaf growth rate), ion balance (Cl^-, Na^+, K^+, and NO_3^-), osmolytes (sugars, proline, glycine betaine, and proteins), membrane leakage (malondialdehyde), and antioxidants (catalase, ascorbate peroxidase, guiacol peroxidase, phenylalanine ammonia lyase, and total phenols). As a result of the screening experiments, four genotypes were selected for molecular analysis: two sensitive and two tolerant. Hardwood cuttings of four grape genotypes [H_6 hybrid (*V. vinifera* cv. GharaUzum × *V. riparia* cv. Kober 5BB), Gharashani, GhezelUzum, and Shirazi] were obtained from Kahriz vineyard (agricultural research center, grape genotypes collection). The cuttings were disinfected

with benomyl (1% w/v), and basal parts were soaked in indole-3-butyric acid (0.1% w/v) for 5–10 s. All cuttings were struck in a mist house (relative humidity 80%) with a heat-bed temperature of 20–30 °C. After 2 weeks, the rooted cuttings were transferred into 2-L pots containing aerated Hoagland solution. Our Hoagland solution was modified and grape-special, containing 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 (Abbaspour, 2008). The pots were protected with aluminum foil to prevent light effects and algae proliferation.

2.2. Salinity treatments

Two-month-old plants were treated with 50 mM NaCl (threshold salinity determined for the genotypes). In screening experiments we used 10–200 mM NaCl treatments for 14 days and concluded that 50 mM salt was sufficient to reduce water potential but did not kill the grapevine plants when they were exposed for 14 days. Our plants were under osmotic stress, and this is seen from water potentials (Figure 1) and MDA contents of roots and leaves (Figure 2). Our sensitive genotypes could not tolerate high salinity (>50 mM NaCl) for more than several days.

Leaf and root tissues were collected at different time points (0, control; 24 h, short-time salinity; 14 days, long-time, regarding tolerance of our sensitive genotypes), immediately frozen in liquid nitrogen, and stored at –80 °C until RNA isolation.

2.3. Determination of water potential and MDA content

Leaf water potential was measured by pressure chamber (Scholander et al., 1965) at different time points. Malondialdehyde (MDA) was determined by TBA reaction, as described by Heath and Packer (1968). The MDA content was calculated using the extinction coefficient of 155 $mM^{-1}cm^{-1}$ and expressed as µmol/g FW.

2.4. RNA isolation, cDNA synthesis, and RT-PCR conditions

Total RNA was extracted from root tissues using Louime et al. (2008) method with a small modification. The RNA concentration was determined by Biophotometer (Eppendorf, Germany). The integrity of RNA was checked on agarose gel containing 0.5X TBE buffer (Tris base, boric acid, and EDTA) by ethidium bromide (0.5 µg/mL) staining. RNA integrity was also electrophoretically verified by OD_{260}/OD_{280} nm absorption ratio >1.90. First-strand cDNA was synthesized from total RNA using a first-strand cDNA synthesis kit (Fermentas), according to the manufacturer's instructions. Oligo dt primers were used for cDNA synthesis. The cycling protocol for 20 µL of reaction mix was 5 min at 65 °C, followed by 60 min at 42 °C, and 5 min at 70 °C to terminate the reaction. Second-strand cDNA synthesis was made with PCR Master kit (Cinnagen Co.). PCR conditions were as follows: initial denaturation at 95 °C for 3 min, followed by 28–30 cycles at 95 °C for 30 s, 53–64 °C for 30 s, and 72 °C for 20 s, with a final extension at 72 °C for 5 min. The *VvEF1* gene (elongation factor 1) was used as internal reference. Forward and reverse primer sequences that were used for RT-PCR are given in the Table. The products of RT-PCR were separated on 1.5% agarose gel and visualized using Gel Logic 212 Pro Imaging System (Carestream, USA). Gene Ruler 50 bp plus (50–1500 bp) was used as the DNA ladder (Fermentas). Experiments were repeated three times. The intensity of the RT-PCR bands was measured using Image J software, 1.43.

2.5. Statistical analysis

Statistical analyses were done using the Statistical Package for Social Sciences (SPSS) for Windows (version 14.0). Error bars on graphs are standard error of mean. A one-way analysis of variance with post tests and the general linear model (GLM) with Tukey's multiple range tests ($P < 0.05$) were used to determine differences between means.

Table. Forward and reverse primers used in RT-PCR experiment.

Genes	Forward primer (5'g3')	Reverse primer (5'g3')
VvEDS1	ACCAAGAAAAGGCCGAGACT	ACTCGAAAGGGAGGGTTTTC
VvNHL1	TCTAAAGTCGATTCAAAATCTCC	ACCAGATGGGATGGAGGGTCC
VvP450	GCTCAACAGGGTCTTCTTTCC	AACGGCGGGAGTAACTATGA
VvCPN21	GGGACAGAGGTGGAGTTCAA	TTTCCTTGCTTGCCTCTGTT
VvChS	GGAAAGGAGCTTGCAGAGAA	TCCAAAGGTCCTAGCACACA
VvPAL	GCCAATCCTGTCACCAAC	CCAAACTGCCTTACCTT
VvEF1-α	TCTGCCTTCTTCCTTGGGTA	GCACCTCGATCAAAAGAGGA

3. Results

3.1. Salinity effects on water potential and MDA content

As shown in Figure 1, salinity decreased water potential in all genotypes. That decrease in sensitive genotypes (Shirazi and GhezelUzum) was higher than in tolerant ones (H6 and Gharashani). It means that Gharashani was better able to retain its water potential under salinity.

The effects of salinity stress on MDA content of genotypes are presented in Figure 2. MDA content increased significantly ($P < 0.05$) in roots and leaves of all genotypes with passing time, but the increase in roots and leaves of H6 and Gharashani (tolerant genotypes) was lower than in Shirazi and GhezelUzum (sensitive genotypes).

3.2. Salinity effects on expression of cell death related genes in grape genotypes

The profile of cell death related gene (*VvEDS1* and *VvNHL1*) expression in leaves and roots of tolerant (H6 and Gharashani) and sensitive (Shirazi and GhezelUzum) grape genotypes (*Vitis* L.) treated with 50 mM NaCl at different time points is presented in Figure 3.

