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Effects of salinity on potassium absorption and expression of K^+ transporter genes at different concentrations of potassium in Grape (*Vitis vinifera L.*)

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Summary

Grapevine is classified as a moderately sensitive plant to salinity. Hydroponically three grape genotypes (*Vitis vinifera* L.) were treated with different concentrations of KCl (0.1, 0.3, 0.5, 1, 5, 10 mM KCl) and NaCl (0, 25, 50,100 mM NaCl). Cl^- and Na^+ contents were significantly increased in different plant organs of all the genotypes under salinity. In this study, sensitive ('GhezelUzum'), tolerant ('Gharashani') and semi-resistant ('Chawga') grape genotypes were selected based on screening experiments under salinity. 'Gharashani' accumulated higher Na^+ and Cl^- in roots compared to the sensitive one. 'Chawga' accumulated high K^+ similar to Na^+ in root and shoot even at high salinity. K_m calculation for K^+ and Na^+ uptake in root and shoot of 'Chawga' showed that K^+ and Na^+ compete to enter the plant through roots. Two KUP/KT/HAK-type potassium transporters are expressed highly in the grapevine during stress. VvK1.1 could play a major role in K^+ loading into grape tissues. The expression of VvKUP1 and VvKUP2 transporters and VvK1.1 channel in roots of 'Chawga' genotype increased significantly ($P < 0.05$) at different KCl concentrations under salinity stress. 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.

K e y w o r d s : salinity; grapevine; potassium absorption; transporters expression.

Introduction

Salinity is one of the most important abiotic stresses and a serious threat to sustainable agriculture. The extent of salinity problem is about 10 % of world land area and 50 % of irrigated areas resulting in 12 Billion US$ loss of agricultural production (FAO 2016). Salt stress is a complex system which affects almost every physiological and biochemical pathway in the plants (Munns and Tester 2008).

Several studies defined that salt tolerance mechanisms in grapevine involve many factors like restriction of ion absorption, translocation from roots to shoots, photosynthesis alteration and solute accumulation (Walker *et al.* 2010). Some of the grapevine rootstocks have been rated as tolerant to salinity because of their ability to restriction Na^+ and/or Cl^- uptake and translocation to aerial parts of the vines. Accumulation of Cl^- and Na^+ in grapevines under saline conditions may result in physiological troubles leading to decline in growth, vegetative biomass, and fruit yield (Walker *et al.* 2010).

Salt tolerance is related to the ability of genotypes to regulate both Cl^- and Na^+ transports in order to escape toxicity. Some genotypes are more effective at regulating Na^+ or Cl^- transport (or both) than others, due to different mechanisms of ions balance. Studies show that high Cl^- accumulation by certain genotypes can cause growth decrease (Teakle and Tyerman 2010). This suggests that Cl^- content of grapevine is certainly an important factor for vine health under salinity.

To retain an optimal intracellular K^+ to Na^+ ratio under salinity, accumulation of excessive content of Na^+ in the cytosol should be inhibited along with preservation of physiological concentrations of cytosolic K^+ at the cellular level. Maintenance of low cytosolic Na^+ may be achieved via several main strategies (Tester and Davenport 2003).

Na^+ competition at transport sites for K^+ entry into the cytoplasm may result in K^+ deficiency. Furthermore, cytoplasmic Na^+ competes for K^+ binding sites and therefore prohibits metabolic activities which critically depend on K^+. Obviously, Na^+ in the cytosol has to be restricted by limiting Na^+ entry and/or function by an effective system for Na^+ efflux into the vacuole (Nieves-Cordones *et al.* 2016b). One of the key traits of salt tolerance in a plant is the ability of plant cells to retain an optimal K^+/Na^+ ratio. Under salinity stress excessive Na^+ accumulation in plant tissue leads to K^+ leakage from the cell (Shabala and Pottosin 2014).

Potassium is an essential element for all living organisms. The important roles of K^+ in plants can be classified into four physiological-biochemical categories: (1) enzyme activation (Marschner 2012); (2) cellular membrane transport operations and translocation of assimilates (Patrick *et al.* 2001); (3) anion neutralization, which is essential for membrane potential stability (Demidchik *et al.* 2014) and (4) osmotic potential regulation which is one of the important mechanisms in the control of plant water status (Nieves-Cordones *et al.*2016a), turgor maintenance and growth. In grapevine, K^+ plays a necessary role in the initiation and control of major fluxes which are necessary for berry growth during maturation (Nieves-Cordones *et al.* 2019). Plant great mul-

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ti-gene families encoding K^+ penetrable transport systems belong to one of the following five families (i) HAK-KUP-KT transporters, (ii) HKT transporters, (iii) cation-proton antiporters (CPA), (iv) Shaker-like K^+ channels, and (v) two pore K (TPKs) channels (Very *et al.* 2014).

Two KUP/KT/HAK-type potassium transporters, *VvKUP1* and *VvKUP2*, and a cation/proton antiporter are related with berry ripening process (Hanana *et al.* 2007) and expressed in the berry skin (Davies *et al.* 2006).

