

## تأثیر تنش شوری بر انباشتگی یون، فتوسنتز و محتوای متابولیت‌های سازگار در ۴ ژنوتیپ

### انگور

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چکیده. تحمل به شوری ۴ ژنوتیپ انگور ( قره اوزوم، حسینی، آق اوزوم و کشمشی) تحت شرایط شوری (۲۵، ۵۰ و ۱۰۰ میلی مولار) مطالعه گردید. فاکتورهای رشدی تحت شرایط شوری به صورت معنی داری ( $P < 0.05$ ) کاهش یافت، در حالی که محتوی یون های سدیم و کلر افزایش یافت. در تمامی تیمارهای شوری انباشتگی کلر نسبت به سدیم بیشتر بود. قره اوزوم کمترین و کشمشی بیشترین محتوی کلر برگ را دارا بود. فتوسنتز، تعرق و هدایت روزنه ای تحت شوری کاهش یافت و بیشترین همبستگی را با محتوی کلر برگ دارا بود. کمترین کاهش در پارامترهای فتوسنتزی در قره اوزوم مشاهده گردید. تحت شوری متوسط (۵۰ میلی مولار) محتوی قند محلول، پرولین و گلايسين بتائين در برگ همه ی ژنوتیپ ها افزایش یافت. نتایج به دست آمده در این مطالعه نشان داد قره اوزوم عملکرد و تحمل بیشتری تحت تنش شوری دارد و کشمشی با سایر ژنوتیپ‌ها حساس تر است.

واژه های کلیدی. اسمولیت، انگور، تحمل شوری، سدیم، کلر

## The effect of salt stress on ion accumulation, photosynthesis and compatible solute contents in four grapevine (*Vitis vinifera*) genotypes

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**Abstract.** Salinity tolerance of four grape genotypes [GharaUzum, Hosseini, AghUzum and Keshmeshi] was studied under various salinity levels (25, 50 and 100 mM NaCl). As a result, growth indices were significantly ( $P < 0.05$ ) reduced by salinity, whereas  $Cl^-$  and  $Na^+$  contents in the plant parts were increased.  $Cl^-$  accumulation exceeded than that of  $Na^+$  in all treatments. Among the genotypes studied, GharaUzum and Keshmeshi had the lowest and highest  $Cl^-$  concentrations in the leaf lamina, respectively. Photosynthesis and transpiration rate as well as stomatal conductance were greatly reduced by salinity and were shown to be highly correlated with leaf  $Cl^-$  content. GharaUzum showed lower reduction in photosynthesis parameters. Soluble sugars, proline and glycine betaine contents increased in the leaf lamina of all the genotypes studied treated with moderate salinity (50 mM). In conclusion, the results showed that GharaUzum and Keshmeshi had the highest and lowest salt stress tolerance among the genotypes studied, respectively.

**Keywords.** chloride, grape, osmolytes, salt tolerance, sodium

## INTRODUCTION

Salinity is an important environmental stress in many regions of the world, causing significant losses in products. The adverse effects of salt stress may relate to accumulation of Na<sup>+</sup> and Cl<sup>-</sup> ions or depletion of K<sup>+</sup> and Ca<sup>2+</sup> ions in plant (Shilpi & Narendra, 2005). Grapevine (*Vitis vinifera*) is classified as being moderately tolerant to salinity (Mass & Hoffman, 1977). Toxic levels of Na<sup>+</sup> are uncommon in leaves because Na<sup>+</sup> is not transported in high amounts from root to leaf (Ehlig, 1960). Hence, Cl<sup>-</sup> is the main toxic ion for grapevines growing under saline conditions (Henderson et al., 2014; Venier et al., 2018). There is general agreement that high Cl<sup>-</sup> accumulation by certain genotypes can cause growth reduction (Alexander & Groot, 1971). However, many grapevine rootstocks have an ability to limit the uptake and/or root to shoot transport of Cl<sup>-</sup> (Walker, 1994).

Photosynthesis and plant growth are the primary processes affected by salinity (Munns et al., 2006). Salinity reduced photosynthesis in grapevine plants. This reduction is due to stomatal limitation (stomatal closure) and non-stomatal limitations of photosynthesis. Stomatal closure can cause the reduction of stomatal conductance, transpiration rate and net photosynthesis (Lu et al., 2009). Salt stress may affect plant physiological and biochemical processes (Greenway & Munns, 1980). One of them is accumulation of different types of compatible solutes such as soluble sugars, betaines and proline (Seraj & Sinclair, 2002). Proline accumulates in larger amounts compare to other amino acids in salt stressed plants (Abraham et al., 2003). In addition to its conventional role in cell osmotic adjustment (Yancey et al., 1982), proline helps stabilizing sub-cellular structures (e.g., membranes and proteins), scavenging free radicals, and buffering cellular redox potential under stress conditions (Ashraf & Orooj, 2006). The involvement of glycine betaine as a protectant against abiotic stress in plants is well known. It has been demonstrated that under salt stress, glycine betaine prevents PSII damage in light (Sakamoto & Murata, 2002) and increased the activation of Rubisco (Nomura et al., 1998). The accumulation of soluble sugars in plants has been widely reported as a response to salinity (Murakeozy et al., 2003). Sugars are involved in the synthesis of other compounds, the production of energy and the stabilization of membranes (Hoekstra et al., 2001).

Grapes are grown in semiarid environments, where drought and salinity are common problems. There is a wide variation for salt tolerance among rootstock genotypes (Walker & Douglas, 1982; Behboudian et al., 1986). In vitro screening for salt tolerance has been investigated in some grape cultivars (Singh et al., 2000; Khawale et al., 2003;

Cavagnaro et al., 2006). In the present study, we examined responses of four different grape genotypes to elevated NaCl levels. The main aim of this study was the evaluation of salt tolerance (NaCl) in different grapevine varieties (West Azarbaijan, Iran) by comparing ion accumulation, compatible solute content, photosynthesis and growth parameters under various saline conditions.

## MATERIALS AND METHODS

### Plant material and treatment

Hard-wood cuttings of four genotype of grapevine (GharaUzum, Hosseini, AghUzum and Keshmeshi) were soaked in IBA 0.1% (w/v) from the basal parts for 5-10 s after disinfection with benomyle (1.5% w/v). All cuttings were placed in a mist chamber (relative humidity 80%) with a heat-bed temperature of 25-35 °C. After the opening leaf buds, rooted cuttings were transferred to hydroponics culture in 2-L pots containing aerated Hoagland nutrient solution. Three replicates for each treatment and 2 plants for each replicate were taken into account. Plants with 3-4 fully expanded leaves were treated for two weeks with NaCl (0, 25, 50 and 100 mM) in 1:2 strength Hoagland solution. After two weeks, plants were harvested and plant parts (leaves, petioles, stems and roots) were weighted separately and dried at 70 °C for 48 h, finely ground.

### Growth analysis

Shoot and root lengths and dry weights were measured on six plants from each treatment at the end of salt stress period.