3.2.1. Expression of *VvEDS1* gene

VvEDS1 gene expression is needed for response to cell death in grape. As shown in Figure 4, after 24 h salinity

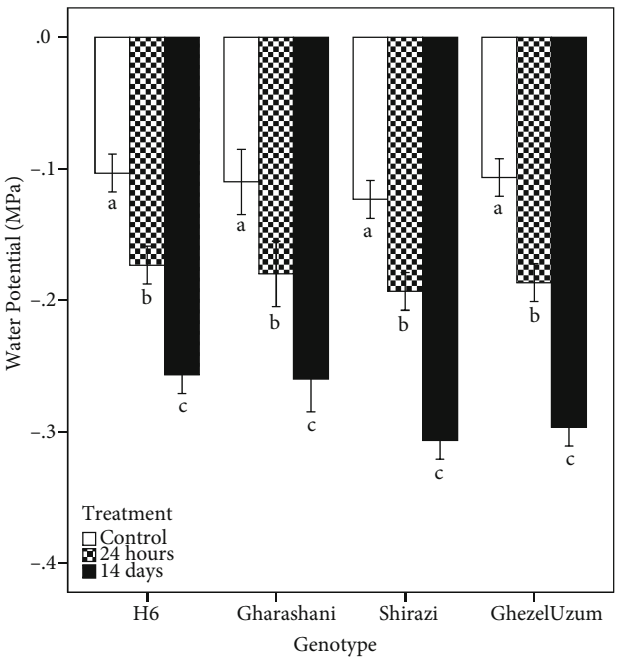

Figure 1. Water potential (MPa) of four grape genotypes [H_6 (*V. vinifera* cv. GharaUzum × *V. riparia* cv. Kober 5BB), Gharashani, Shirazi, GhezelUzum] after 0, 24 h, and 14 days treatment with 50 mM NaCl. Bars are the means (n = 3) ± standard error ($P < 0.05$, one-way ANOVA). Different letters above the columns indicate significant difference between the treatments according to Tukey's test.

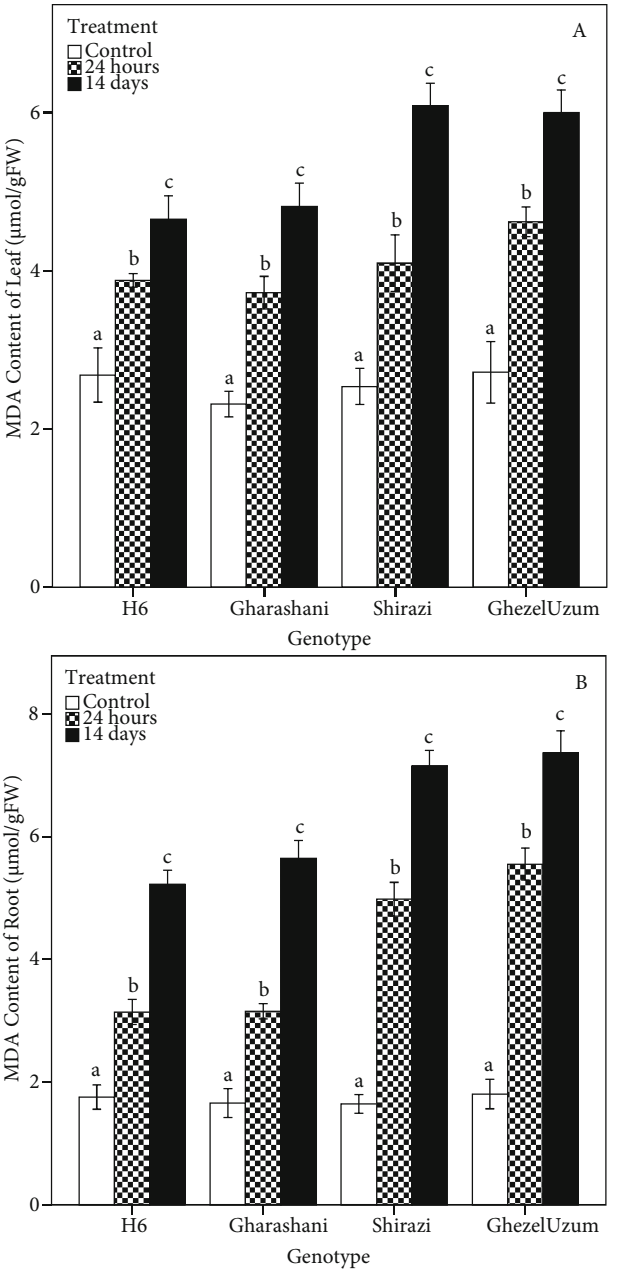

Figure 2. MDA content (µmol g FW^{-1}) in roots (A) and leaves (B) of four grape genotypes [H_6 (*V. vinifera* cv. GharaUzum × *V. riparia* cv. Kober 5BB), Gharashani, Shirazi, GhezelUzum] after 0, 24 h, and 14 days treatment with 50 mM NaCl. Bars are the means (n = 3) ± standard error ($P < 0.05$, one-way ANOVA). Different letters above the columns indicate significant difference between the treatments according to Tukey's test.

($P < 0.05$), except for H6, the leaves of all genotypes accumulated *VvEDS1* transcripts compared to the control.

We observed a significant decrease in roots of all genotypes after 24 h salinity, except in Shirazi, which showed no significant change in *VvEDS1* transcripts compared to the control.

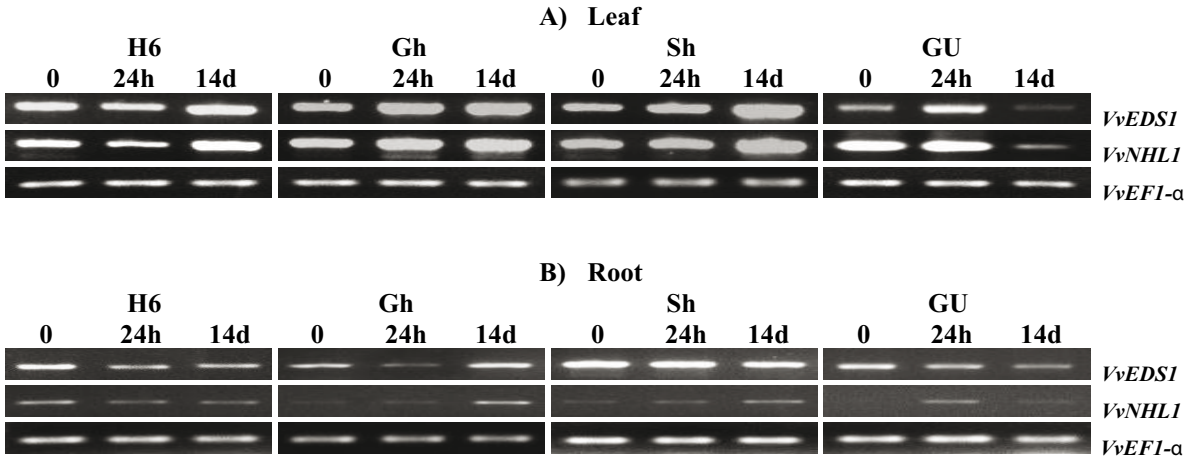

Figure 3. Expression profile of genes related to cell death in leaves (A) and roots (B) of four grape genotypes [H_6 (*V. vinifera* cv. GharaUzum × *V. riparia* cv. Kober 5BB), Gh: Gharashani, Sh: Shirazi, GU: GhezelUzum] after 0, 24 h, and 14 days treatment with 50 mM NaCl.

