# Expression of related proteins and aquaporin genes in grape (*Vitis vinifera* L.) under salinity stress

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## Abstract

Due to worldwide increasing of salinity, the identification of genes conferring tolerance to plants is important. The aim of this study was to investigate salinity effects on the expression of three genes-related to proteins and aquaporin in grape (*Vitis vinifera* L.). Based on screening study on 18 grape genotypes, H6 and Gharashani that showed lower decrease in water potential, leaf area, leaf growth rate and relative water content under salinity, were selected as tolerant genotypes compared to sensitive ones (Shirazi and GhezelUzum). Plants were treated with 50 mM NaCl as a critical concentration for 0, 24 hours and 14 days. High expression of *VvFS41* gene in the sensitive genotypes under salinity showed that these genotypes used all of their capacity to survive under stress condition. The expression of *VvPHP1* showed no regular status. Expression of *VvPIP2.2* decreased in roots of all genotypes under salinity and the determination of *VvPIP2.2* role seems be difficult in these genotypes. Based on the results, sensitive genotypes showed higher changes in proteins and aquaporin genes under salinity, but tolerant genotypes was more stable. Our findings showed a significant difference between tolerant and sensitive genotypes and highlighted a strong relationship between the accumulation of specific transcripts and stress tolerance.

**Key words:** Salinity, Grape, Stress proteins, Transcripts, Aquaporin genes.

**Abbreviations**

MIP: membrane intrinsic protein, PIP: plasma membrane intrinsic proteins, TIP: tonoplast intrinsic proteins, EF1: Elongation Factor 1.

## INTRODUCTION

### Salinity effects on gene expression in grape

Recent studies have shown that salinity and drought stresses induced great changes in grape gene expression (Jellouli *et al.*, 2007). Comparative gene expression analysis could be a useful approach for understanding the mechanisms of tolerance and susceptibility (Kozian and Kirschbaum, 1999). Grapevine (*Vitis vinifera* L.) is a most widely cultivated perennial plant in the world, and viticulture has high economic importance. Due to the recent identification of the grapevine genome, this plant may become a model for fruit tree genetics and abiotic stress tolerance by biotechnology approaches (Troggio *et al.*, 2008).

Grapevine is well known for its high content of interfering substances, which prevent the application of standard RNA isolation protocols. During abiotic stresses such as salt stress, unfavorable secondary metabolites accumulate significantly. It is a major challenge to obtain sufficient amounts of high-quality RNA from grapevine, especially when cultivated under abiotic stress conditions (Daldoul *et al.*, 2009). Roots absorb water and nutrients from soil and are the first organ to perceive abiotic stresses like drought and salinity. Grape roots also accumulate some defense compounds (Cushman and Bohnert, 2000). Despite importance of roots, the expression of genes in roots has rarely been studied.

Molecular information is needed to determine the gene expression profile. Previous genetics and functional genomic studies have provided some molecular knowledge about plant salt tolerance. Some important genes encoding proteins for ion channels, signaling factors and anti oxidative enzymes have been recognized. That information is useful for improvement of grape quality (Deluc *et al.*, 2007).

**Salinity effects on expression of related genes**
*VvFS41* gene encodes a putative S1-like ribosomal protein that in conjunction with rRNA make up ribosomal subunits involved in translation process. They are also being responsible for the stabilization of rRNA structures by filling the gaps between RNA domains (Gueydan *et al.*, 2002).

Within the diverse Hsp gene family, Hsp 70 is the most studied member. The encoded and highly conserved 70 KDa protein-member plays a key role in stress response in plants (Vierling, 1991). It is reported that Hsp mRNA increases in the plant cytosol in response to different types of stresses, especially higher temperature (Cronje *et al.*, 2004). Hsp proteins act as chaperone proteins and assist in the translocation or degradation of damaged proteins (Bukau and Horwich, 1998). The diversity that characterizes this heat shock protein family is thought to reflect a molecular adaptation to environmental and developmental conditions that plants must tolerate during their life cycle (Waters, 1995). When different Hsp families are compared, it appears that they exhibit unequal rates of molecular evolution, as has been suggested for some of the subclasses of the stress 70 family (Waters *et al.*, 1996). Functioning as molecular chaperones in vitro and in vivo, HSPs can prevent irreversible protein aggregation and maintain denatured proteins in a folding-competent state under abiotic stress conditions (Mu *et al.*, 2013).

**Salinity effects on expression of aquaporin gene**
Aquaporins are members of the membrane intrinsic protein (MIP) family that are highly hydrophobic proteins. Major intrinsic proteins (MIPs) are integral membrane proteins most of which have been shown to function as water channels across membranes. In many membranes, MIPs constitute the major membrane protein. Plant MIPs have been classified into three different subfamilies. Two of the subfamilies, plasma membrane intrinsic proteins (PIPs) and tonoplast intrinsic proteins (TIPs), are named according to the subcellular location of the proteins. The third group of MIPs shows similarity with NOD26, a nodulin expressed in peribacteroid membrane surrounding the symbiotic nitrogen-fixing bacteria in nodules of soybean roots (Johanson

and Gustavsson, 2002). In grapevine, there are few studies characterizing and identifying the roles of the MIPs in water transport (Vandeleur *et al.*, 2009). Gene expression studies in various plant species have shown variable responses of aquaporin isoforms to water stress, with both up and down regulation of genes (Alexandersson *et al.*, 2005). PIP2 aquaporin involved in radial water movement, controls water absorption and usage efficiency and alters transgenic plants drought and salt tolerance (Wang *et al.*, 2015).