### Gas exchange measurements

Gas exchange measurements were carried out after 12 days of salt treatment. The net photosynthetic rate, transpiration rate and stomatal conductance of upper fully-expanded leaves on the young shoot were measured using hcm-1000 portable photosynthesis system (WALZ, Germany). During the measurements, photosynthetic photon flux density was set to 1500 μmol/m<sup>2</sup> s, temperature of leaf cuvette was 30-33°C, the air relative humidity was 40-50% and the area of cuvette that caught full illumination was 5 cm<sup>2</sup>.

### Ion analysis

Ground samples (100 mg) of plant part (lamina, petiole, stem and root) were weighed into 15 ml plastic centrifuge tubes and 10 ml of deionized water was added. The tubes were placed in a boiling water bath for approximately 1 h. Sample tubes were centrifuged at 5000 rpm, the supernatant poured into new tubes and the volume made up to 10 ml by addition of deionized water. In the extract, the sodium and potassium concentrations were measured by flame photometer (Fater electronic 405) and chloride by silver ion titration method

using a chloride meter (Corning 926). Nitrate concentration was determined by salicylic sulfuric acid method (Cataldo et al., 1975). Briefly, 0.5 ml aliquots were mixed with 0.8 ml of 5% (w/v) salicylic acid in concentrated H<sub>2</sub>SO<sub>4</sub>. After 20 min at room temperature, 19 ml of 2N NaOH were added slowly to raise the pH above 12. The samples were cooled to room temperature and absorbance at 410 nm was determined by using a spectrophotometer (UV-visible, WPA S2100).

#### Compatible solute content

Free proline content in the leaves and roots was determined following the method of Bates et al., (1973). Plant roots and leaves were homogenized in 10 ml of 3% sulfosalicylic acid and the homogenates were filtered through Filter Paper S&S 604. The filtrates (2 ml) were treated with 2 ml of ninhydrin (3% v/v) and 2 ml of glacial acetic acid, followed by 4 ml of toluene. The absorbance of the colored solutions was measured at 520 nm.

Glycine-betaine of leaves was measured in dried leaf powder spectrophotometrically after reaction with KI-I2 at 520 nm, according to the method of Grieve & Grattan (1983).

Total soluble sugars were estimated by phenol-sulfuric acid method (Dubios et al., 1956). Ground samples of roots and leaves were homogenized with ethanol and filtered through Filter Paper S&S 604. The filtrates were treated with 5% phenol and 98% sulfuric acid. After 1 h, the absorbance of the mixtures was measured at 485 nm.

#### Statistical analysis

Analysis of variance was performed by the statistical program SPSS version 16 and one-way ANOVA was used to determine the significance of the results between different treatments in each genotype and then Tukey's multiple range tests ( $P < 0.05$ ) were performed. A GLM (General Linear Model) analysis was used to determine differences among genotypes and interactions between genotypes and salinity treatments. Correlation ( $P < 0.01$ ) among measured factors also calculated.

## RESULTS

Growth parameters were affected by salinity. Shoot and root lengths were significantly ( $P < 0.05$ ) reduced at all salinity levels (Fig. 1). Keshmeshi showed higher shoot and root length decrease (65.2% and 66.54%, respectively) than the others. The results also showed that dry weights were significantly reduced by salinity levels (Fig. 1). Hosseini had higher reduction in shoot and root dry weight (50.41% and 46.83%, respectively) as compare with the other genotypes. Analysis of variance showed that difference in growth

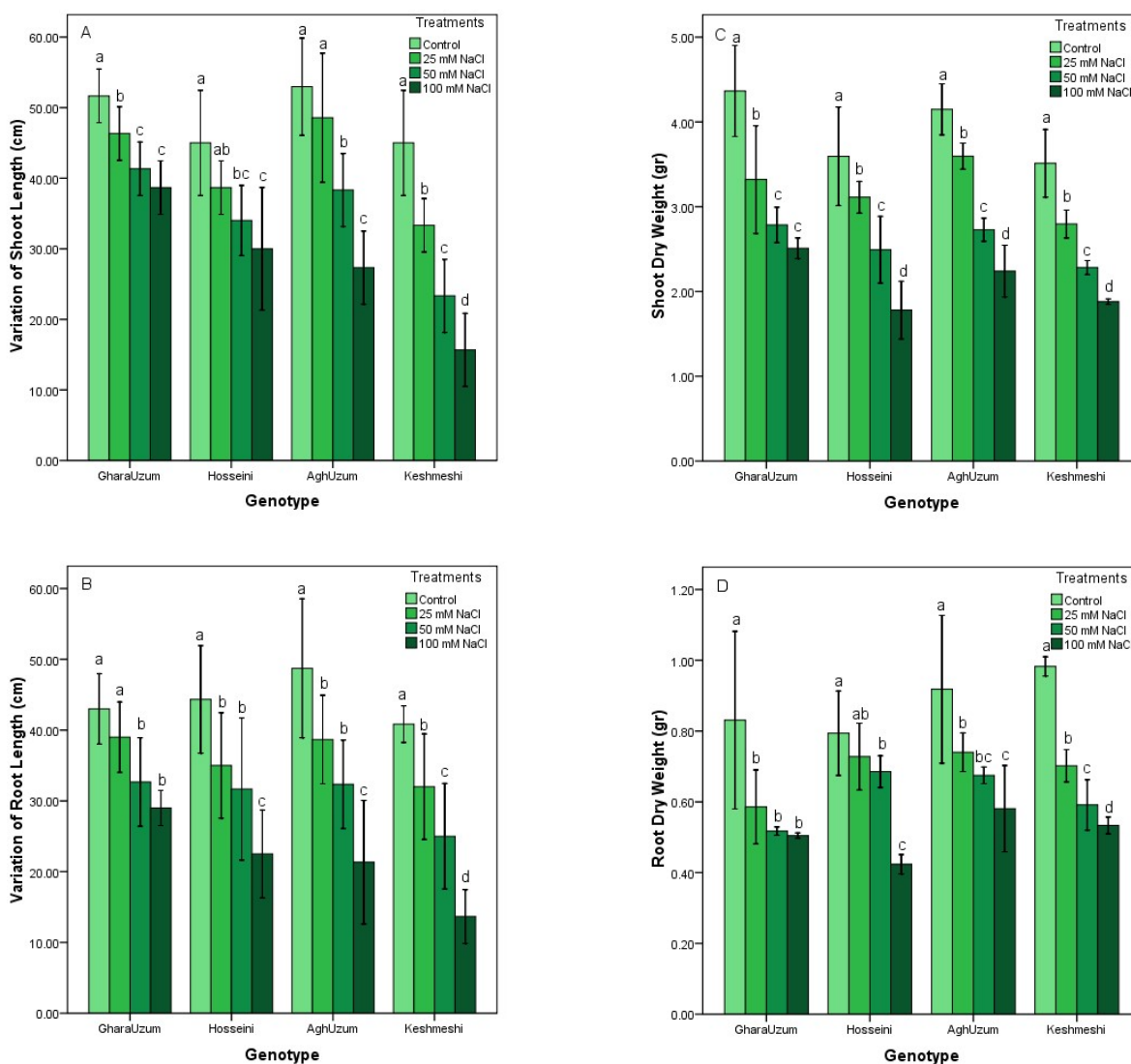
parameters among genotypes and salinity treatments was significant ( $P < 0.05$ ).