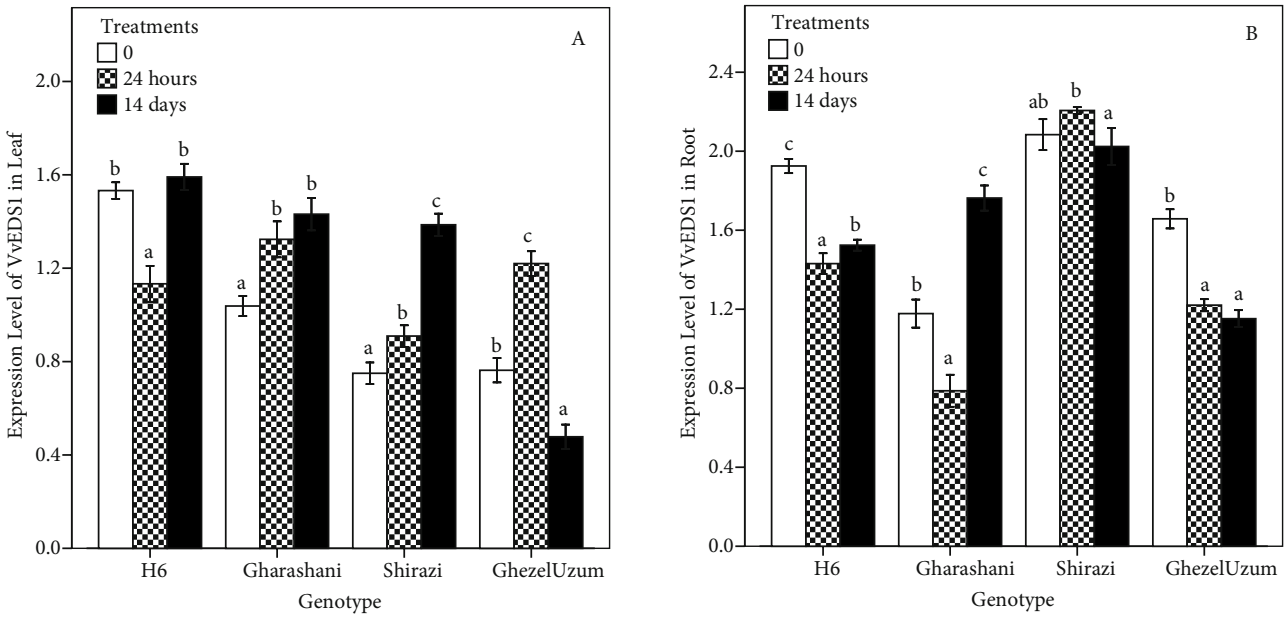

Figure 4. Expression level of *VvEDS1* gene in leaves (A) and roots (B) of four grape genotypes [H_6 (*V. vinifera* cv. GharaUzum × *V. riparia* cv. Kober 5BB), Gharashani, Shirazi, GhezelUzum] after 0, 24 h, and 14 days treatment with 50 mM NaCl. Bars are the means (n = 3) ± standard error ($P < 0.05$, one-way ANOVA). Different letters above the columns indicate significant difference between the treatments according to Tukey's test.

GLM analysis showed that the difference in expression of *VvEDS1* gene among all genotypes, and also among time points, was significant ($P < 0.05$).

3.2.2. Expression of *VvNHL1* gene

Overexpression of *VvNHL1* gene was related to cell death. The expression of *VvNHL1* gene in leaves and roots of tolerant and sensitive genotypes under salinity is presented in Figure 5. When compared to the expression of *VvEDS1* and *VvNHL1* genes, it can be concluded that they showed the same status in leaves of all genotypes.

In short-time salinity (24 h) *VvNHL1* transcripts accumulated in leaves of all genotypes, except for H6. In long-time salinity (14 days) transcripts upregulated in leaves of all genotypes, except for GhezelUzum. However, accumulation of transcripts was not significant in H6 genotype.

The roots of all genotypes showed an increase in expression of *VvNHL1* gene compared to the control; H6 showed a decrease.

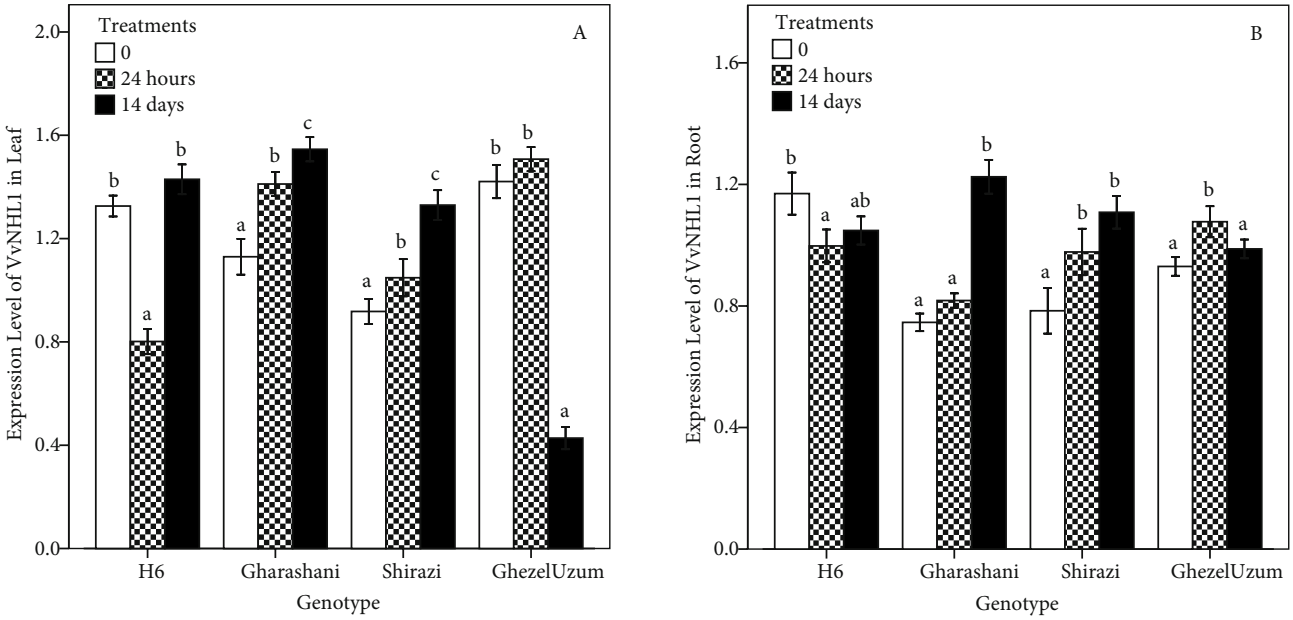

Figure 5. Expression level of *VvNHL1* gene in leaves (A) and roots (B) of four grape genotypes [H_6 (*V. vinifera* cv. GharaUzum × *V. riparia* cv. Kober 5BB), Gharashani, Shirazi, GhezelUzum] after 0, 24 h, and 14 days treatment with 50 mM NaCl. Bars are the means (n = 3) ± standard error ($P < 0.05$, one-way ANOVA). Different letters above the columns indicate significant difference between the treatments according to Tukey's test.