The most studied members of the KT/KUP/HAK family belong to the two largest groups: I and II. In growing grapevine fruits (*Vitis vinifera*), the expression of *VvKUP1* and *VvKUP2* potassium transporter genes is dependent on their developmental phase. It is likely that these transporters are required for the potassium-driven cell development in young grape berries (Davies *et al.* 2006). Another inwardly rectifying K^+ channel, *VvK1.1*, involved in K^+ uptake from soil into roots, is up regulated in berries under drought stress conditions (Cuéllar *et al.* 2013).

Other types of potassium transporters have also been shown to be expressed in grape berries. A Shaker-type potassium channel has been cloned from grapevine (Pratelli *et al.* 2002). It is expressed at low levels in a range of tissues including pre-veraison berries. They showed that this channel is expressed in stomatal guard cells and may play a role in the control of transpiration and water movement in berries. Both *VvKUP*1 and *VvKUP2* were most highly expressed in grapevine reproductive tissues (berries, flowers, and seeds).

In *Arabidopsis*, K^+-selective channels, nonselective cation channels, and probably the high affinity K^+ transporter *KUP4* are located at the plasma membrane of root epidermis cells. In addition, four other root KUP/HAK transporters might contribute to root K^+ uptake. *AtKUP4* would be involved in a specific K^+ transport process essential for root-hair elongation (Rigas *et al.* 2001). Furthermore, *AKT1* is mainly expressed in Arabidopsis roots, where it increases inward K^+ channel activity involved in K^+ uptake from the soil (Xu *et al.* 2006).

In previous experiments, 18 grape genotypes were screened from the viewpoint of salt tolerance parameters (Mohammadkhani *et al*. 2014). The genotypes with high ('GhezelUzum') and low ('Gharashani') Cl^- and Na^+ accumulation capacity were selected for further experiments. In addition, in screening experiments, they used 0-100 mM NaCl treatments for 14 d and concluded that 50 mM salt was sufficient to reduce the water potential, but the grapevine plants was not killed, when exposed to it for 14 d.

In this study, three grape genotypes were compared to determine ions (K^+ and Na^+) accumulation capacity. The genotype with the highest K^+ accumulation capacity was selected for absorption kinetics experiments and molecular analyses. Molecular studies in the genotype with the highest capacity ('Chawga') was conducted to show K^+ transporters (KUPs and *K1.1*) expression in root cells under salinity.

Material and Methods

Plant materials and growth conditions: Hardwood cuttings of three genotypes of grapevine ('Gharashani', 'GhezelUzum' and 'Chawga') (Mohammadkhani *et al*. 2012) were collected from Kahriz vineyard (Agricultural Research Center, grape genotypes collection). The cuttings were disinfected with benomyle (1 % w/v), and then the basal parts soaked in 0.1 % (w/v) IBA (Indole-3-butyric acid) 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 two weeks, the rooted cuttings were transferred into the pots (2L) containing 1/4 strength modified Hoagland solution (Walker *et al*. 2004). Plants with 4-5 fully expanded leaves were then treated with NaCl (0, 25, 50, 100 mM NaCl) in full strength Hoagland solution for 2 weeks. NaCl was added to the nutrient solution at the desired concentration incrementally (during 3 d) until the final desired concentrations were reached. The pots were covered with aluminum foil to avoid light effects and alga proliferation. Plants were harvested after 2 weeks and plant parts were weighed separately and dried at 70 °C for 48 h.

Salinity and potassium treatments: Two-month old plants were treated for 14 d at different potassium levels (0.1, 0.3, 0.5, 1, 5, 10 mM KCl) with 50 mM NaCl (threshold salinity determined for the genotypes). According to the screening study (Mohammadkhani *et al*. 2014), 50 mM salt was sufficient to reduce water potentials and not killing the grapevine plants when exposed for several days. Leaf and young root segments were collected at different time points (0, 24 h and 14 d), frozen in liquid nitrogen immediately and stored at -80 °C until RNA isolation.

Ions content analysis: Hundred mg of ground tissues of all treatments were weighed and transfered into 15 mL plastic centrifuge tubes containing 10 mL deionized water (Abbaspour *et al.* 2014). The tubes were placed in a boiling water bath for approximately 1 h. Samples were centrifuged at 5,000 rpm. The supernatant was transferred into new tubes and the volume made up to 10 mL by adding deionized water. Sodium and potassium concentrations were measured by a flame photometer (Fater 405, Iran).

RNA isolation, cDNA synthesis and RT-PCR: For PCR experiment, treated root tissues (only 0.1 and 10 mM KCl) at 50 mM NaCl (threshold salinity determined for the local genotypes) were collected at different time points (0, 24 h and 14 d), frozen in liquid nitrogen immediately and stored at -80 °C until RNA isolation.