Salinity is one of the important abiotic stresses that reduces plant growth and yield via osmotic stress and ionic toxicity. Salinity of soil and water resources is a serious threat in many parts of the country. Estimated land area affected by salinity varies between 16 to 23 Mha. Grape (275,000 ha) is the second tree crop after pistachio in Iran. Grape plants have medium sensitivity to salinity. Soil salinity affected viticulture as a strategic agricultural product in the fields around Urmia Salt Lake. Iran is one of the top 10 countries with highest grapes production in the world with 2,252,480 tons production annually. World table grape production reaches 16.5 million tons (Siadat *et al.*, 1997). In this study the expression of genes associated with salinity tolerance were compared in four grape genotypes. In previous experiments we screened 18 grape genotypes from the view point of salt tolerance parameters (Mohammadkhani *et al.*, 2012; Mohammadkhani *et al.*, 2014). The 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 gene expression related to stress and aquaporin in roots and leaves of tolerant and sensitive grape genotypes under salinity.

## MATERIALS AND METHODS

**Plant materials and growth conditions**
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 and Natural Resources Research Center, grape genotypes collection, Urmia, West Azarbayjan). The cuttings were disinfected with benomyle (1% w/v) and then basal parts soaked in Indole-3-butyric acid 0.1% (w/v) for 5-10 s. All cuttings were placed in a mist house (relative humidity 80%) with a heat-bed temperature of 20-30ºC. After two weeks, the rooted cuttings were transferred into pots containing aerated Hoagland solution. The pots were protected with aluminum foil to avoid light

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**Table 1.** Mean values for some physiological factors in four grape genotypes (*Vitis vinifera* L.) at different salinity time points (0, 24 hours and 14 days under 50 mM NaCl). Data are the means ± standard Error (n=3, One Way ANOVA). Different letters within a column indicate significant differences (P<0.05).

| Genotype & Salinity (mM NaCl) | Water Potential (MPa) | Leaf Area (cm$^2$) | Leaf Growth Rate (cm$^2$/day) | Relative Water Content (RWC) |
|---|---|---|---|---|
| **H6** | | | | |
| 0 | -0.10±0.003 c | 1343.14±5.21 c | 23.31±0.46 c | 91.06±0.51 b |
| 24 hours | -0.17±0.003 b | 1225.31±26.87 b | 18.71±0.66 b | 85.58±0.52 a |
| 14 days | -0.26±0.003 a | 1111.49±25.96 a | 7.58±0.24 a | 84.28±0.32 a |
| **Gharashani** | | | | |
| 0 | -0.11±0.006 c | 1305.26±7.10 c | 22.15±0.63 c | 80.80±0.25 c |
| 24 hours | -0.18±0.006 b | 1166.00±26.91 b | 13.88±0.37 b | 74.84±0.24 b |
| 14 days | -0.26±0.006 a | 1046.09±4.31 a | 4.74±0.24 a | 70.29±0.28 a |
| **Shirazi** | | | | |
| 0 | -0.12±0.003 c | 1487.04±28.61 c | 19.58±0.61 c | 92.29±0.77 c |
| 24 hours | -0.19±0.003 b | 1193.50±21.80 b | 12.56±0.29 b | 84.68±0.41 b |
| 14 days | -0.31±0.003 a | 1048.02±23.12 a | 2.21±0.13 a | 74.08±0.36 a |
| **GhezelUzum** | | | | |
| 0 | -0.12±0.003 c | 1614.99±3.29 c | 25.63±0.19 c | 83.74±1.15 c |
| 24 hours | -0.19±0.003 b | 1398.20±22.21 b | 18.90±0.62 b | 79.00±0.54 b |
| 14 days | -0.31±0.003 a | 1059.74±6.96 a | 1.13±0.03 a | 68.71±0.00 a |

effects and alga proliferation.

**Salinity Treatments**

Two month-old plants were treated with 50 mM NaCl (threshold salinity determined for the genotypes). According to our screening study 50 mM NaCl was sufficient to reduce water potentials, but did not kill the grapevine plants when exposed for several days. We used 10- 200 mM salinity for 14 days in screening experiments and concluded 50 mM NaCl was a concentration that reduced water potentials, but did not kill plants for 14 days. Our plants were exposed to osmotic stress and decrease of relative water content. Water potentials and some physiological factors are showed in Table 1. Leaf and root tissues were collected at different time points (0, 24 hours and 14 days), frozen in liquid nitrogen immediately and stored at -80°C until RNA isolation.