Cl<sup>-</sup> content significantly ( $P < 0.05$ ) increased in parallel to increasing salt treatments at all genotypes (Fig. 2). Among the different parts of the plants, roots and petioles showed the highest Cl<sup>-</sup> contents. AghUzum accumulated higher Cl<sup>-</sup> than others in petioles and shoots and Keshmeshi had higher contents in lamina, however Hosseini showed higher Cl<sup>-</sup> in roots than other genotypes. For all genotypes, Cl<sup>-</sup> content was higher than Na<sup>+</sup> in all plant's parts and all salt treatments. Analysis of variance showed that difference in toxic ion contents among genotypes, treatments and genotype × treatment was significant ( $P < 0.05$ ).

Na<sup>+</sup> contents of vine tissue (root, petioles, lamina and shoot) increased significantly ( $P < 0.05$ ) with increasing salinity in the nutrient solution (Fig. 3). Roots accumulated higher amounts of Na<sup>+</sup>. GharaUzum showed a lower Na<sup>+</sup> content in shoots than other genotypes at all salinity treatments. At 50 and 100 mM of NaCl, GharaUzum had lower Na<sup>+</sup> accumulation compare to others in lamina, while at the same salinity level AghUzum and Keshmeshi showed higher Na<sup>+</sup> contents in shoots and lamina. At all salinity treatments, Keshmeshi accumulated higher Na<sup>+</sup> than other genotypes in petiole and root.

Unlike for Na<sup>+</sup>, K<sup>+</sup> contents of roots, lamina and shoots decreased with increasing salinity treatments in all genotypes (Table 1). GharaUzum showed lower reduction in K<sup>+</sup> contents of root and shoot when compared to control plants (19.34% and 43.62% of control, respectively), while AghUzum showed higher reduction than others in shoots and roots (62.20% and 57.75% of control, respectively). The results showed some fluctuations in NO<sub>3</sub><sup>-</sup> concentration among genotypes (Table 2). However, different salinity levels induced a decrease in root and shoot NO<sub>3</sub><sup>-</sup> content in all genotypes.

Salt stress had a significant ( $P < 0.05$ ) effect on gas exchange parameters. Comparing the means obtained from statistical analysis showed that stomatal conductivity and the rate of transpiration and photosynthesis were decreased by increasing salinity levels (Fig. 4). GharaUzum showed lower reduction in photosynthesis rate (53.33%), while Keshmeshi showed higher reduction than other genotypes (93.55%). In Keshmeshi, the transpiration rate and stomatal conductance decreased 97.93% and 98.62% under treatment of 100 mM NaCl, respectively when compared to control plants (Fig. 4). This genotype showed higher reduction compare to others. GLM analysis showed that difference in gas exchange parameters among genotypes, treatments and genotype × treatments was significant.



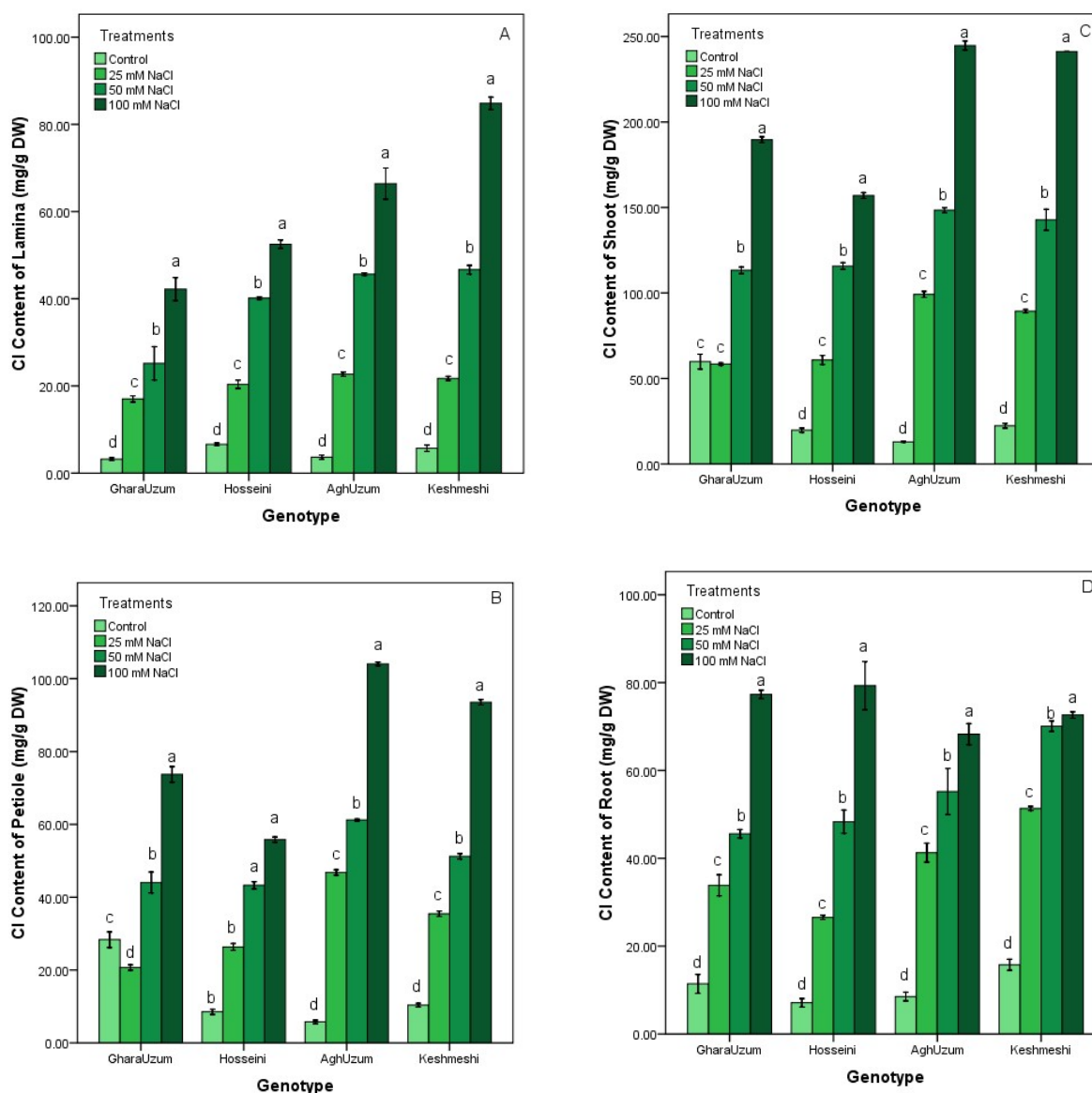
**Figure 1.** Effect of different NaCl concentrations (0, 25, 50 and 100 mM) on shoot (A) and root (B) length (cm) and dry weight of shoot (C) and root (D) (gr) in four grape (*Vitis vinifera* L.) genotypes. Bars are the means  $\pm$  standard error ( $n=3$ , one-way ANOVA). Different letters within column indicate significant differences ( $P<0.05$ ) according to Tukey's multiple range tests.