Total RNA was extracted from root tissues using Louime *et al.* (2008) method with a small modification. The RNA concentration was determined by Nanodrop. The integrity of RNA was checked on agarose gel. First strand cDNA was synthesized from total RNA using a first strand cDNA synthesis Kit (Yekta Tajhiz, Iran) 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 follows: initial denaturation at 95 °C for 3 min, followed by 28-30 cycles at 95 °C for 30 s, 57-58 °C for 30 s and 72 °C for 30 s and final extension at 72 °C for 10 min. The *VvEF1* gene (Elongation Factor 1) was used as internal reference. Forward and reverse primers sequences are shown in Tab. 1. The products of RT-PCR were separated on 1.5

% agarose gel which contained Ethidium Bromide (0.5 $\mu g \cdot mL^{-1}$) and visualized using Ingenius3 (Syngen, UK). The experiment was repeated three times. The intensity of the RT-PCR bands was measured using Image J software1.43.

Statistical analysis: All statistical analyses were performed using the Statistical Package for Social Sciences (SPSS) for Windows (Version 14.0). Our experimental design was Complete Randomized Design (CRD). The mean values of three replicates and the Standard Error of the means were calculated. 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 ($P < 0.05$) was applied. To determine differences between genotypes, GLM (General Linear Model) analysis was used. Nonlinear regression curve fit and K_m levels (Michaelis-Menten) were calculated by Graphpad Prism 5 software.

Results

Effects of salinity on mineral contents: Sodium significantly ($P < 0.05$) increased in roots of all the genotypes with increasing salinity. At 50 mM NaCl, the roots of 'Gharashani' showed a higher Na^+ concentration (42.89 $mg \cdot g^{-1}$ DW) than two other genotypes (36.3 $mg \cdot g^{-1}$ DW and 32.8 $mg \cdot g^{-1}$ DW) (Tab. 2). Unlike sodium, potassium concentration decreased with increasing salinity treatments in roots, except in 'Chawga' which showed a significant ($P < 0.05$) K^+ concentration increase under salinity in roots (49.92 $mg \cdot g^{-1}$ DW) and especially in shoots (160 $mg \cdot g^{-1}$ DW) (Tab. 2).

Sodium, also significantly ($P < 0.05$) increased in different parts (shoot and root) of 'Chawga' with increasing salinity in growth medium (Tab. 2). In all treatments sodium concentration in shoot was higher than that in root (Tab. 2).

Sodium uptake: Na^+ content increased significantly ($P < 0.05$) in roots of all the genotypes under 50 mM salinity. Na^+ content of sensitive genotype ('GhezelUzum') was higher than that of the tolerant genotype ('Gharashani') in all the plant organs (Tab. 2). GLM analysis showed that with increasing of K^+ concentrations (0.1, 0.3, 0.5, 1, 5, 10 mM KCl) in roots of three grape genotypes: 'Chawga', 'Gharashani' and 'GhezelUzum' (*Vitis vinifera* L.) under 50 mM NaCl, sodium concentration decreased in roots. At 10 mM KCl, the roots of 'Chawga' showed a lower Na^+ concentration than two the other genotypes (7.33 DW % for 'Gharashani' and 11.7 DW % for 'GhezelUzum') (Fig. 1).

Potassium uptake: Unlike for sodium, the potassium concentration of roots and shoots decreased with increasing salinity treatments in all genotypes, except in 'Chawga' which showed a significant ($P < 0.05$) increase in the K^+ concentration of root (67.24 $mg \cdot g^{-1}$ DW) and particularly shoot (174.29 $mg \cdot g^{-1}$ DW) at 100 mM salinity (Tab. 2).

Table 1

Forward and revers primers used in RT-PCR experiment (Davies *et al.* 2006)

Genes	Forward Primer (5'3')	Reverse Primer (5'3')
VvKUP1	TGAGCTTTGAAACATGGGAAGACT	TTCTTGTTACCAAGCCTTCCGG
VvKUP2	ATGCTTCCTGCCATTTCCACATA	GGTTGGCATGGTTTATATCGTCTG
VvK1.1	TTGTTGAAACGTGGTCTGGA	GCCCTGCCCCATAATCTAGT
VvEF1	TCTGCCTTCTTCCTTGGGTA	GCACCTCGATCAAAAGAGGA

Table 2

Root and shoot Na^+,Cl^- and K^+ contents ($mg \cdot g^{-1}$ DW) in three table grapes (*Vitis vinifera* L.) at different salinity levels (0, 25, 50 and 100 mM NaCl)