GLM analysis showed that the difference in expression of *VvNHL1* gene in leaves and roots of Shirazi and GhezelUzum, and also in roots of Gharashani and Shirazi, was not significant. In leaves, the difference in *VvNHL1* transcripts among time points was not significant, whereas in roots it was significant ($P < 0.05$).

3.3. Salinity effects on expression of photosynthesis-related genes in grape genotypes

The profile of photosynthesis-related genes (*VvP450* and *VvCPN21*) in leaves and roots of tolerant (H6 and Gharashani) and sensitive (Shirazi and GhezelUzum) grape genotypes (*Vitis* L.) at different time points under salinity (50 mM NaCl) is presented in Figure 6.

3.3.1. Expression of *VvP450* gene

VvP450 gene is a part of the photosynthesis electron transport chain. The expression of *VvP450* gene in leaves and roots of tolerant and sensitive grape genotypes is presented in Figure 7. The expression of *VvP450* gene increased in leaves of H6 and GhezelUzum but decreased in Gharashani, whereas the leaves of Shirazi showed no significant change compared to the control.

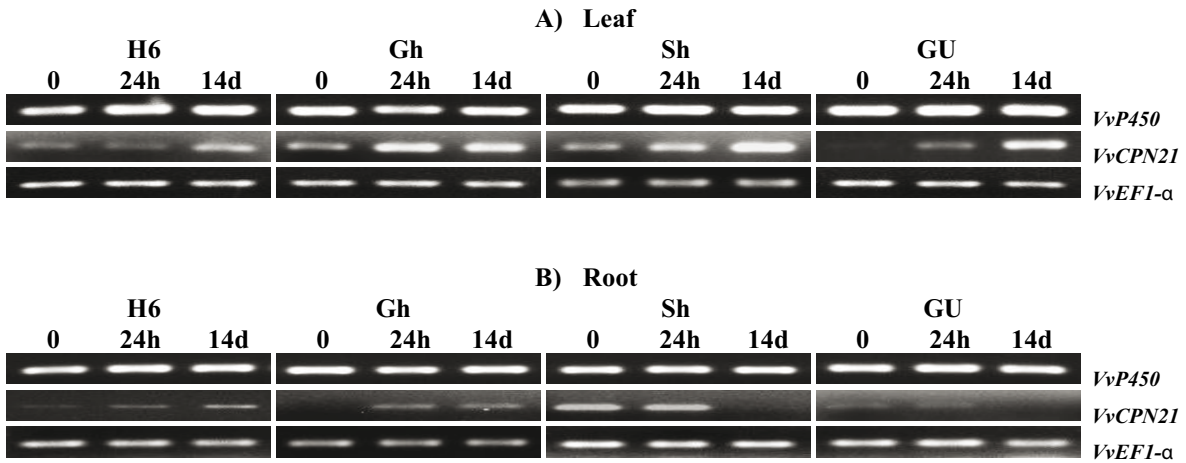

Figure 6. Expression profile of genes related to photosynthesis in leaves (A) and roots (B) of four grape genotypes [H_6 (*V. vinifera* cv. GharaUzum × *V. riparia* cv. Kober 5BB), Gh: Gharashani, Sh: Shirazi, GU: GhezelUzum] after 0, 24 h, and 14 days treatment with 50 mM NaCl.

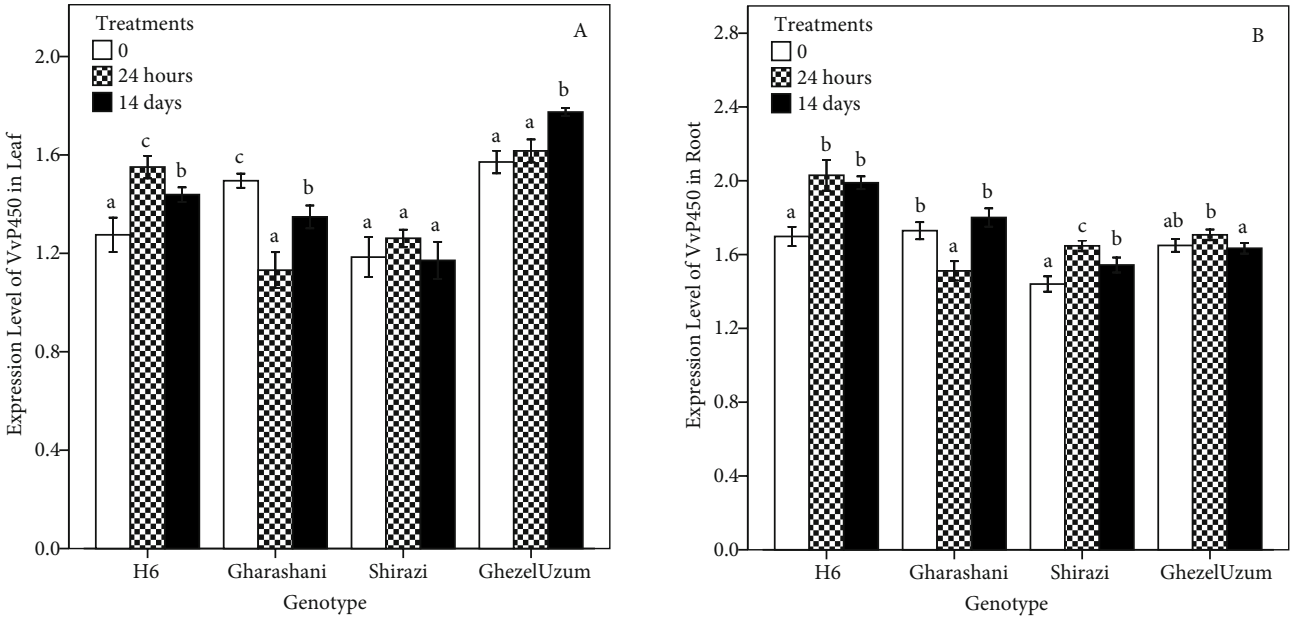

Figure 7. Expression level of *VvP450* gene in leaves (A) and roots (B) of four grape genotypes [H_6 (*V. vinifera* cv. GharaUzum × *V. riparia* cv. Kober 5BB), Gharashani, Shirazi, GhezelUzum] after 0, 24 h, and 14 days treatment with 50 mM NaCl. Bars are the means (n = 3) ± standard error ($P < 0.05$, one-way ANOVA). Different letters above the columns indicate significant difference between the treatments according to Tukey's test.

In long-time salinity the roots of H6 and Shirazi genotypes accumulated *VvP450* transcripts, but the roots of Gharashani and GhezelUzum showed no significant change compared to the control.

GLM analysis showed that the difference in accumulation of *VvP450* transcripts in leaves of all genotypes was significant ($P < 0.05$), but in roots the difference between GhezelUzum and Gharashani genotypes was not significant. The difference in expression of *VvP450* gene between 24-h and 14-days salinity in leaves and roots was not significant.