The soluble sugar content in roots and laminae of genotypes increased with increasing NaCl concentrations (Table 3). Higher and lower accumulation of sugar contents were observed in AghUzum and GharaUzum, respectively. In addition, salinity significantly affected proline content ( $P<0.05$ ) in plant parts. Compare to other genotypes Keshmeshi accumulated higher amounts of proline in laminae and roots. Also, GharaUzum had a lower proline accumulation (Table 3). Glycine betaine had a regular increase in all treatments and all genotypes. Among the genotypes, GharaUzum showed higher increase compare to others at 50 and even at 100 mM NaCl. Keshmeshi and AghUzum had lower glycine betaine content in compare to GharaUzum and Hosseini. Analysis of variance showed that difference in

compatible solutes among genotypes, treatments and genotypes  $\times$  treatments was significant ( $P<0.05$ ).

## DISCUSSION

Growth reduction is a common response in woody plants to salt stress (Vijayan et al., 2003) and is observed even at low salinities, often before the appearance of visible symptoms. In our study, shoot and root lengths and dry weights of plants were decreased as compared to control with increasing salinity treatments. Growth parameters of salt-sensitive genotypes like Keshmeshi decreased more than others under NaCl stress, whereas GharaUzum showed lower decrease in plant lengths and dry weights.



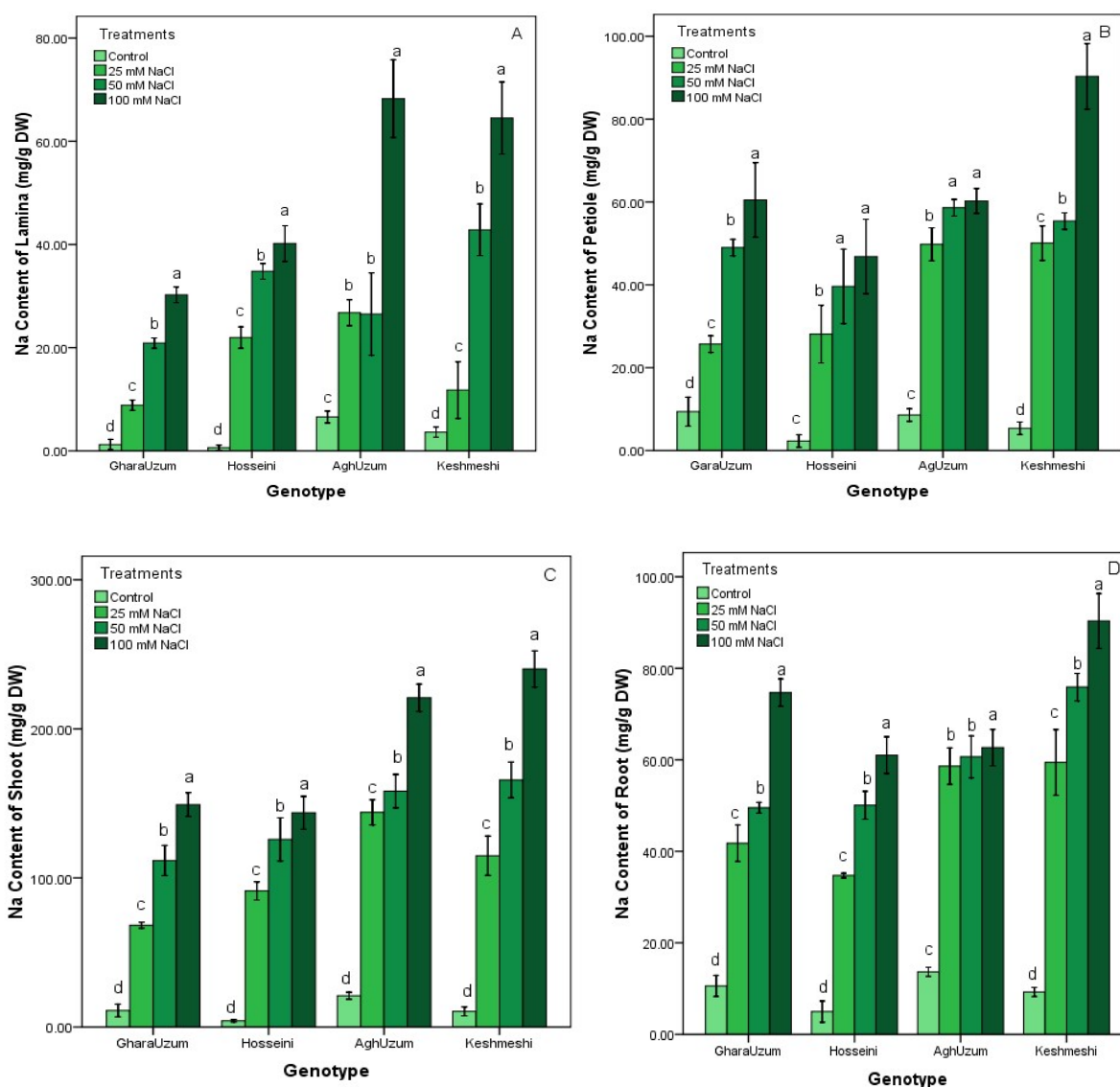
**Figure 2.** Cl<sup>-</sup> concentrations (mg/g DW) in laminas (A), petioles (B), shoots (C) and roots (D) of four grape (*Vitis vinifera* L.) genotypes at three different salinity levels (25, 50 and 100 mM NaCl). Bars are the means  $\pm$  standard error (n=3, one-way ANOVA). Different letters within column indicate significant differences (P<0.05) according to Tukey's multiple range tests.

Salinity reduced the plant growth by reducing both leaf area and photosynthesis rates (Misra et al., 1997; Munns, 2002; saed, 2015).

Photosynthesis reduction may be related to the accumulation of chloride in roots and shoots of grapevine. Therefore, salt tolerance in grapevine is associated with the ability to limit the uptake or transport of ions (mainly Cl<sup>-</sup> and Na<sup>+</sup>) from root to aerial parts. Keshmeshi had higher Cl<sup>-</sup> and Na<sup>+</sup> accumulation compare to other genotypes and showed the highest growth and photosynthesis inhibition. In this study, all genotypes had higher Cl<sup>-</sup> than Na<sup>+</sup> content in salt conditions. It seems that salinity damage

is caused by chloride ions because grapevines have modest capacity to exclude Cl<sup>-</sup> (Christensen et al., 1978). Cl<sup>-</sup> decreased photosynthesis rate through its inhibition of NO<sub>3</sub><sup>-</sup> uptake by roots (Banuls et al., 1990). McClure et al., (1986) showed a NO<sub>3</sub><sup>-</sup>/Cl<sup>-</sup> antagonism in grapevines. In all genotypes studied here, a decrease in nitrate accumulation was observed with increasing Cl<sup>-</sup> contents. Furthermore, a significant negative correlation (P<0.01, r>-0.8) was found between NO<sub>3</sub><sup>-</sup> and Cl<sup>-</sup> in all parts of the plants.

Salinity tolerance is related to the maintenance of net photosynthesis rate and stomatal conductance (Lakshmi et al., 1996; Mousavian & Abbaspour, 2017).