**RNA isolation, cDNA synthesis and RT-PCR conditions**

Total RNA was extracted from leaves and 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. First-strand cDNA was synthesized from total RNA using a first strand cDNA synthesis Kit (Fermentas) according to the manufacturer's instructions. The cycling protocol for 20 µl 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 up with PCR Master Kit (Cinnagen Co.). PCR conditions were as following protocol: initial denaturation at 95°C for 3 min, followed by 28-30 cycles at 95°C for 30 s, 58-64°C for 30 s and 72°C for 20 s and final extension at 72°C for 5 min. The *VvEF1* gene (Elongation Factor 1) was used as internal reference. Forward and reverse primers sequences are showed in Table 2. The products of RT-PCR were separated on 1.5% agarose gel containing Ethidium Bromide (0.5 µg/ml) and visualized using Gel Logic 212 pro Imaging System (Carestream, USA). Gene Ruler 50 bp plus (50-1500 bp) was used as DNA ladder (Fermentas). Experiments wererepeated three times. The intensity of the RT-PCR bands was measured using Image J software 1.43.

**Statistical analysis**

Statistical analyses were done using SPSS (Version 14.0). Error bars on graphs are standard error of mean. One-way analysis of variance with post tests and two-way analysis of variance (General Linear Model) with Tukey's multiple range tests (*P*<0.05) was used to dete-

**Table 2.** Forward and revers primers used in RT-PCR experiment.

| Genes | Forward Primer (5'→3') | Reverse Primer (5'→3') |
|---|---|---|
| *VvFS41* | GAGACATCCTCACCCTGCTC | GACTATGACTGTTTTATCCTGA |
| *VvPHP1* | CATCCATCACCAACCCATTT | CCAACATGCAGTTCACCATC |
| *VvPIP2.2* | TCCGCCAAGGACTATCATGAC | CGCAATCAGAGCCCTGTAGAA |
| *VvEF1-α* | TCTGCCTTCTTCCTTGGGTA | GCACCTCGATCAAAAGAGGA |

**Table 3.** Mean values and analysis of variance for expression level of genes in four grapes (*Vitis vinifera* L.) at different salinity time points (0, 24 hours and 14 days under 50 mM NaCl). Data are the means ± standard Error (One Way ANOVA).

| Salinity & Genotype | *VvFS41* Expression in Leaves | *VvFS41* Expression in Roots | *VvPHP1* Expression in Leaves | *VvPHP1* Expression in Roots | *VvPIP2.2* Expression in Leaves | *VvPIP2.2* Expression in Roots |
|---|---|---|---|---|---|---|
| **Salinity (50 mM NaCl)** | | | | | | |
| 0 | 1.19±0.01 b[a] | 1.13±0.01 b | 1.36±0.01 b[a] | 1.54±0.01 c | 1.07±0.01 a | 1.33±0.01 b |
| 24 h | 1.05±0.01 a | 1.03±0.01 a | 1.23±0.01 a | 1.23±0.01 b | 1.32±0.01 c | 0.88±0.01 a |
| 14 d | 1.24±0.01 c | 1.52±0.01 c | 1.20±0.01 a | 1.01±0.01 a | 1.17±0.01 b | 0.85±0.01 a |
| **Genotypes** | | | | | | |
| H6 | 1.19±0.01 c | 1.80±0.01 d | 1.13±0.01 a | 1.73±0.02 d | 0.84±0.02 a | 0.975±0.01 b |
| Gharashani | 1.06±0.01 b | 1.40±0.01 c | 1.60±0.01 c | 1.00±0.02 a | 1.47±0.02 c | 1.35±0.01 c |
| Shirazi | 0.99±0.01 a | 0.62±0.01 a | 1.10±0.01 a | 1.07±0.02 b | 1.22±0.02 b | 0.77±0.01 a |
| GhezelUzum | 1.42±0.01 d | 1.08±0.01 b | 1.22±0.01 b | 1.24±0.02 c | 1.20±0.02 b | 0.99±.22 b |
| **Analysis of Variance (mean square)** | | | | | | |
| Genotype | 0.321*[b] | 2.25* | 0.478*[b] | 0.974* | 0.613* | 0.516* |
| Salinity | 0.114* | 0.826* | 0.082* | 0.841* | 0.182* | 0.884* |
| Genotype × Salinity | 0.200* | 0.117* | 0.313* | 0.106* | 0.149* | 0.234* |

Salinity means calculated for all genotype combinations and genotype means calculated for all salinity treatments.
[a]Different letters within columns indicate significant differences at $P<0.05$ according to the Tukey's test.
[b]* indicate significance level at $P<0.05$ according to the Tukey's test.

rmine differences between means.

## RESULTS

Figure 1 showed the profile of protein related genes (*VvFS41* and *VvPHP1*) in leaves and roots of tolerant (H6 and Gharashani) and sensitive (Shirazi and GhezelUzum) grape genotypes (*Vitis vinifera* L.) in different time points treated by 50 mM NaCl. Tolerant and sensitive genotypes were selected based on the

screening experiments. Table 1 shows some physiological parameters (water potential, leaf area, leaf growth rate and relative water content) in studied genotypes. All of these factors decreased with time upon treatment by 50 mM NaCl. That decrease was higher in sensitive genotypes (Shirazi and GhezelUzum) compared to tolerant ones (H6 and Gharashani). It means that sensitive genotypes showed lower water potential, leaf area, leaf growth rate and relative water content to control. The mean values and analysis of variance of expression lev-

el of studied genes in Table 3. Table 3 shows the difference among genotypes, salinity treatments and genotype× salinity was significant about all studied genes.