Genotype and salinity (mM NaCl)	Na^+ content of root ($mg \cdot g^{-1}$ DW)	Na^+ content of shoot ($mg \cdot g^{-1}$ DW)	Cl^- content of root ($mg \cdot g^{-1}$ DW)	Cl^- content of shoot ($mg \cdot g^{-1}$ DW)	K^+ content of root ($mg \cdot g^{-1}$ DW)	K^+ content of shoot ($mg \cdot g^{-1}$ DW)
GhezelUzum						
0	2.16 ± 0.1 a	4.06 ± 0.09 a	6.61 ± 0.28 a	9.06 ± 0.49 a	66.49 ± 1.89 a	150.21 ± 0.65 a
25	27.6 ± 0.37 b	94.53 ± 1.14 b	22.32 ± 0.42 b	36.11 ± 0.52 b	51.44 ± 0.62 b	148.22 ± 0.66 b
50	36.3 ± 0.57 c	178 ± 1.84 c	26.88 ± 0.36 c	44.92 ± 0.87 c	44.08 ± 0.96 c	144.23 ± 1.19 c
100	75.15 ± 1.47 d	208 ± 0.52 d	50.83 ± 0.97 d	129.22 ± 0.83	27.80 ± 1.20 d	139.25 ± 0.85 c
Gharashani						
0	4.66 ± 0.26 a	9.38 ± 0.37 a	5.69 ± 0.77 a	8.26 ± 0.41 a	55.23 ± 0.49 a	143.12 ± 1.40 a
25	38.33 ± 0.50 b	89.06 ± 0.14 b	15.52 ± 0.05 b	25.83 ± 0.24 b	42.00 ± 0.22 b	125.22 ± 0.85 b
50	42.89 ± 0.36 c	105.23 ± 0.13 c	16.86 ± 0.20 b	40.46 ± 0.57 c	34.69 ± 1.27 c	104.24 ± 0.58 c
100	60.18 ± 0.52 d	126.20 ± 0.69 d	22.23 ± 0.47 c	43.77 ± 0.81 d	25.53 ± 1.43 d	202.1 ± 0.99 d
Chawga						
0	8.51 ± 0.32 a	25.33 ± 0.55 a	7.27 ± 0.13 a	12.04 ± 0.4 a	9.39 ± 0.24 a	21.34 ± 0.67 a
25	26.79 ± 0.23 b	80.38 ± 0.46 b	12.67 ± 0.21 b	33.69 ± 0.9 b	45.88 ± 0.12 b	132.02 ± 1.81 b
50	32.8 ± 0.18 c	92.86 ± 0.35 c	16.62 ± 0.36 c	43.42 ± 0.56 c	49.92 ± 0.57 b	160 ± 2.01 c
100	42.88 ± 0.13 d	134.20 ± 1.24 d	19.99 ± 0.36 d	64.89 ± 0.91 d	67.24 ± 3.5 c	174 ± 2.29 d

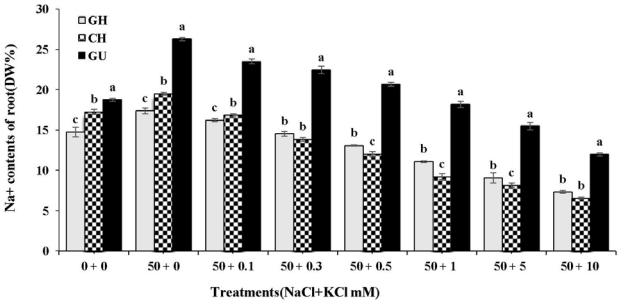

Fig. 1: Na^+ contents in roots of three grape genotypes 'Chawga' (CH), 'Gharashani' (GH) and 'GhezelUzum' (GU) (*Vitis vinifera* L.) at different potassium levels (0.1, 0.3, 0.5, 1, 5, 10 mM KCl) at 50 mM NaCl after 14 d treatment.

However, GLM analysis showed that in roots of all genotypes, K^+ accumulation was decreased with increasing potassium ingredient at 50 mM NaCl but in 'Chawga' K^+ accumulation highly increased than the other genotypes (11.93 DW % for 'Gharashani' and 17.69 DW % for 'GhezelUzum') (Fig. 2).

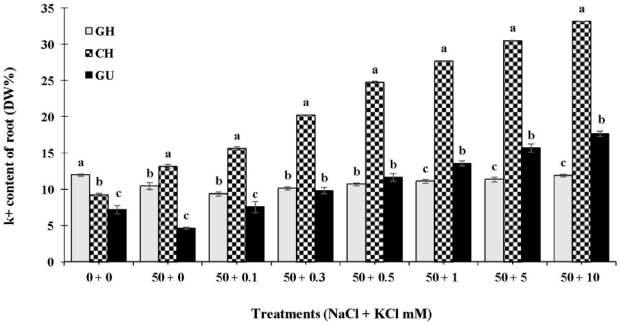

Fig. 2: K^+ contents in roots of three grape genotypes 'Chawga' (CH), 'Gharashani' (GH) and 'GhezelUzum' (GU; *Vitis vinifera* L.) at different potassium levels (0.1, 0.3, 0.5, 1, 5, 10 mM KCl) at 50 mM NaCl after 14 d treatment.

At 10 mM KCl, the roots of 'Gharashani' showed a lower K^+ concentration (11.93 DW %) than other genotypes (33.19 DW % for 'Chawga' and 17.69 DW % for 'GhezelUzum') (Fig. 2).