3.3.2. Expression of *VvCPN21* gene

VvCPN21 gene belongs to a chloroplast chaperonin. As shown in Figure 8, *VvCPN21* transcripts in leaves of all genotypes treated with 50 mM NaCl accumulated significantly ($P < 0.05$) compared to the control. However, in H6 genotype gene expression decreased in short-time and increased in long-time salinity.

After 14 days salinity the roots of tolerant and sensitive genotypes showed inverse status; hence, *VvCPN21* transcripts upregulated in tolerant and downregulated in sensitive genotypes.

GLM analysis showed that the difference in *VvCPN21* transcripts was significant ($P < 0.05$) in leaves of all genotypes, but the difference among Gharashani, H6, and GhezelUzum was not significant in the roots. In both leaves and roots the difference among salinity treatments was significant.

3.4. Salinity effects on expression of phenylpropanoid pathway genes in grape genotypes

The profile of phenylpropanoid pathway genes (*VvChS* and *VvPAL*) in leaves and roots of tolerant (H6 and Gharashani) and sensitive (Shirazi and GhezelUzum) grape genotypes (*Vitis* L.) treated with 50 mM NaCl at different time points is presented in Figure 9.

3.4.1. Expression of *VvChS* gene

VvChS gene belongs to chalcone synthase, which is a key enzyme in the flavonoid synthesis pathway. As shown in Figure 10, *VvChS* transcripts first upregulated (24 h treatment) and then decreased (14 days salinity), except for Shirazi, which showed no significant increase after 24 h salinity. The increase in chalcone synthase gene in the leaves of tolerant genotypes was higher than in sensitive ones.

Under salinity, *VvChS* transcripts downregulated in roots of tolerant genotypes, whereas sensitive genotypes showed first an increase and then a decrease. After 14 days of treatment with 50 mM NaCl, *VvChS* transcripts decreased significantly ($P < 0.05$) in roots of GhezelUzum, but roots of Shirazi genotype showed no significant change.

GLM analysis showed that the difference in accumulation of *VvChS* transcripts between H6 and GhezelUzum genotypes was not significant in leaves, but the difference among all genotypes in roots was significant. In addition, the difference among time points in both roots and leaves was significant ($P < 0.05$).

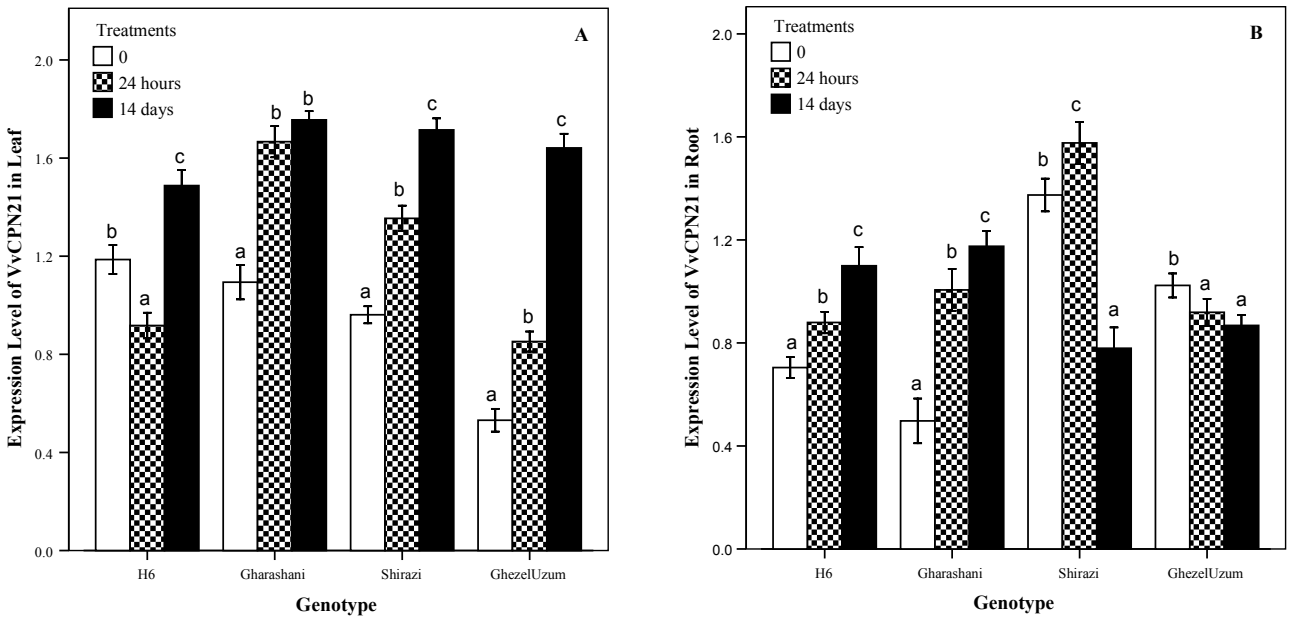

Figure 8. Expression level of *VvCPN21* gene in leaves (A) and roots (B) of four grape genotypes [H_6 (*V. vinifera* cv. GharaUzum × *V. riparia* cv. Kober 5BB), Gharashani, Shirazi, GhezelUzum] after 0, 24 h, and 14 days treatment with 50 mM NaCl. Bars are the means (n = 3) ± standard error ($P < 0.05$, one-way ANOVA). Different letters above the columns indicate significant difference between the treatments according to Tukey's test.

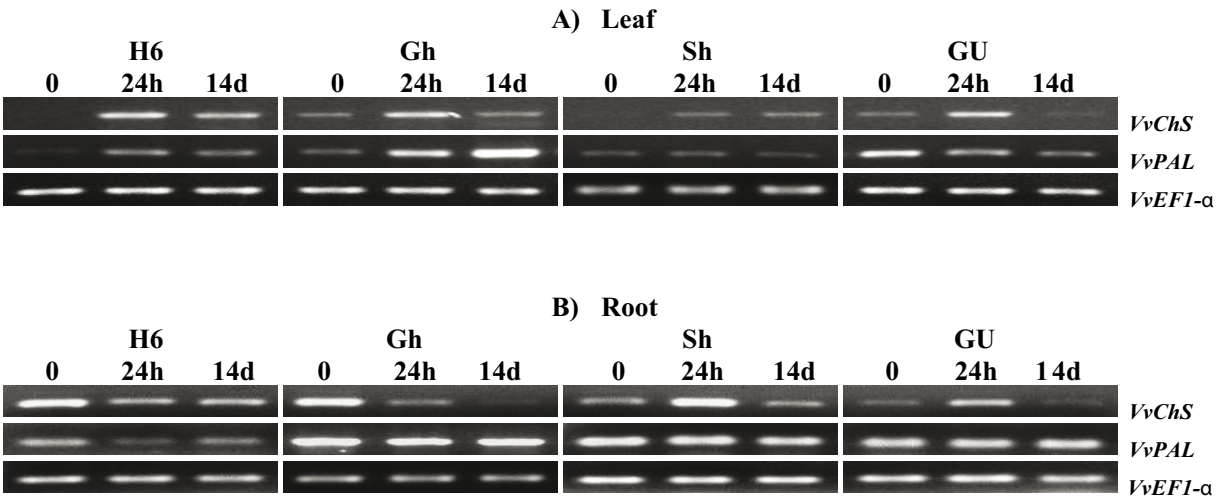

Figure 9. Expression profile of phenylpropanoid pathway genes in leaves (A) and roots (B) of four grape genotypes [H_6 (*V. vinifera* cv. GharaUzum × *V. riparia* cv. Kober 5BB), Gh: Gharashani, Sh: Shirazi, GU: GhezelUzum] after 0, 24 h, and 14 days treatment with 50 mM NaCl.