**Salinity Effects on expression of VvFS41 gene**
*VvFS41* gene codes a ribosomal protein that interferes in translation. Figure 2 shows the expression of *VvFS41* gene in leaves and roots of tolerant and sensitive genotypes under salinity. The expression of *VvFS41* gene decreased in leaves of tolerant genotypes, but sensitive genotypes showed no significant changes ($P<0.05$) under under short-term salinity (24 hours) and increase in long-term treatment(14 days). After 14 days salinity *VvFS41* transcripts accumulated in roots of all genotypes. GLM analysis showed that the difference in *VvFS41* transcripts was significant ($P<0.05$) among genotypes and also among treatments.

**Salinity Effects on expression of VvPHP1 gene**
*VvPHP1* gene codes a heat sock protein that interferes in plant responses to environmental stimuli. In long-term salinity (14 days) leaves of tolerant genotypes showed no significant changes inexpression of *VvPHP1*, but *VvPHP1* transcripts increased in Shirazi and decreased in GhezelUzum (Figure 3). *VvPHP1* transcripts down-regulated in roots of all genotypes, the decrease in sensitive genotypes was higher than the tolerant ones. GLM analysis showed that the difference in *VvPHP1* transcripts was not significant between Shi-

razi and H6, also the difference between 24 hours and 14 days treatments was not significant in leaves. In roots the difference was significant among all genotypes and also among salinity treatments.

**Salinity effects on expression of aquaporin gene**
Figure 4 shows the expression profile of aquaporin gene (*VvPIP2.2*) in leaves and roots of tolerant (H6 and Gharashani) and sensitive (Shirazi and GhezelUzum) grape genotypes (*Vitis vinifera* L.) under salinity.

**Salinity Effects on expression of VvPIP2.2 gene**
*VvPIP2.2* gene is one of the aquaporin related genes. As Figure 5 shows, *VvPIP2.2* transcripts accumulated significantly ($P<0.05$) in leaves of H6 and GhezelUzum genotypes, but decreased in Gharashani. In Shirazi genotype the gene expression (24 hours treatment) increased first and then (14 days treatment) decreased. The roots of all genotypes showed decreases in gene transcripts compared to control, the decrease in Gharashani genotype was higher than others. GLM analysis showed that the difference in expression of *VvPIP2.2* gene was not significant ($P<0.05$) in leaves between GhezelUzum and Shirazi genotypes, but the difference among all treatments was significant. In roots the difference between H6 and GhezelUzum genotypes and also between 24 hours and 14 days salinity was not significant ($P<0.05$).
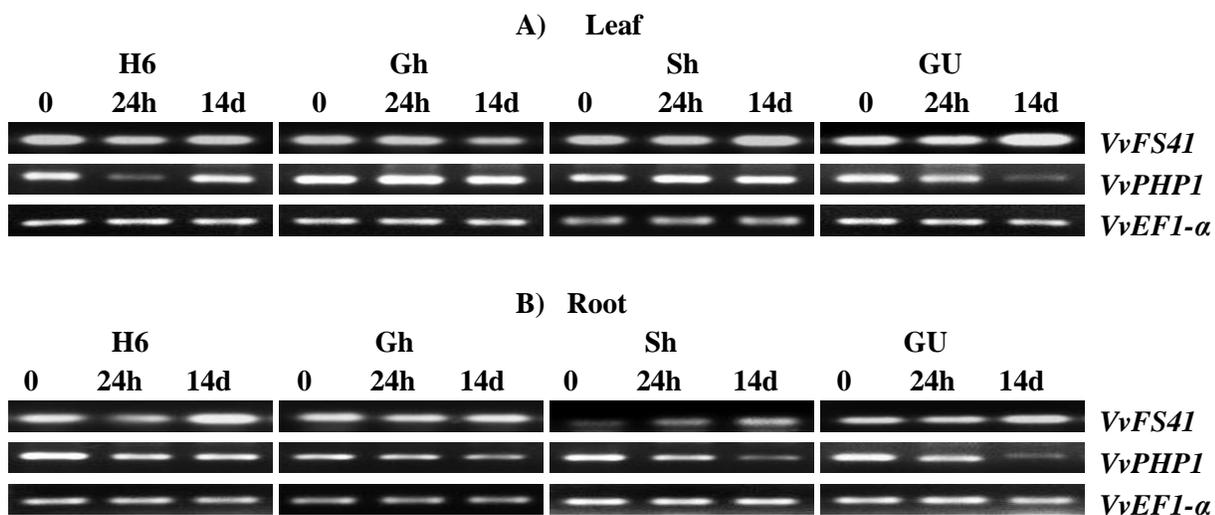