Chloride uptake: All genotypes showed high Cl^- accumulation in different plant parts under salt treatments when compared to control plants (Tab. 2). GhezelUzum showed the highest shoot Cl^- accumulation at 50 mM NaCl compared to 'Gharashani' with the lowest Cl^- content.

The Cl^- concentration in shoot increased with increasing salinity in all genotypes. However, at 50 and 100 mM of NaCl 'Gharashani' accumulated lower Cl^- in shoot than GhezelUzum and 'Chawga' (Tab. 2). In all genotypes, Cl^- accumulation in shoots was nearly two-fold higher than in roots.

Serious damages such as leaf chlorosis and burning were observed in 'GhezelUzum' at high NaCl concentration. 'Gharashani' (16.86 mg·g^{-1} DW) and 'Chawga' (16.62 mg·g^{-1} DW) showed lower Cl^- contents in root at 50 mM NaCl than 'GhezelUzum' genotype (26.88 mg·g^{-1} DW). GLM analysis showed that with increasing of different concentrations of potassium at 50 mM NaCl, Cl^- accumulation decreased in all genotypes. At 10 mM KCl, the roots of 'Gharashani' showed a lower Cl^- concentration (0.9 DW %) than other genotypes (2.18 DW % for 'GhezelUzum' and 1.53 DW % for 'Chawga') (Fig 3).

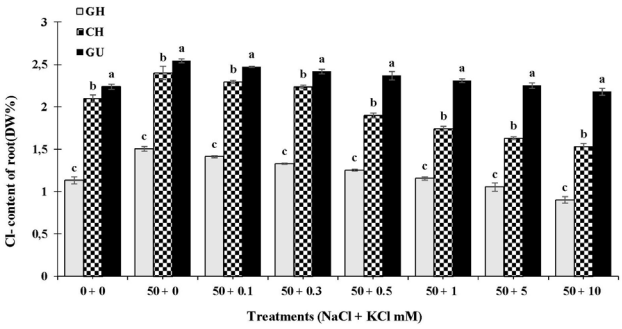

Fig. 3: Cl^- contents in roots of three grape genotypes 'Chawga' (CH), 'Gharashani' (GH) and 'GhezelUzum' (GU; *Vitis vinifera* L.) at different potassium levels (0.1, 0,3, 0.5, 1, 5, 10 mM KCl) at 50 mM NaCl after 14 d treatment.

Nonlinear regression curves for Na^+ and K^+ uptake rate: The results of nonlinear regression analysis for sodium and potassium uptake rate in 'Chawga' genotype showed that K^+ was accumulated in roots of 'Chawga' with K_m = 62.48 compared to Na^+ with K_m = 68.56 while shoots experienced K^+ accumulation with K_m = 47.23 compared to Na^+ with K_m = 81.42 after 14 d treatment under different NaCl concentrations (0-100 mM). The K_m=56.39 and K_m=22.56 were calculated for Cl^- accumulation in shoots and roots of 'Chawga', respectively (Fig. 4).

Expression profile of K^+ transporter genes: In roots and leaves of 'Chawga', expression of three K^+ genes (*VvKUP1*, *VvKUP2* transporters and *VvK1.1* channel) increased under salinity (Fig. 5). Expression of all these genes increased in roots at distinct time points (24 h and 14 d) under 50 mM salinity (Fig. 5).

Potassium uptake gene *VvKUP1* belongs to a potassium transporter family. The expression of *VvKUP1* gene increased after 24 h though it was nearly 4 times less after 14 d. However, the root of 'Chawga' genotype showed significant difference ($P < 0.05$) between control and 14-d salinity treatment (Fig. 5).

The expression profiles of K^+ transporter genes (*VvKUP1*, *VvKUP2* and *VvK1.1*) in root of 'Chawga', after 0, 24 h treatment at 0.1 and 10 mM KCl under 50 mM NaCl have been shown in Fig. 6. Transcription of all the genes increased in root under salinity compared to control. Expression of all the genes increased after 24 h, especially, there was a high increase at 10 mM KCl concentration under 50 mM salinity (Fig 6).

Discussion

It is generally considered that the excess amount of Na^+ leads to nutrient imbalance and thereby causing specific ion toxicity (Munns *et al.* 2016). High concentration of Na^+ and Cl^- ions in soil solution reduces the uptake of K^+ ions, causing K^+ deficiency in plants which results in chlorosis and then necrosis in leaves (Gopal and Dube 2003). Salt sensitive species have no ability to control Na^+ transport. The sodium ion appears to be accumulated more rapidly to a toxic