**Figure 3.** Na<sup>+</sup> concentrations (mg/g DW) in laminas (A), petioles (B), shoots (C) and roots (D) of four grape (*Vitis vinifera* L.) genotypes at three different salinity levels (25, 50 and 100 mM NaCl). Bars are the means  $\pm$  standard error (n=3, one-way ANOVA). Different letters within column indicate significant differences ( $P < 0.05$ ) according to Tukey's multiple range tests.

Keshmeshi (salt sensitive genotypes) maintain lower NO<sub>3</sub><sup>-</sup> content under high salinity conditions and showed higher reduction in photosynthesis rate compare to other genotypes (93.55%).

There were significant correlations ( $P < 0.01$ ) between photosynthesis rate and plant ion content (Cl<sup>-</sup>, Na<sup>+</sup>, K<sup>+</sup> and NO<sub>3</sub><sup>-</sup>). Photosynthesis rate showed negative correlations with sodium and chloride contents in different plant parts ( $P < 0.01$ ,  $r > -0.8$ ).

Similar to net photosynthesis, transpiration rate and stomatal conductance decreased with increasing salinity treatment in all genotypes. According to Misra et al., (2002), transpiration rate of plants decreases under osmotic stress through different mechanisms such as stomatal conductivity reduction. It seems this

mechanism is an adaptive process that conserves water for the later stages of plant growth. Keshmeshi had the general decline in transpiration rate and stomatal conductance with increasing salinity compare to other genotypes.

Under saline conditions in some plants, particularly in grapevines, sodium remains in the roots and stems and chloride accumulated in the shoots. Therefore, control of Cl<sup>-</sup> transport and Cl<sup>-</sup> exclusion from shoots is correlated with salt tolerance (Mohammadkhani et al., 2013). In our study, GharaUzum and Keshmeshi had respectively lower and higher Cl<sup>-</sup> accumulation in petioles and lamina at 100 mM NaCl. Higher Cl<sup>-</sup> concentrations in petioles and lamina reflect the poor capacity of Keshmeshi for Cl<sup>-</sup> exclusion.

**Table 1.** Effect of different NaCl concentrations (0, 25, 50 and 100 mM) on K<sup>+</sup> content (mg/gDW) in four grape (*Vitis vinifera* L.) genotypes.

Genotype and Salinity (mM NaCl)	K <sup>+</sup> Content of root (mg/g DW)	K <sup>+</sup> Content of shoot (mg/g DW)	K <sup>+</sup> Content of Lamina (mg/g DW)
<b>GharaUzum</b>			
0	54.62±1.06a	173.21±2.20a	57.03±1.74a
25	29.97±0.35b	166.13±0.35b	55.42±0.69a
50	31.98±0.70b	147.94±0.35c	51.81±0.69ab
100	30.79±0.70b	139.76±0.46d	47.00±1.38b
<b>Hosseini</b>			
0	45.77±0.92a	131.20±1.14a	26.51±0.46a
25	40.69±0.70b	116.45±0.69b	25.16±0.35a
50	38.02±0.53b	108.16±0.93c	23.02±0.13b
100	25.29±0.46c	88.36±0.83d	21.95±0.35b
<b>AghUzum</b>			
0	57.03±1.06a	151.09±0.81a	31.84±0.13a
25	46.17±0.23b	134.24±0.58b	27.97±0.13b
50	25.02±0.13c	97.70±0.35c	25.69±0.23c
100	24.09±0.46c	57.11±0.40d	14.26±0.11d
<b>Keshmeshi</b>			
0	84.71±1.06a	186.30±1.20a	61.29±0.70a
25	57.41±0.23b	153.11±1.18b	45.37±0.83b
50	47.64±0.26c	115.40±0.26c	40.96±0.00c
100	40.16±0.92d	89.92±0.80d	29.97±0.58d

Data are the means ± standard error (n=3, one-way ANOVA). Different letters within column indicate significant differences ( $P<0.05$ ) according to Tukey's multiple range tests.

**Table 2.** Effect of different NaCl concentrations (0, 25, 50 and 100 mM) on NO<sub>3</sub><sup>-</sup> content (mg/gDW) in four grape (*Vitis vinifera* L.) genotypes.

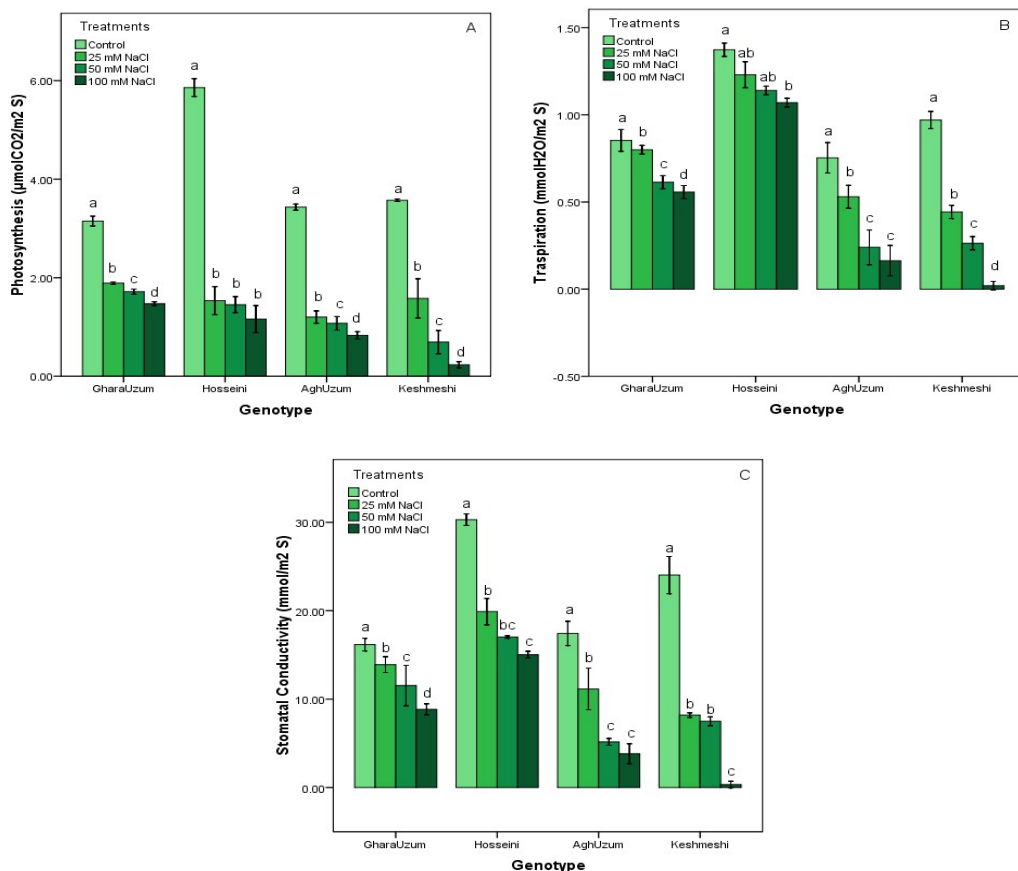
Genotype and Salinity (mM NaCl)	NO <sub>3</sub> <sup>-</sup> Content of root (mg/g DW)	NO <sub>3</sub> <sup>-</sup> Content of shoot (mg/g DW)	NO <sub>3</sub> <sup>-</sup> Content of Lamina (mg/g DW)
<b>GharaUzum</b>			
0	6.98±0.69a	8.88±0.07a	0.96±0.01ab
25	2.44±0.13b	5.03±0.13b	0.85±0.00b
50	5.87±0.10a	4.91±0.13b	1.03±0.05ab
100	1.01±0.01b	3.47±0.06c	1.06±0.06a
<b>Hosseini</b>			
0	5.15±0.05a	8.33±0.09a	0.79±0.02ab
25	5.06±0.24a	4.03±0.07b	1.05±0.08a
50	0.90±0.05b	3.93±0.18b	0.63±0.01b
100	1.06±0.05b	3.46±0.21b	0.56±0.08b
<b>AghUzum</b>			
0	2.81±0.00b	3.81±0.04a	0.73±0.09a
25	3.03±0.00a	2.73±0.01b	0.45±0.00b
50	2.75±0.01b	2.46±0.11b	0.38±0.01b
100	0.25±0.02c	1.38±0.00c	0.38±0.00b
<b>Keshmeshi</b>			
0	4.31±0.05a	6.85±0.02a	1.88±0.01a
25	3.32±0.19b	4.10±0.03b	1.18±0.00b
50	2.24±0.09c	3.36±0.04c	0.84±0.01c
100	0.77±0.00d	2.19±0.01d	0.33±0.01d