**Figure 1.** Expression profile of genes related to proteins in leaves **(A)** and roots **(B)** of four grape genotypes [H$_6$ (*V. vinifera* cv. GharaUzum *x V. riparia* cv. Kober 5BB), Gh: Gharashani, Sh: Shirazi and GU: GhezelUzum] after 0, 24 hours and 14 days treated by 50 mM salinity.
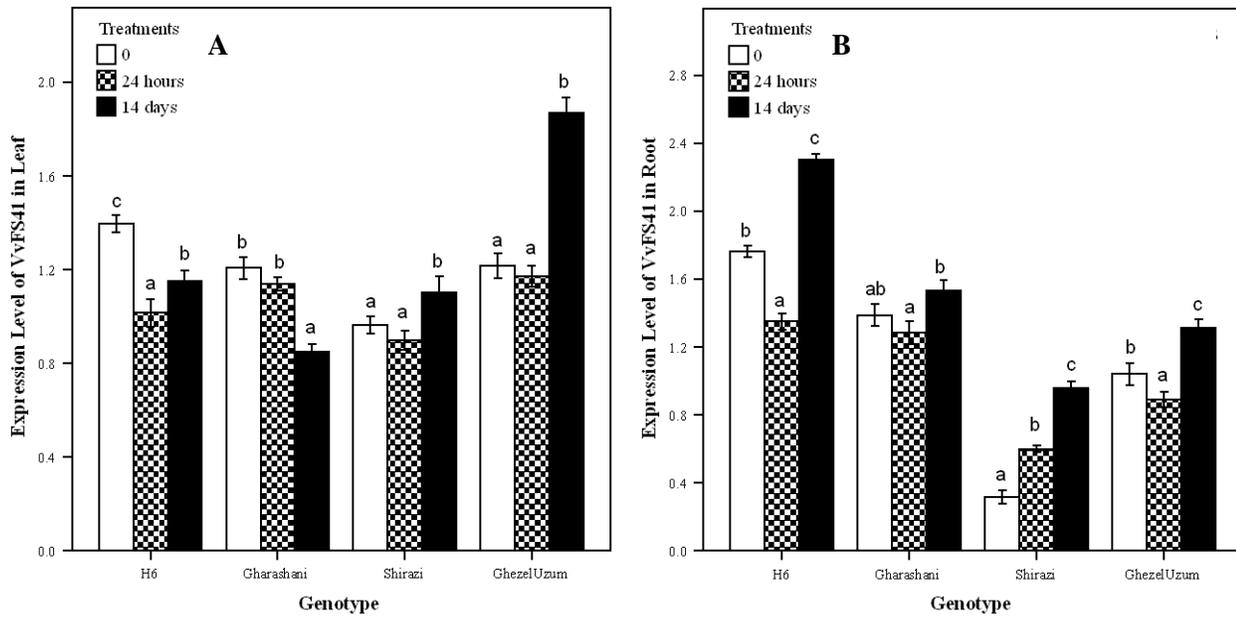
**Figure 2.** Expression level of *VvFS41* gene in leaves **(A)** and roots **(B)** of four grape genotypes [H₆ (*V. vinifera* cv. GharaUzum *x V. riparia* cv. Kober 5BB), Gharashani, Shirazi, GhezelUzum] after 0, 24 hours and 14 days treated by 50 mM NaCl. Bars are the means (n=3) ± Standard Error (*P<0.05*, One Way ANOVA). Different letters above the columns indicate significant difference between the treatments according to Tukey's test.
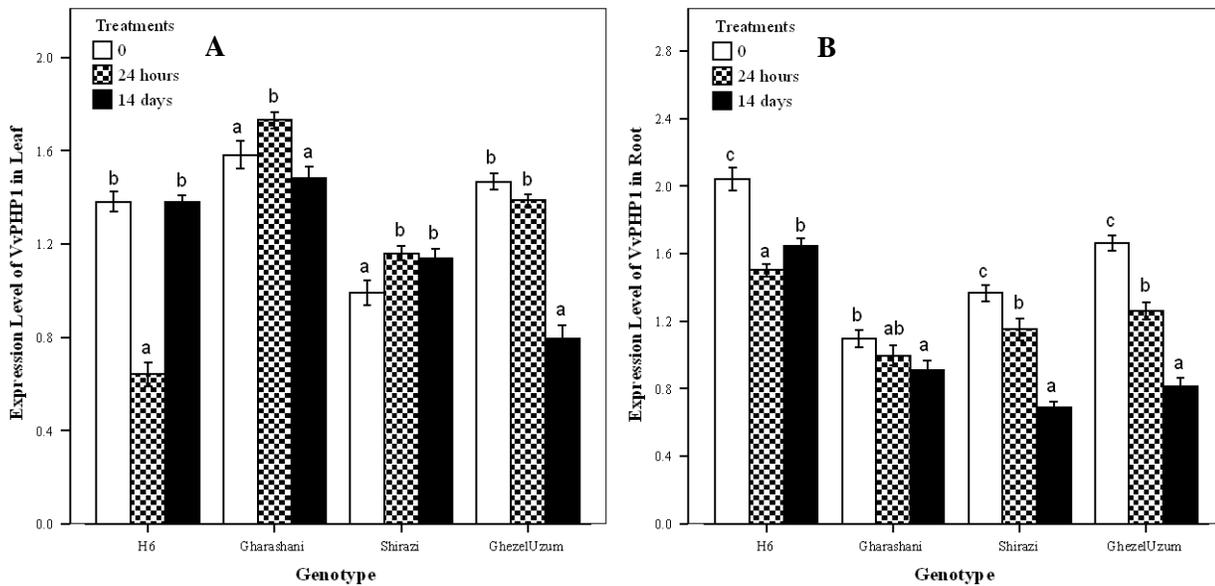