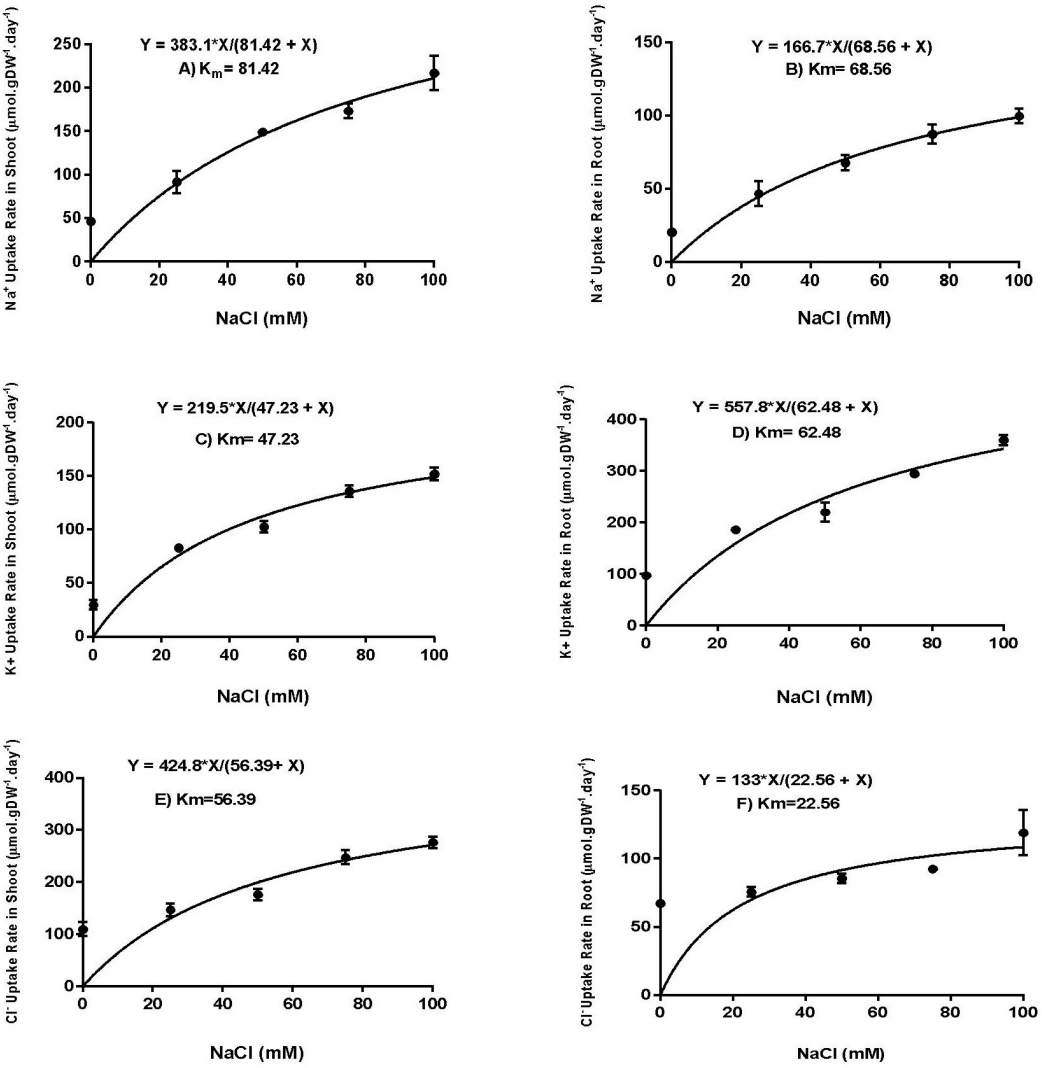

Fig. 4: Nonlinear regression curve fit (Michaelis-Menten) for Na^+ uptake rate (µmol·gDW^{-1}·day^{-1}) in shoot (**A**) and root (**B**) and K^+ uptake rate (µmol·gDW^{-1}·day^{-1}) in shoot (**C**) and root (**D**) and Cl^- uptake rate (µmol·gDW^{-1}·day^{-1}) in shoot (**E**) and root (**F**) of 'Chawga' genotype at different salinity levels (0-100 mM NaCl).

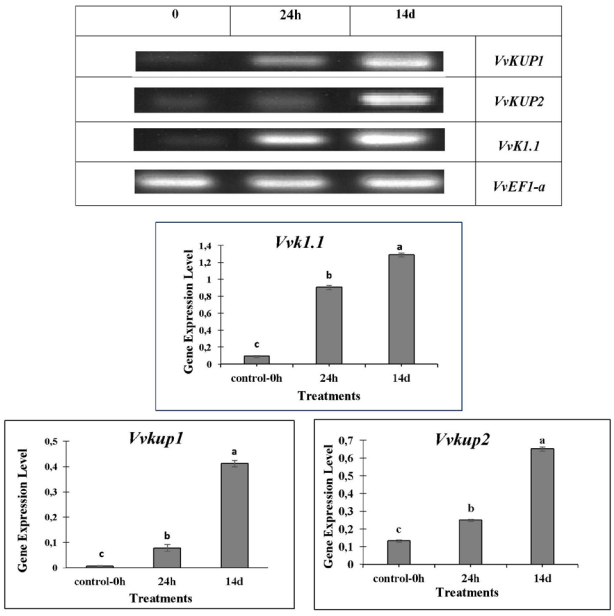

Fig. 5: Expression profile of K^+ transporter genes in root of 'Chawga' (*Vitis vinifera* L.) after 0, 24 h and 14 d treated by 50 mM NaCl. Graphs show relative expression of K^+ transporter genes in root of 'Chawga' (*Vitis vinifera* L.).