3.4.2. Expression of *VvPAL* gene

VvPAL gene belongs to phenylalanine ammonia lyase, which is a key enzyme in the phenylpropanoids pathway. As shown in Figure 11, *VvPAL* transcripts upregulated in leaves of tolerant genotypes and downregulated in GhezelUzum; however, Shirazi showed no significant change.

The roots of all genotypes showed first a decrease and then an increase, except for Shirazi, which showed the inverse. In long-time salinity the expression of *VvPAL* gene decreased in H6 and Shirazi, but Gharashani and GhezelUzum genotypes showed no significant change.

GLM analysis showed that the difference in expression of *VvPAL* gene was significant ($P < 0.05$) among genotypes and also among treatments.

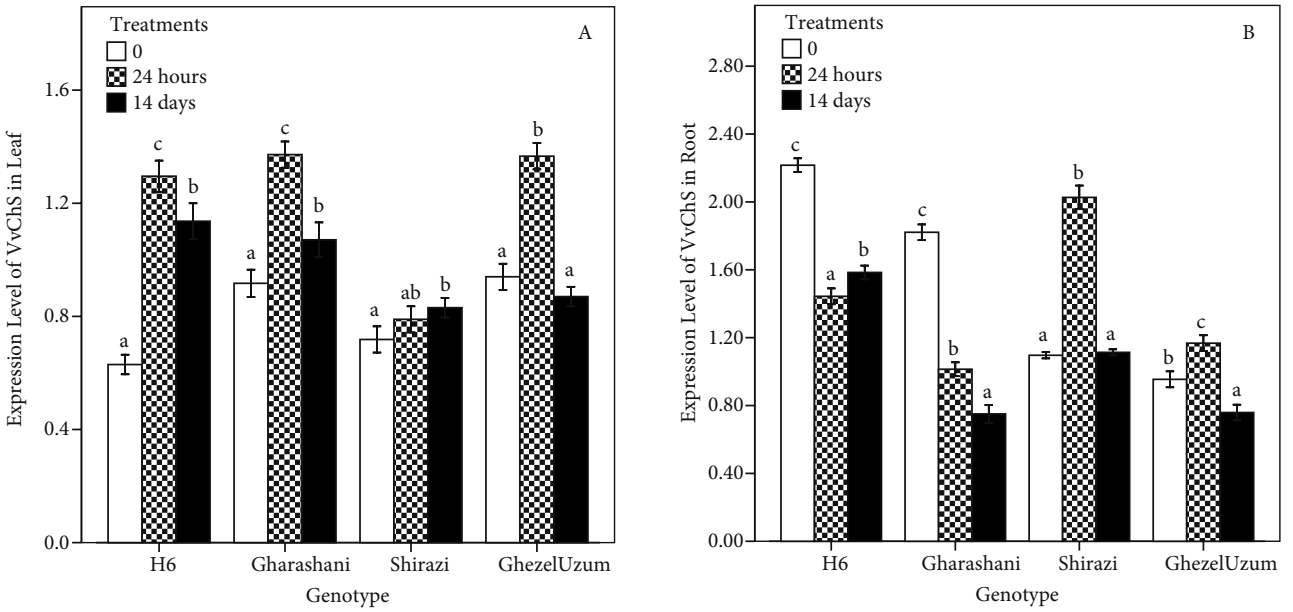

Figure 10. Expression level of *VvChs* gene in leaves (A) and roots (B) of four grape genotypes [H_6 (*V. vinifera* cv. GharaUzum × *V. riparia* cv. Kober 5BB), Gharashani, Shirazi, GhezelUzum] after 0, 24 h, and 14 days treatment with 50 mM NaCl. Bars are the means (n = 3) ± standard error ($P < 0.05$, one-way ANOVA). Different letters above the columns indicate significant difference between the treatments according to Tukey's test.

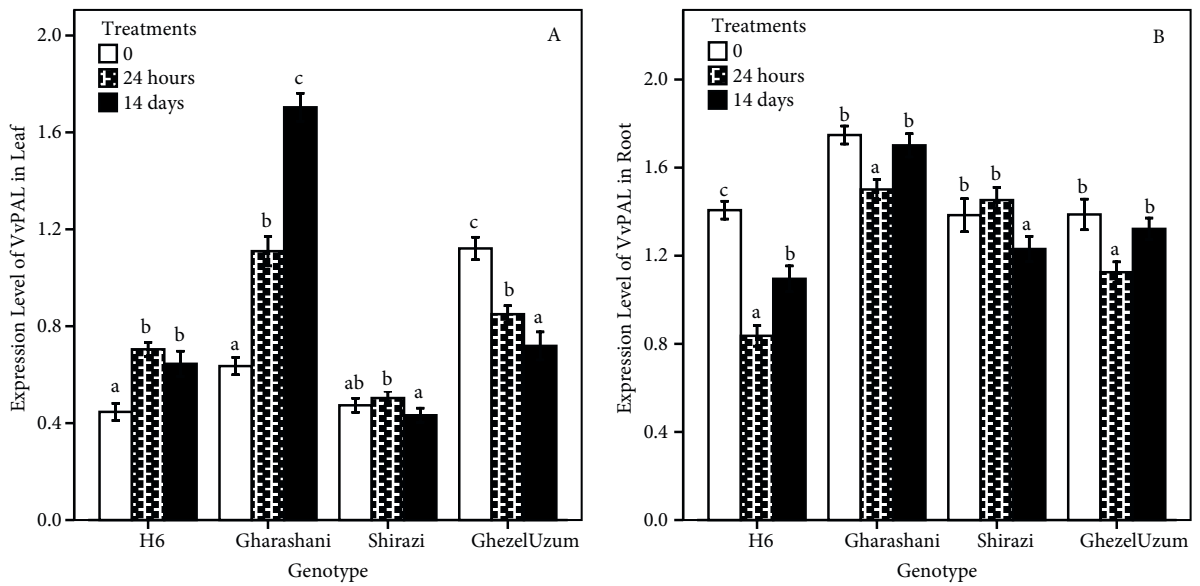

Figure 11. Expression level of *VvPAL* gene in leaves (A) and roots (B) of four grape genotypes [H_6 (*V. vinifera* cv. GharaUzum × *V. riparia* cv. Kober 5BB), Gharashani, Shirazi, GhezelUzum] after 0, 24 h, and 14 days treatment with 50 mM NaCl. Bars are the means (n = 3) ± standard error ($P < 0.05$, one-way ANOVA). Different letters above the columns indicate significant difference between the treatments according to Tukey's test.