Data are the means ± standard error (n=3, one-way ANOVA). Different letters within column indicate significant differences ( $P<0.05$ ) according to Tukey's multiple range tests.

In the salt stress, the NO<sub>3</sub><sup>-</sup> concentration is reduced in all vine parts. The decrease of nitrate uptake results in a lower transport rate to the top and probably due to NO<sub>3</sub><sup>-</sup>Cl<sup>-</sup> antagonism (McClure et al., 1986). The results obtained here showed that increased salinity levels resulted in a considerable reduction in NO<sub>3</sub><sup>-</sup> concentrations in all vine parts, except for GharaUzum. Among our genotypes, GharaUzum exhibited the opposite pattern: the NO<sub>3</sub><sup>-</sup>

content in laminae increased with increasing salinity. GharaUzum had lower Cl<sup>-</sup> and higher NO<sub>3</sub><sup>-</sup> accumulation in laminae compared to the other genotypes under salinity.

Like Cl<sup>-</sup>, Na<sup>+</sup> was accumulated in all part of four genotypes under salinity. Keshmeshi had the higher Cl<sup>-</sup> and Na<sup>+</sup> accumulation than other genotypes in shoot. It shows lack or poor exclusion system in this genotype so that Cl<sup>-</sup> and Na<sup>+</sup> could be transported to lamina, whereas



**Figure 4.** Photosynthesis rate [A] ( $\mu\text{molCO}_2/\text{m}^2 \text{ s}$ ), Transpiration [B] and Stomatal conductivity [C] ( $\text{mmol}/\text{m}^2 \text{ s}$ ) in four grape (*Vitis vinifera* L.) genotypes at three different salinity levels (25, 50 and 100 mM NaCl). Bars are the means  $\pm$  standard error (n=3, one-way ANOVA). Different letters within column indicate significant differences ( $P < 0.05$ ) according to Tukey's multiple range tests.

**Table 3.** Effect of different NaCl concentrations (0, 25, 50 and 100 mM) on sugar, proline, glycine betaine content (mg/gDW) in four grape (*Vitis vinifera* L.) genotypes.

Genotype and Salinity (mM NaCl)	Sugar Content (mg/gDW) of		Proline Content ( $\mu\text{g}/\text{gDW}$ ) of		Glycine betaine content of Lamina (mg/gDW)
	Lamina	Root	Lamina	Root	
<b>GaraUzum</b>					
0	17.80 $\pm$ 0.03c	3.39 $\pm$ 0.08d	0.20 $\pm$ 0.02d	0.27 $\pm$ 0.01d	4.48 $\pm$ 0.01d
25	21.30 $\pm$ 0.19b	6.47 $\pm$ 0.07c	0.42 $\pm$ 0.01c	0.57 $\pm$ 0.04c	6.04 $\pm$ 0.04c
50	21.86 $\pm$ 0.39b	8.56 $\pm$ 0.06b	0.53 $\pm$ 0.00b	0.78 $\pm$ 0.00b	7.98 $\pm$ 0.04b
100	23.03 $\pm$ 0.02a	9.25 $\pm$ 0.05a	1.31 $\pm$ 0.02a	0.92 $\pm$ 0.02a	12.26 $\pm$ 0.11a
<b>Hosseini</b>					
0	18.81 $\pm$ 0.06d	6.05 $\pm$ 0.00d	0.52 $\pm$ 0.01d	0.78 $\pm$ 0.00d	5.33 $\pm$ 0.01c
25	21.17 $\pm$ 0.23c	9.00 $\pm$ 0.03c	0.76 $\pm$ 0.03c	1.06 $\pm$ 0.03	6.73 $\pm$ 0.04b
50	21.95 $\pm$ 0.05b	10.47 $\pm$ 0.02b	1.15 $\pm$ 0.02b	1.31 $\pm$ 0.00b	7.09 $\pm$ 0.46b
100	23.16 $\pm$ 0.08a	10.97 $\pm$ 0.09a	1.56 $\pm$ 0.01a	1.65 $\pm$ 0.00a	10.14 $\pm$ 0.03a
<b>AgUzum</b>					
0	18.90 $\pm$ 0.41c	5.43 $\pm$ 0.00c	0.51 $\pm$ 0.00d	0.50 $\pm$ 0.00c	3.10 $\pm$ 0.20d
25	22.34 $\pm$ 0.03b	8.51 $\pm$ 0.19bc	0.63 $\pm$ 0.01c	0.57 $\pm$ 0.01c	4.64 $\pm$ 0.18c
50	23.06 $\pm$ 0.38b	10.21 $\pm$ 0.11b	0.76 $\pm$ 0.01b	0.96 $\pm$ 0.05b	6.12 $\pm$ 0.07b
100	30.40 $\pm$ 0.98a	17.10 $\pm$ 1.47a	1.53 $\pm$ 0.01a	1.37 $\pm$ 0.04a	8.11 $\pm$ 0.08a
<b>Keshmeshi</b>					
0	19.91 $\pm$ 0.14d	4.77 $\pm$ 0.02d	0.67 $\pm$ 0.02c	0.77 $\pm$ 0.04d	2.69 $\pm$ 0.09d
25	21.04 $\pm$ 0.13c	6.65 $\pm$ 0.07c	0.80 $\pm$ 0.02c	0.95 $\pm$ 0.00c	4.83 $\pm$ 0.03c
50	22.30 $\pm$ 0.02b	8.76 $\pm$ 0.33b	1.83 $\pm$ 0.35b	1.48 $\pm$ 0.06b	6.65 $\pm$ 0.03b
100	23.65 $\pm$ 0.32a	11.81 $\pm$ 0.07a	2.84 $\pm$ 0.07a	2.46 $\pm$ 0.00a	7.81 $\pm$ 0.12a

Data are the means  $\pm$  standard error (n=3, one-way ANOVA). Different letters within column indicate significant differences ( $P < 0.05$ ) according to Tukey's multiple range tests.

the GharaUzum showed lower  $\text{Cl}^-$  and  $\text{Na}^+$  accumulation than others. There were high significant positive correlations ( $P < 0.01$ ,  $r > 0.9$ ) between  $\text{Cl}^-$  and  $\text{Na}^+$  in laminae of all genotypes.