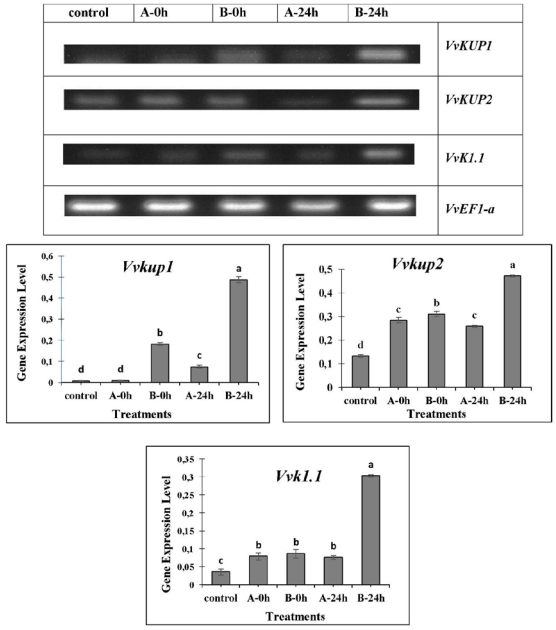

Fig. 6: Expression profile of K+ transporter genes in root of 'Chawga' (*Vitis vinifera* L.) after 0, 24 h treated at 0.1 mM KCl (**A**) and 10 Mm KCl (**B**) under 50 mM NaCl. Graphs show relative expression of K^+ transporter genes in root of 'Chawga' (*Vitis vinifera* L.).

level than Cl^-, therefore most studies have focused on Na^+ exclusion and the control of Na^+ transport within the plant (Munns and Tester 2008). Maintenance of required K^+ level in plant cell under saline conditions depends on selective uptake of K^+, cellular compartmentation of Na^+ and K^+ and distribution in the leaf tissues (Tester and Davenport 2003).

The present results showed significant differences between genotypes in accumulation of Na^+ and K^+ into the root and shoot. Similar differences between grapevine rootstocks in accumulation of K^+ have been previously reported (Demidchik *et al.* 2014). Among our genotypes, 'Chawga' exhibited the reverse pattern of K^+ uptake with nearly similar Na^+ and K^+ accumulation in roots and shoots compared to the other genotypes under salinity. Commonly, under saline conditions, K^+ concentration in all grape tissues as well as many glycophytes is reduced due to competition with Na^+ (Shabala and Pottosin 2014).

Downton (1977) reported Na^+ accumulation in the root system of grapevine. The general decline in potassium concentrations with increasing salinity can be due to replacement by sodium ion which has been elevated with increasing sodium in nutrient solutions (Almeida *et al.* 2017).

Previous studies (Fisarakis *et al.* 2001 and 2005, Walker *et al.* 2004) demonstrated higher accumulation of Cl^- than Na^+ in grapevine under salinity. The results of the present study is consistent with this indication, as Cl^- accumulation exceeded that of Na^+ in all vine parts under all saline treatments. It seems that all the genotypes studied here are adapted to their ecological conditions and are capable of tolerating high concentrations of salinity. In the all genotypes, sodium concentration in shoot was higher than in root.

The results of this study is consistent with the results obtained by Mohammadkhani *et al*. (2015). They showed that 'Chawga' is an exceptional genotype with a high K^+ uptake rate despite of higher Na^+ concentration under salinity. Cuin *et al.* (2008) reported similar data in some wheat lines. Most authors agreed that K^+/Na^+ homeostasis is a key feature of plant salinity tolerance. It has been found that a salt-tolerant barley cultivar was better compared with a salt-sensitive variety at maintaining root cytosolic K^+ under saline conditions. The findings of this study indicate that increasing salinity levels lead to a considerable reduction in K^+ concentrations in all vine parts, except for 'Chawga'. The general decline in K^+ concentrations with increasing salinity level is due to K^+ replacement by higher sodium content in nutrient solutions (Almeida *et al.* 2017).

Uptake and distribution of K^+ in plant cells is carried out by a variety of transporter proteins categorized into several families with varing structures and transport mechanisms that include: shaker-type voltage-dependent, the tandem-pore (TPK), and the two-pore channels (TPC) (Hedrich 2012), the carrier-like families KT/HAK/KUP, HKT uniporters and symporters and cation-proton antiporters families (CPA) (Li *et al.* 2018). In *A. thaliana* , the 12 members of this family are present in different tissues and are probably involved in many diverse physiological functions in plants. Among them, *AtKUP2*, 4, 6, and 7 are involved in cell enlargement, K^+ translocation, and long-distance K^+ transport (Santa-María *et al.* 2018).