**Figure 3.** Expression level of *VvPHP1* gene in leaves **(A)** and roots **(B)** of four grape genotypes [H₆ (*V. vinifera* cv. GharaUzum *x V. riparia* cv. Kober 5BB), Gharashani, Shirazi, GhezelUzum] after 0, 24 hours and 14 days treated by 50 mM NaCl. Bars are the means (n=3) ± Standard Error (*P<0.05*, One Way ANOVA). Different letters above the columns indicate significant difference between the treatments according to Tukey's test.

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A) Leaf



B) Root
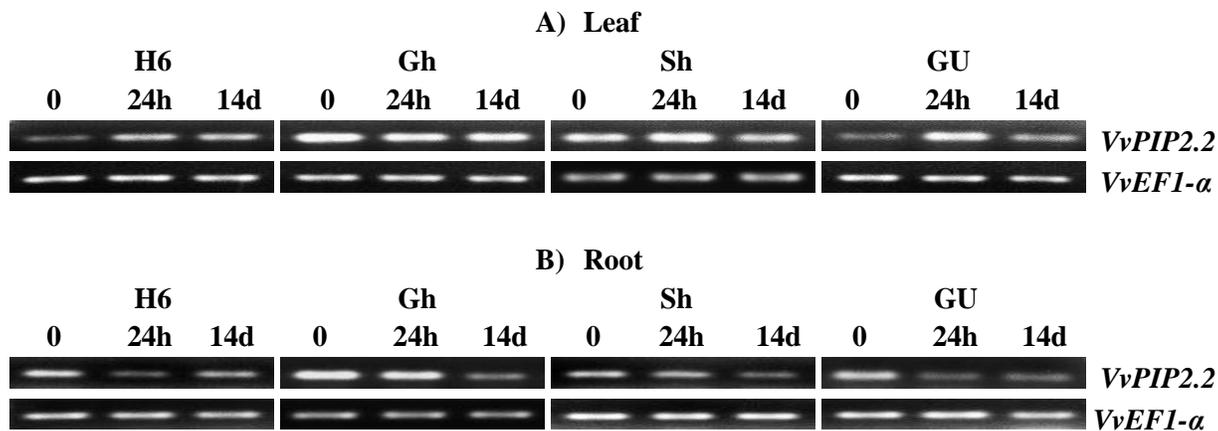


**Figure 4.** Expression profile of Aquaporin gene in leaves **(A)** and roots **(B)** of four grape genotypes [H₆ (*V. vinifera* cv. GharaUzum *x V. riparia* cv. Kober 5BB), Gh: Gharashani, Sh: Shirazi and GU: GhezelUzum] after 0, 24 hours and 14 days treated by 50 mM salinity.
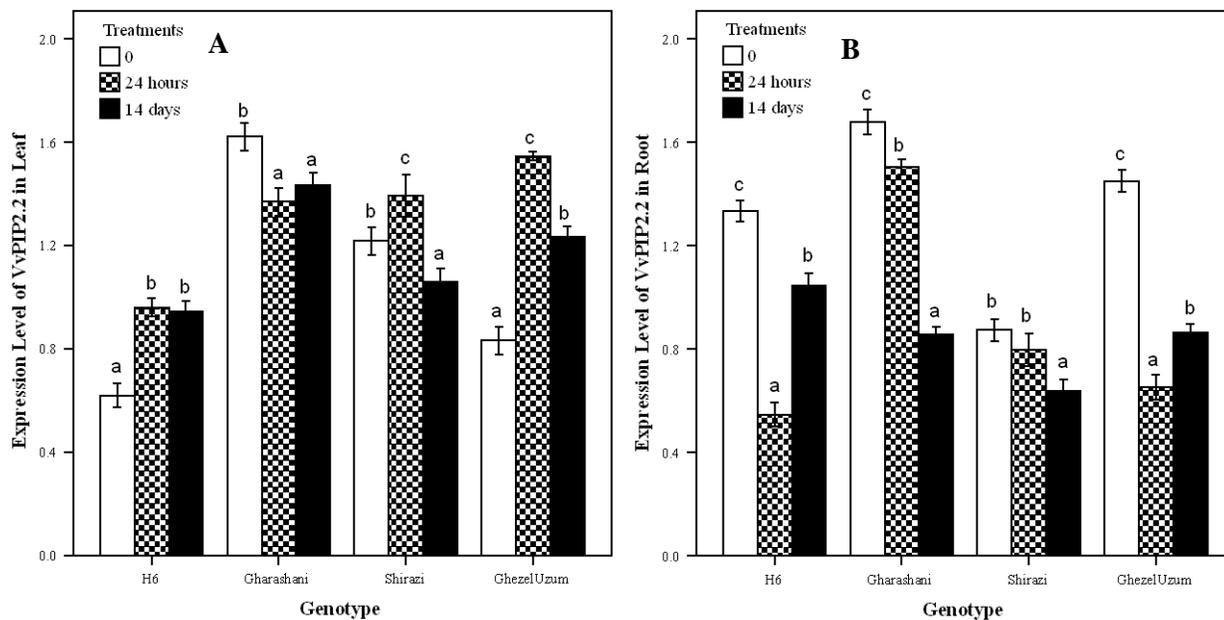