Establishment of ion homeostasis is an essential requirement for plants to survive under salt stress conditions. Maintenance of high  $\text{K}^+$  concentrations in salt-tolerant genotypes may be one of the mechanisms underlying their superior salt tolerance (Tester & Davenport, 2003). Under saline conditions, the  $\text{K}^+$  content is reduced in all grape tissues, like in many glycophytes (Greenway & Munns, 1980). Our results showed that increased salinity levels resulted in a considerable reduction in  $\text{K}^+$  concentrations in all vine parts, although the reduction in GharaUzum was lower than others. This genotype maintained higher  $\text{K}^+$  contents in salinity. The correlation between  $\text{Na}^+$  content with  $\text{K}^+$  in laminae was negative ( $P < 0.01$ ,  $r > -0.8$ ) in all genotypes.

During adaptation, plants may respond to environmental stresses by accumulating compatible solutes such as proline, glycine betaine and soluble sugar (Bohnert et al., 1995). The conventional role of these compatible solutes is cell osmotic adjustment (Yancey et al., 1982). Many plant species accumulate significant amounts of soluble sugar in response to high salinity. Although some researchers have reported positive correlations between the capacity for soluble sugars accumulation and salinity tolerance (Taji et al., 2002). Others have challenged the value of these solutes as positive indicator for resistance to salt stress. Mohammadkhani et al., (2013) reported that hyper accumulation of soluble sugars is not essential for improving salinity tolerance in grapevines and it is just a symptom of salt stress. The soluble sugar content in roots and laminae increased with increasing salinity in all of our genotypes. Our results were consistent to Mohammadkhani et al., (2013) that the accumulation of soluble sugar was not associated with salt tolerance. GharaUzum that had lower toxic ion accumulation and are assumed to be salt tolerant did not necessarily show higher sugar content than others.

One of the earliest biochemical processes of many plant species exposed to salt stress is the accumulation of proline. Nevertheless, its role as an adaptive process in salt stress is still a matter of debate (Kumar et al., 2003). Many studies show the important osmoprotective role of proline under stress conditions (Stewart & Lee, 1974). However, some researchers consider an increased proline content simply as a stress effect, rather than a cause of stress tolerance (Moftah & Michel, 1987). Proline content of lamina and roots of all our genotypes

increased with increasing salt treatments (Table 2). Hosseini and Keshmeshi had higher proline contents in roots and laminae than others.

The osmoprotective role of glycine betaine against abiotic stress in plants is well known. Here the glycine betaine content increased in the lamina of all four genotypes under salinity. The accumulation of glycine betaine was higher than that of proline under salinity. This means that our genotypes accumulate glycine betaine better than proline in the leaves.

Given that glycine betaine accumulation is induced by abiotic stress, it is reasonable to assume that increased levels of glycine betaine led to increased stress tolerance (Martinez et al., 2005). Our results confirm this observation. GharaUzum and Keshmeshi showed higher and lower glycine betaine content than the other genotypes. GharaUzum accumulated lower levels of toxic ions and seems to be a salt-tolerant genotype. There was a positive significant correlation ( $P < 0.05$ ,  $r > 0.7$ ) between toxic ions and glycine betaine in our studied genotypes.

Salinity decreased plant growth because of high accumulation of  $\text{Na}^+$  and  $\text{Cl}^-$  in all plant parts. The higher accumulation of  $\text{Cl}^-$  than  $\text{Na}^+$ , particularly in shoots, indicated that all genotypes studied here were poor  $\text{Cl}^-$  excluders. There was a high negative correlation between  $\text{Cl}^-$  and  $\text{Na}^+$  contents with photosynthesis rate in lamina. Among the genotypes, GharaUzum showed a higher glycine betaine content in lamina and lower toxic ions contents when compared to the others. Taken together, GharaUzum and Keshmeshi showed, respectively, higher and lower ability to inhibit excessive  $\text{Na}^+$  and  $\text{Cl}^-$  transport to shoot.

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## REFERENCES

- Abraham, E., Rigo, G., Szekely, G., Nagy, R., Koncz, C. & Szabados, L. 2003. Light-dependent induction of proline biosynthesis by abscisic acid and salt stress is inhibited by brassinosteroid in *Arabidopsis*. Plant Molecular Biology 51: 363-372.
- Alexander, D.Mc.E. & Groot Obbink, J. 1971. Effect of chloride in solution culture on growth and chloride uptake of Sultana and Salt Creek grapevines. Australian Journal Experimental Agriculture. Anim. Husb 11: 357-361.
- Ashraf, M. & Orooj, A. 2006. Salt stress effects on growth, ion accumulation and seed oil concentration in an arid zone traditional medicinal plant ajwain (*Trachyspermum ammi* [L.] Sprague). Journal of Arid Environments 64: 209-220.