Transcription of *KUP2*, mostly expressed in rapidly growing tissues with playing a role in cell expansion, decreased in the shoots of plants treated under salinity. The *KUP2* down regulation may reflect salinity-induced lack of turgor and reduction in growth (Osakabe *et al.* 2013). Low external K^+ concentration upregulates several genes encoding K^+ transporters, including some HAK/KT/KUP genes (Santa-Maria *et al.* 2018).

The present study showed that under salinity stress the expression of *VvKUP1* and *VvKUP2* increased 6 and 5 fold in roots of 'Chawga' respectively, after 14 d exposure to 50 mM NaCl, compared to control plants. These upregulations could be due to direct contact of roots with salt solutions leading to no opportunity for salt escape.

The results obtained in this study were consistent with Mohammadkhani *et al.* (2015) who found that under salinity stress the expression of *VvKUP1* and *VvKUP2* increased in roots and leaves of 'Chawga', although leaves were not affected as much as roots. Also,they showed that salt stress increased the transcript levels of *VvK1.1* in roots and leaves of 'Chawga'.

The observed enhancement in expression of *VvKUP1* in 'Chawga' is verified by Su *et al.* (2002) and Elumalai *et al.* (2002) who reported upregulation of *VvKUP1* transcripts under salinity after 24 h salinity treatment. However, the results were not consistent with Elumalai *et al.* (2002) in which a decline in mRNA accumulation of *KUP2* gene under salinity was reported. Our data showed a significant increase ($P < 0.05$) in *VvKUP2* transcripts compared to control.

Like other shaker-type channels, *VvK1.1* is voltage dependent. The voltage dependence is independent of the external K^+ concentration, a feature that classically reported in inwardly rectifying plant shaker-type channels (Very and Sentenac 2003). In roots, expression of *VvK1.1* in cortical cells suggests a role in K^+ uptake from the soil solution, as shown for the *A.thaliana AKT1* channel by using a mutant line disrupted in the encoding gene (Xu *et al.* 2006). The results for expression of *VvK1.1* in 'Chawga' roots were in agreement to Cuéllar *et al.* (2013) as they reported a decrease in transcript of *VvK1.1* in roots of grape under drought stress. All of the studied genotypes showed enhancement in *VvK1.1* transcript in roots after 14 d imposing salinity compared to control. When the roots of 'Chawga' genotype were treated with 50 mM under incremental K^+ concentrations (0.1 and 10 mM of KCl) at 0 and 24 h time points, transcription of this genes increased in root compared to control.

Potassium uptake transporter 1 (*VvKUP1*) is referred to as a dual- or high-affinity transporter depending on the expression system (Davies *et al.* 2006). It has been found that hypersensitivity to salt stress was frequently associated with poor K^+ absorption in Arabidopsis and tomato mutants and the maintenance of high K^+ levels and a low Na^+/K^+ ratio in the cytoplasm could also be essential for salt tolerance (Shabala and Pottosin 2014).

Sun *et al.* (2015) demonstrated that the high K^+ content, which is the result of low K^+ efflux and high expression of the K^+ transporters genes *AtHAK5*, *AtKUP1* and *AtCHX17*, plays an important role in the salinity response of these tolerant accessions. Based on our observations, exposure of

'Chawga' root tissues to high concentrations of K^+ resulted in high expression of *VvKUP1*, *VvKUP2* and *VvK1.1* under salinity. These data suggest that the tolerant accessions were better pre-conditioned to survive salinity stress through more responsive regulation of K^+ homeostasis and more transcription of key stress responsive genes.

Perhaps one of the reasons for the high potassium content in 'Chawga' genotype is the high expression of these genes in root, which distinguishes 'Chawga' from the other grapevine genotypes.

Considering significantly higher root and shoot K^+ accumulation in 'Chawga', we suggest that studyies on kinetics of ion uptake might be useful to understand the regulation mechanisms of sodium and potassium transport.

The K_m for K^+ uptake of root was nearly similar to K_m calculated for Na^+, so K^+ concentration of root was nearly similar to Na^+ under 0-100 mM NaCl showing that potassium competes with sodium for entering to plant through root system. Based on the expression levels of these two *VvKUP* transporter genes (especially *VvKUP1*) and the accumulation of potassium, it seems these two transporters are involved in uptake of potassium, especially in roots.

Conclusion

In conclusion, salinity increased Na^+ concentrations in all plant organs. Among the genotypes, the rate of Na^+ accumulation in 'Chawga' was lower than others. 'Gharashani' and 'GhezelUzum' showed, respectively, higher and lower ability to inhibit excessive Na^+ and Cl^- transport to shoots.

Salinity resulted in increased expression of *VvKUP1* and *VvKUP2* transporter profiles and *VvK1.1* channel increased significantly ($P < 0.05$) in roots of 'Chawga' and it seems that K^+ combined with salinity caused increases in expression of these genes in root.

Comparative gene expression analysis could be a useful approach for understanding the mechanisms of tolerance and susceptibility (Troggio *et al.* 2008). Therefore, 'Chawga' was selected to study because of high K^+ accumulation even at high salinity and we suggest more physiological and molecular experiments on 'Chawga' genotype.

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