**Figure 5.** Expression level of *VvPIP2.2* gene in leaves **(A)** and roots **(B)** of four grape genotypes [H₆ (*V. vinifera* cv. GharaUzum *x V. riparia* cv. Kober 5BB), Gharashani, Shirazi, GhezelUzum] after 0, 24 hours and 14 days treated by 50 mM NaCl. Bars are the means (n=3) ± Standard Error (*P<0.05*, One Way ANOVA). Different letters above the columns indicate significant difference between the treatments according to Tukey's test.
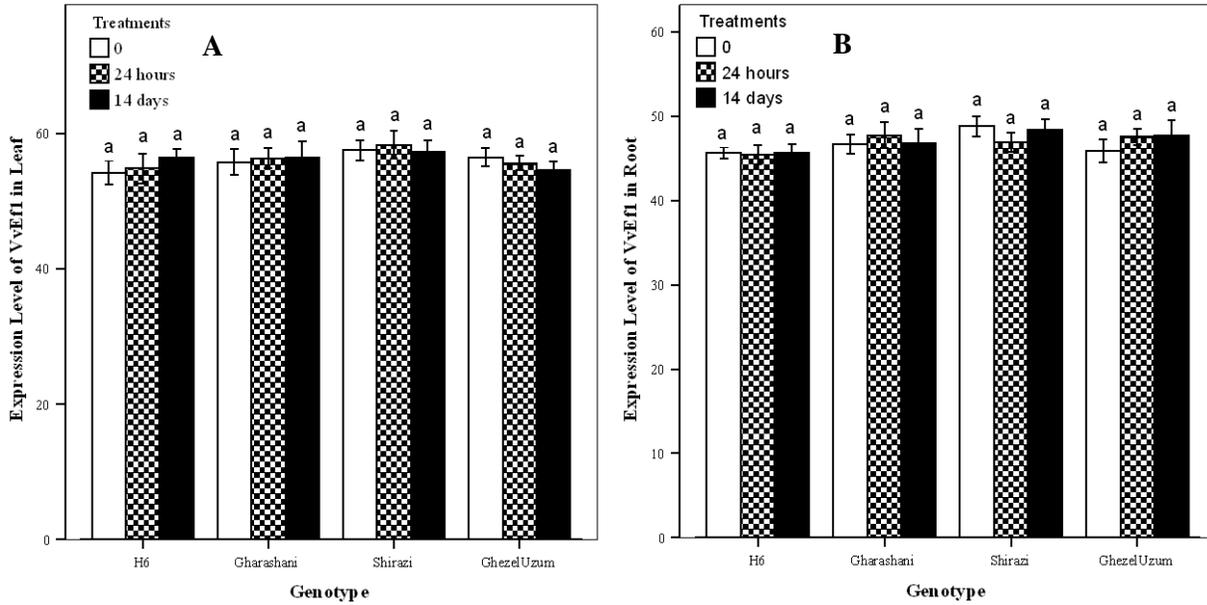
**Figure 6.** Expression level of *VvEF1* gene in leaves **(A)** and roots **(B)** of four grape genotypes [H₆ (*V. vinifera* cv. GharaUzum ×
*V. riparia* cv. Kober 5BB), Gharashani, Shirazi, GhezelUzum] after 0, 24 hours and 14 days treated by 50 mM NaCl. Bars are the
means (n=3) ± Standard Error (*P<0.05*, One Way ANOVA). Different letters above the columns indicate significant difference
between the treatments according to Tukey's test.

Figure 6 shows the expression level of VvEF1 gene
in leaves and roots of four grape genotypes treated by
50 mm NaCl for 0, 24 hours and 14 days. The differ-
ence among salinity treatments was not significant
(P<0.05).

## DISCUSSION

### Salinity effects on genes expression
Recent and important works have focused on the tran-
scriptome dynamics during grapevine development
since most of the physiological and biochemical chang-
es described are determined by gene transcriptional
variation (Zenoni *et al.*, 2010). Those studies identified
key genes whose expression programmes the cell me-
tabolism via the regulation of hormones production and
signal transduction. Some abiotic stress-responsive
genes play important roles in salt tolerance. The current
work studied the changes in expression of some im-
portant genes in tolerant and sensitive grape genotypes.