4. Discussion

Salt stress, one of the most important abiotic stresses, can cause plants to receive and transmit signals to cellular machinery, thereby activating adaptive responses; this is of fundamental importance to biology (Lu et al., 2015).

4.1. Salinity effects on water potential and MDA content

The decrease in water potential and increase in MDA content indicated that our genotypes were under osmotic stress.

Restriction of extension growth caused by salinity is the result of decreased water uptake by roots due to an imbalance between water uptake and transpiration. This, in turn, depresses water potential and collapses the water potential gradient between the expanding cells and water source (xylem) that drives extension (Munns and Tester, 2008).

In the present study, under long-time salinity stress (14 days) we observed a drastic decrease in water potential compared to the control plants, especially in sensitive plants. Our results were consistent with the Çulha Erdal and Çakırlar (2014) study that reported a decrease of water content in safflower cultivars under salinity.

Lipid peroxidation is correlated with oxidative damage under abiotic stresses (Bor et al., 2003). The increase in MDA content is an index of oxidative stresses such as salinity (Hernández and Almansa, 2002; Çulha Erdal and Çakırlar, 2014; Priya et al., 2015). Our results showed a significant ($P < 0.05$) increase in MDA content in roots and leaves of salt-treated plants, particularly in sensitive genotypes. Previous studies also reported that lipid peroxidation under salt stress was lower in salt-tolerant plants such as *Beta maritima* (Bor et al., 2003). In the present study, the increase in MDA content in roots was higher than in leaves. Perhaps because our roots were in salt solutions and could not escape salinity, they showed greater damage than the leaves.

There were significant negative correlations ($P < 0.05$) between water potential and MDA content.

4.2. Salinity effects on gene expression in grape

Recent and important works have focused on transcriptome dynamics during grapevine development, since most of the physiological and biochemical changes are determined by gene transcriptional variation (Zenoni et al., 2010). These studies identified key genes the expression of which programmed cell metabolism via the regulation of signal transduction. Some abiotic stress responsive genes play significant roles in salt tolerance. The current work determined the changes in expression of some important genes in tolerant and sensitive grape genotypes.

4.3. Salinity effects on expression of cell death related genes

In this study, the expressions of *VvNHL1* and *VvEDS1* were studied as defense genes.

EDS1 and *PAD4* are needed for the expression of hypersensitive response cell death. Further studies should be done to reveal the exact role of *VvEDS1* in grapevine defense pathways (Chong et al., 2008). *EDS1* is also needed to maintain the stress response program after ROS production. Hence, *EDS1* seems to function as a 'master' regulator of cell death in response to 'stress' signals (Ochsenbein et al., 2006).

An increase in *EDS1* transcripts would not be an integral part of a stress-response network activated by singlet oxygen. Signaling pathways that activate abiotic stress responses could have a common origin with *EDS1* and play a central role during biotic and abiotic stress responses. In this case, the function of *EDS1* would not be limited to defense against pathogens, but could also provide cross-protection against a variety of other stress conditions (Wagner et al., 2004).

In the present study, after 14 days salinity, *VvEDS1* transcripts decreased in all genotypes, except for Gharashani, which showed a significant increase ($P < 0.05$). Because Gharashani was a tolerant genotype, *VvEDS1* transcript accumulation could probably increase genotype tolerance to salinity. This was consistent with the Chini et al. (2004) study that reported that increased expression of *EDS1* caused drought tolerance. In the present study Gharashani was the only genotype that *VvEDS1* transcripts upregulated into its roots and leaves under long-term salinity (14 days). The other tolerant genotype (H6) showed no significant increase in expression of *VvEDS1* and even showed a decrease in roots. It seems that *VvEDS1* gene does not play a key role in defense mechanisms of H6 genotype.

Stress responses of plants could be caused by perception of ROS as a signal that activates a genetic stress response program. The *EDS1* protein seems to be involved in controlling the singlet-oxygen–mediated visible stress responses. *EDS1* seems to control recovery after plants have been exposed to environmental conditions. *EDS1* encodes a protein that shares similarity in its amino terminal portion with the catalytic motifs of eukaryotic lipases, suggesting that hydrolytic activity of *EDS1* may contribute to its biological activity (Wagner et al., 2004).

NHL genes belong to a multigenic family whose members could have different functions not strictly related to plant defense. *NHL* genes are a marker of hypersensitive response in tobacco and are also activated at a late stage of leaf senescence. Thus, overexpression of several *NHL* genes could be associated with abnormal cell death regulation. *VvNHL1* is constitutively expressed in *Vitis vinifera*. One hypothesis is that *VvNHL1* could promote cell death (Pontier et al., 1999). Kamei et al. (2005) reported upregulation of *NHL* gene under salinity as a part of a defense response in *Arabidopsis*.

This study showed that abundance of *VvNHL1* transcripts was similar to that of *VvEDS1* in leaves of all genotypes and in roots of tolerant genotypes. Our results verified Pontier et al. (1999), who found that *VvNHL1* and *VvEDS1* work together as a part of grape defense responses because of similar expression patterns. The *VvNHL1* gene accumulated in leaves and roots of Gharashani and Shirazi genotypes; the increase in Gharashani was higher than

Shirazi. Gene function and the contribution between *VvNHL1* and *VvEDS1*—as defense genes even under abiotic stress—was likely different in tolerant and sensitive genotypes. The function of these genes may be different in Gharashani and H6, tolerant genotypes, because *VvNHL1* gene showed no significant increase in leaves and roots of H6.

4.4. Salinity effects on expression of photosynthesis-related genes

A cytochrome P450 (CYP450), or *VvP450* that was cloned from grape, belongs to a very large superfamily of hemoproteins. They are usually part of electron transfer chains that lead to the synthesis of various fatty acid conjugates, plant hormones, and defense compounds. Terpenoids, the largest class of characterized natural plant compounds, are often substrates for plant cytochrome P450 (Ehlting et al., 2008; Costenaro-da-Silva et al., 2010). This gene may be producing the signals that activate fruit development due to its involvement in the synthesis of plant secondary products (Carmona et al., 2008).

In our study, after 24 h salinity *VvP450* transcripts increased in leaves and roots of all genotypes, except Gharashani, which showed a significant decrease ($P < 0.05$) compared to the control. Because *VvP450* gene codes a part of the electron transport chain in grape and in light of our photosynthesis results (Mohammadkhani, 2013), all genotypes showed a decrease in photosynthesis rates under salinity. Only Gharashani genotype showed a positive significant correlation ($P < 0.05$) between *VvP450* transcripts in leaves and photosynthesis rate under salt stress. Other genotypes showed no correlation.