- Bohnert, H.J., Nelson, D.E. & Jensen, R.G.** 1995. Adaptations to environmental stresses. *Plant Cell* 7: 1099-1111.
- Banuls, J., Legaz, F. & Primo-Millo, E.** 1990. Effect of salinity on uptake and distribution of chloride and sodium in some citrus scion-rootstock combinations. *Journal of Horticultural Sciences* 65: 715-724.
- Cataldo, D.A., Haroon, M., Schrader, L.E. & Youngs, V.L.** 1975. Rapid colorimetric determination in plant tissues by nitration of salicylic acid. *Communications in Soil Science and Plant Analysis* 6: 71-80.
- Cavagnaro, J.B., Ponce, M.T., Guzman, J. & Cirrincione, M.A.** 2006. Argentinean cultivars of *Vitis vinifera* grow better than European ones when cultured *in vitro* under salinity. *Biology of The Cell* 30: 1-7.
- Christensen, L.P., Kasimatis, A.N. & Jensen, F.L.** 1978. Grapevine Nutrition and Fertilization in the San Joaquin Valley. University of California, Berkeley, Agriculture & Natural Resources, Publication No. 4087.
- Dubois, M., Gilles, K.A., Hamilton, J.K., Roberts, P.A. & Smith, F.** 1956. Colorimetric method for determination of sugars and related substances. *Analytical Chemistry* 28: 350-356.
- Ehlig, C.F.** 1960. Effects of salinity on four varieties of table grapes grown in sand culture. *In: Proceedings of the American Society for Horticultural Science*, Magness J.R. (ed.), vol. 76, pp. 323-331.
- Greenway, H. & Munns, R.** 1980. Mechanisms of salt tolerance in nonhalophytes. *Annual Review of Plant Physiology* 31: 149-190.
- Grieve, C.M. & Grattan, S.R.** 1983. Rapid assay for determination of water soluble quaternary ammonium compounds. *Plant Soil* 70: 303-307.
- Henderson, S.W., Baumann, U., Blackmore, D.H., Walker, A.R., Walker, R.R. & Gilliam, M.** 2014. Shoot chloride exclusion and salt tolerance in grapevine is associated with differential ion transporter expression in roots. *BMC Plant Biology* 14: 273.
- Hoekstra, F.A., Golovina, E.A. & Buitink, J.** 2001. Mechanisms of plant desiccation tolerance. *Trends in Plant Science* 6: 431-438.
- Khawale, R.N., Singh, S.K., Patel, V.B. & Singh, S.P.** 2003. Changes due to *in vitro* sodium chloride induced salinity in grape (*Vitis vinifera* L.). *Indian Journal of Plant Physiology* 28: 378-382.
- Kumar, S.G., Reddy, A.M. & Sudhakar, C.** 2003. NaCl effects on proline metabolism in two high yielding genotypes of mulberry (*Morus alba* L.) with contrasting salt tolerance. *Plant Science Journal* 165: 1245-1251.
- Lakshmi, A., Ramanjulu, S., Veeranjanyulu, K. & Sudhakar, C.** 1996. Effect of NaCl on photosynthesis parameters in two cultivars of mulberry. *Photosynthetica* 32: 285-289.
- Lu, K.X., Cao, B.H., Feng, X.P., He, Y. & Jiang, D.A.** 2009. Photosynthetic response of salt-tolerant and sensitive soybean varieties. *Photosynthetica* 47: 381-387.
- Maas, E.V. & Hoffman, G.J.** 1977. Salt crop tolerance Current assessment. *Journal of The Irrigation and Drainage Division* 103: 115-134.
- Martinez, J.P., Kinet, J.M., Bajji, M. & Lutts, S.** 2005. NaCl alleviates polyethylene glycol-induced water stress in the halophyte species *Atriplex halimus* L. *Journal of Experimental Botany* 56: 2421-2431.
- McClure, P.E., Omholt, T.E. & Pace, G.M.** 1986. Anion uptake in maize roots: interactions between chlorate and nitrate. *Physiologia Plantarum* 68: 107-112.
- Misra, A.N., Sahu, S., Misra, M., Mohapatra, P., Meera I. & Das, N.** 1997. Sodium chloride induced changes in leaf growth, and pigment and protein contents in two rice cultivars. *Biologia Plantarum* 39: 257-262.
- Misra, A.N., Biswal, A.K. & Misra, M.** 2002. Physiological, biochemical and molecular aspects of water stress responses in plants and the biotechnological applications. *Proceedings of The National Academy of Science* 72 B: 115-134.
- Moftah, A.E. & Michel, B.E.** 1987. The effect of sodium chloride on solute potential and proline accumulation in soybean leaves. *Plant Physiology* 83: 238-240.
- MohammadKhani, N., Heidari, R., Abbaspour, N. & Rahmani, F.** 2013. Comparative study of salinity effects on ionic balance and compatible solutes in nine Iranian table grape (*Vitis vinifera* L.) genotypes. *Journal International Des Sciences De La Vigne Et Du Vin* 47: 99-114.
- Mousavian, M. & Abbaspour, N.** 2017. Effects of salinity on some morphological and physiological parameters in four canola (*Brassica napus* L.) cultivars. *Nova Biologica Reperta* 4: 98-106. (In Persian).
- Munns, R.** 2002. Comparative physiology of salt and water stress. *Plant, Cell and Environment* 25: 239-250.
- Munns R., James R.A. & La" uchli A.** 2006. Approaches to increasing the salt tolerance of wheat and other cereals. *Journal of Experimental Botany* 57: 1025-1043.
- Murakeozy, E.P., Nagy, Z., Duhaze, C., Bouchereau, A. & Tuba, Z.** 2003. Seasonal changes in the levels of compatible osmolytes in three halophytic species of inland saline vegetation in Hungary. *Journal of Plant Physiol* 160: 395-401.
- Nomura, M., Hibino, T., Takabe, T., Sugiyama, T., Yokota, A., Miyake, H. & Takabe, T.** 1998. Transgenically produced glycinebetaine protects ribulose 1,5-bisphosphate carboxylase/oxygenase from inactivation in *Synechococcus* sp. PCC7942 under salt stress. *Plant and Cell Physiology* 39: 425-432.
- Owais, S.J.** 2015. Morphological and physiological responses of six grape genotypes to NaCl salt stress. *Pakistan Journal of Biology Sciences* 18: 240-246.
- Sakamoto, A. & Murata, N.** 2002. The role of glycine betaine in the protection of plants from stress: clues from transgenic plants. *Plant, Cell and Environment* 25: 163-171.

- Seraj, R. & Sinclair, T.R.** 2002. Osmolyte accumulation: can it really help increase crop yield under drought conditions? *Plant, Cell and Environment* 25: 333-341.
- Shilpi, M. & Narendra, T.** 2005. Cold, salinity and drought stresses: an overview. *Archives of Biochemistry and Biophysics* 444: 139-158.
- Singh, S.K., Sharma, H.C., Goswami, A.M., Dutta, S.P. & Singh, S.P.** 2000. *In vitro* growth and leaf composition of grapevine cultivars as affected by sodium chloride. *Biologia Plantarum* 6: 283-286.
- Stewart, G.R. & Lee, J.A.** 1974. The role of proline accumulation in halophytes. *Planta* 120: 279-289.
- Taji, T., Ohsumi, C., Iuchi, S., Seki, M., Kasuga, M., Kobayashi, M., Yamaguchi-Shinozaki, K. & Shinozaki, K.** 2002. Important roles of drought and cold-inducible genes for galactinol synthase in stress tolerance in *Arabidopsis thaliana*. *The Plant Journal* 29: 417-426.
- Tester, M. & Davenport, R.** 2003. Na<sup>+</sup> tolerance and Na<sup>+</sup> transport in higher plants. *Annals of Botany* 91: 503-527.
- Venier, M., Aguero, C.B., Bermejillo, A., Filippini, M.F., Hanana, M., Walker, M.A., Blumwald, E. & Dandekar, A.M.** 2018. Analysis of salinity tolerance of *Vitis vinifera* 'Thompson Seedless' transformed with *AtNHX1*. *Vitis* 57: 143-150.
- Vijayan, K., Chakraborti, S.P. & Ghosh, P.D.** 2003. In vitro screening of mulberry (*Morus* spp.) for salinity tolerance. *Plant Cell Reports* 22: 350-357.
- Walker, R.R.** 1994. Grapevine responses to salinity (Réaction de la vigne à la salinité). *Bulletin de l'O I V (Organisation Internationale de la Vigne et du Vin)* 67: 634-661.
- Walker, R.B. & Douglas, T.J.** 1982. Effect of salinity on uptake and distribution of chloride, sodium and potassium ions in citrus plants. *Australian Journal of Agricultural Research* 34: 145-153.
- Yancey, P.H., Clark, M.E., Hand, S.C., Bowlus, R.D. & Somero, G.N.** 1982. Living with water stress: evolution of osmolyte systems. *Science* 217: 1214-1222.

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