Gueydan *et al.* (2002) showed that *VvFS41* is mostly
expressed during fruit development, it is in consistent
with the fact that most RNA and protein synthesis and
processing occur during cell division. *VvFS41* gene
transcripts increased in roots of our genotypes after 14

days salinity, whereas leaves of tolerant genotypes
showed a decrease and sensitive genotypes showed a
significant increase (*P<0.05*) in *VvFS41* transcripts
compared to control. Considering that *VvFS41* gene is
responsible for protein synthesis and RNA processing,
it seems that higher gene transcripts in sensitive geno-
types under salinity shows that these genotypes have
used all of their capacity to survive. Plants synthesize
new proteins to increase their stress tolerance. The ex-
pression of *VvFS41* gene in tolerant and sensitive geno-
types showed a significant difference (*P<0.05*), espe-
cially in leaves.

*VvPHP1* gene encodes a heat shock protein 70
(Hsp70), related to the plant hypersensitive response
(HSR). The encoded and highly conserved 70 kDa pro-
tein plays a key role in stress response in plants. It is
reported that Hsp mRNA increases in plant in response
to different types of stresses (Rajan and D'Silva, 2009).
It is believed that Hsp proteins act as chaperones of
denatured proteins as well as helping in the degradation
of damaged proteins (Bukau and Horwich, 1998). Mu
*et al.* (2013) reported that overexpression of gene en-
coding Hsp enhances tolerance to salinity in Arabidop-
sis.

It seems that *VvPHP1* gene does not play an important role in our genotypes. Because unlike the previous reports about increase in expression of *VvPHP1* under salinity (Rajan and D'Silva, 2009; Mu *et al.*, 2013), in our study *VvPHP1* transcripts down regulated compared to control in roots of all genotypes under salinity. In long- term salinity (14 days) leaves of tolerant genotypes showed no significant changes (*P<0.05*) in *VvPHP1* gene transcripts. A decrease was observed in leaves of GhezelUzum, whereas Shirazi showed a significant increase (*P<0.05*) compared to control. Therefore, the expression of *VvPHP1* showed no regular trend in our studied genotypes and because of that our results were not consistent with previous reports about the expression manner of *VvPHP1* gene under salinity stress.

## Salinity effects on expression of aquaporin gene

Vandeleur *et al.* (2009) reported that *VvPIP1.1* and *VvPIP2.2* genes were highly expressed in roots of Chardonnay grape plants when well-watered. *VvPIP2.2* appeared to be constitutively expressed regardless of treatment, with only low changes in the level of expression, but relative expression of *VvPIP1.1* changed significantly. These changes also correlated with the different expression pattern of *VvPIP1.1* between cultivars in response to water stress. The significant increase in *VvPIP1.1* expression was observed in Chardonnay under water stress, whereas there was no significant change in Grenache. The two grapevine cultivars showed contrasting responses to water stress. These responses were associated with changes in the expression of *VvPIP1.1* and *VvPIP2.2*. Wang *et al.* (2015) reported that Arabidopsis plants expressing PIP2 gene showed tolerance to drought and salt stress.

The results of studies on PIP expression in roots under drought conditions showed that among the 37 PIP genes studied, 15 were down regulated, 13 up regulated, and nine were unaltered (Ruiz-Lozano *et al.*, 2009). Hence, based on expression studies it is difficult to determine the roles of PIP genes during drought stress. There is evidence that each PIP gene could have a specific function under stress. For example, Jang *et al.* (2007) found that the overexpression of a certain PIP aquaporin gene induced tolerance to some environmental stresses but sensitivity to others. Similarly, Aharon *et al.* (2003) found that the overexpression of a foreign PIP aquaporin gene in transgenic tobacco improved plant vigor under favorable growth conditions but not under drought or salt stress conditions. Different regulation of PIP protein abundance in root tissues under drought stress has also been observed. Variable responses of aquaporins to water stress at the transcript level depends on species, type of water stress, degree of water stress, and plant organ (Tyerman *et al.*, 2002). Temmei *et al.* (2005) demonstrated that there is an interaction between aquaporins from the PIP1 subclass and the PIP2 subclass.

Commonly a decrease in abundance of PIP2 proteins has been recorded (Ruiz-Lozano *et al.*, 2009), but an accumulation of PIP1 proteins under drought conditions has also been found (Aroca *et al.*, 2007). Our results in roots verified Ruiz-Lozano *et al.* (2009) report. The expression of *VvPIP2.2* decreased in roots of all genotypes under salinity, that decrease in Gharashani genotype was higher than others (47% compare to control). Inversely, leaves of all genotypes showed increases in *VvPIP2.2* transcripts after 24 hours of salinity, except Gharashani that showed a decrease compared to control. Considering contradictory reports about changes in the expression of aquaporin genes under salinity and according to our results on roots and leaves of Gharashani genotype that showed higher decrease in *VvPIP2.2* transcripts, it seems difficult to determine the role and function of *VvPIP2.2* gene in our genotypes. However, our results were consistent with some previous reports.

In conclusion, our findings highlighted 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 economical interest that is more exposed to salt stress. Our findings help clarify the relationship between various physiological factors and gene expression patterns in studied grapes under salinity. Identification of genes conferring tolerance to environmental stresses and transfer them to sensitive plants can help us to improve salinity tolerance in plants.

## ACKNOWLEDGMENT

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