It is interesting that the leaves and roots of H6 genotype showed a significant increase ($P < 0.05$) compared to the control. Because *VvP450* codes proteins of the photosynthesis electron transport chain, we expected to detect it only in leaves; however, expression and significant changes ($P < 0.05$) in gene transcripts were observed in roots as well. The changes in *VvP450* transcripts in leaves were higher than in roots. Nomura and Bishop (2006) reported cytochrome P450 has a role in synthesis of steroidal hormones in plants. Perhaps changes in *VvP450* transcripts under salinity were related to that hormones synthesis.

Hanania et al. (2007) suggested that a key protein determining seedlessness in grapes is the chloroplast chaperonin 21 (ch-Cpn21). They suggested that downregulation of ch-Cpn21 is correlated with the seedless phenotype, indicating that absence or lower levels of ch-Cpn21 may lead to seed abortion.

In this study, *VvCPN21* was selected for two reasons: (1) it belongs to a chaperonin, and the chaperones had a key role in plant tolerance to abiotic stresses, and (2) chloroplast chaperonin 21 had a functional role in rubisco (ribulose-1,5-bisphosphate carboxylase oxygenase) activity (Baneyx et al., 1995).

Our results showed that the leaves of all genotypes accumulated *VvCPN21* transcripts, and the increase in sensitive genotypes (Shirazi and GhezelUzum) was higher. In long- term salinity (14 days) the roots of tolerant genotypes showed an increase, and sensitive genotypes showed a decrease in expression of *VvCPN21*. Therefore, chloroplast chaperonin 21 may be a chaperone that plays a role in the salinity tolerance (Baneyx et al., 1995) of our grape genotypes, in particular because an increase in *VvCPN21* transcripts was only observed in roots of tolerant genotypes; perhaps because the roots were subjected to higher salinity. The detection of *VvCPN21* transcripts was expected only in leaves, but observed in roots as well. However, the expression of *VvCPN21* in leaves was higher than in roots. There was a significant negative correlation ($P < 0.05$) between *VvCPN21* transcripts in leaves and photosynthesis rate and chlorophyll content (Mohammadkhani, 2013) in all genotypes under salinity.

4.5. Salinity effects on expression of phenylpropanoid pathway genes

Chalcone synthase is a key enzyme in flavonoid biosynthesis, and its content is severely influenced by plant growth conditions. In grape genes, transcripts related to anthocyanin synthesis (like *VvChS*) increased at the end of fruit development. Fruit begins anthocyanin synthesis at the end of development. Further, anthocyanin biosynthesis was induced under environmental stimuli such as drought conditions (Castellarian et al., 2007).

Theoretically, there are many ways to regulate CHS activity in vivo, from metabolic control to the control of CHS gene transcription. Under stress conditions a plant is expressing a number of genes as a part of defense responses. Among these genes, CHS is commonly induced in different plant species under abiotic stresses (Dao et al., 2011).

VvChS gene transcripts increased in leaves of our genotypes under salinity, although GhezelUzum showed no significant change compared to control under long-term (14 days) salinity. Our results verified the Dao et al. (2011) study that reported abiotic stresses increased the expression of *VvChS*. Moreover, our results were consistent with the Dehghan et al. (2014) results that reported an increase in expression of *ChS* gene after 24 h salinity. In the present study gene transcripts downregulated in roots of tolerant genotypes, and they first increased and then decreased in sensitive genotypes. Different expression of *VvChS* in roots of our tolerant and sensitive genotypes under salinity probably was related to tolerance of the genotype to stress. This means that sensitive genotypes that showed low tolerance to salinity try to activate defense mechanisms by increasing *VvChS* transcripts. However,

the leaves of tolerant genotypes showed higher *VvChS* gene transcripts compared to those of sensitive ones.

The leaves and roots of tolerant genotypes showed different expression of the *VvChS* gene. Considering the expression of *VvChS* decreased or showed no significant change in roots under salinity, it seems that the expression of the key gene belonging to the phenylpropanoid pathway (*VvChS*) in leaves of our studied genotypes was related to plant tolerance.

Phenylalanine ammonia-lyase (PAL, EC 4.3.1.5) converts l-phenylalanine to *trans*-cinnamic acid, a precursor of various phenylpropanoids such as phenolic acids and flavonoids. PAL is considered a key enzyme between primary (shikimate pathway) and secondary (phenylpropanoid) metabolism, regulating the flux of phenylalanine to the biosynthesis of phenolic compounds. Due to the nature and defense-related function of these products, PAL activity under stress has been considered part of the defense mechanism. Some reports have suggested that PAL activity is affected by promoting the transcription or translation of the *PAL* gene (Dixon and Paiva, 1995). Solecka and Kacperska (2003) have shown an increase in PAL activity and accumulation of phenylpropanoids in plants acclimated to cold. PAL activity could be induced by different biotic and abiotic stresses (Lafuente et al., 2004).

In our study the expression profile of *VvPAL* in leaves of tolerant genotypes was consistent with Lafuente et al. (2004); gene transcripts increased in leaves of tolerant genotypes (H6 and Gharashani) under salinity. The increase in phenylalanine ammonia lyase transcripts in leaves of tolerant genotypes probably showed the ability of our plants to use that enzyme as a part their defense mechanisms. Under salinity, roots of our genotypes showed no significant change in *VvPAL* transcripts, and some genotypes showed a decrease. This was verified in Dehghan et al. (2014), results that reported a decrease in *PAL* gene under salinity. The expression profile of the *VvPAL* gene in roots did not show significant correlation with plant tolerance to salinity.

In conclusion, our tolerant genotypes showed a higher water potential and a lower MDA content. The expression of these genes in grape showed a significant difference between tolerant (H6 and Gharashani) and sensitive genotypes (Shirazi and GhezelUzum).

The expression of *VvNHL1* in leaves of all genotypes and in roots of tolerant genotypes was similar to *VvEDS1*. Probably these genes associated in defense responses, because they showed similar expression profiles (Prior et al., 1999).

Considering that *VvP450* gene codes a protein of the photosynthesis electron transport chain and *VvCPN21* gene is a chloroplast chaperonin that plays a key role in rubisco activity, their detection was expected only in leaves; however, they were also expressed in roots.

The expression of *VvChS* and *VvPAL* increased significantly ($P < 0.05$) in leaves of tolerant genotypes under salinity. Expression of genes belonging to phenylpropanoid key enzymes in leaves of our studied genotypes was related to plant tolerance to salinity. Different biotic and abiotic stresses affect PAL enzyme activity and anthocyanin production (Castellarin et al., 2007).

To summarize, our findings highlight a strong relationship between the accumulation of specific transcripts and salinity tolerance in grape. Transcriptional induction of genes in response to salt stress has been recognized as an adaptive mechanism of plants against salinity (Cushman and Bohnert, 2000). Different expression of genes in salt-sensitive and tolerant grape genotypes, combined with previous studies of salt-induced responses in specific cultivars (Tattersall et al., 2007), provides useful information for salt tolerance in grape, a crop of major economic interest that is exposed to salt stress.

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