



Salinity Effects on Potassium Accumulation and Transporters Expression in Grape (*Vitis vinifera* L.)

Nayer Mohammadkhani^{1*}, Reza Heidari² and Nasser Abbaspour²

1. Shahid Bakeri High Education Center of Miandoab, Urmia University, Urmia, Iran

2. Department of Biology, Faculty of Science, Urmia University, Urmia, Iran

Abstract

Hydroponically grown ten grape genotypes (*Vitis vinifera* L.) were treated with different concentrations of NaCl. Chawga genotype accumulated K⁺ in its root and shoot even at high salinity. The correlation between Na⁺ and K⁺ concentrations in root and lamina of all genotypes was negative ($P < 0.05$, $r^2 = -0.841$) except for Chawga ($P < 0.01$, $r^2 = 0.998$). K_m calculated for K⁺ and Na⁺ uptake into root and shoot of Chawga showed that K⁺ and Na⁺ compete to enter the plant, especially through roots. Chawga accumulated K⁺ in plant parts in spite of external higher Na⁺ due to increasing salinity. 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. Under salinity stress the expression of *VvKUP1* and *VvKUP2* transporter and *VvK1.1* channel increased significantly ($P < 0.05$) in roots and leaves of Chawga genotype, but that increase was higher in roots than in leaves.

Keywords: salt; grapevine; potassium uptake; transporters expression

Mohammadkhani, N., R. Heidari and N. Abbaspour. 2015. 'Salinity effects on potassium accumulation and transporters expression in grape (*Vitis vinifera* L.)'. *Iranian Journal of Plant Physiology* 5 (4), 1483- 1494.

Introduction

Soil salinity is one of the most serious environmental threats for plant survival and crop yield. It affects 19.5% of irrigated land and 2.1% dry land agriculture across the globe (FAO, 2000).

In grapevine as in other plants, K⁺ is an essential macronutrient and a major osmoticum. Potassium plays key roles in the osmoregulation of cells and has a dominant effect on the membrane

potential of the cell which to a large extent determines the uptake of many different cations, anions, and sugars (Lang, 1983).

Plant genotypes, including grapevines, differ in the uptake, transport, accumulation and requirement of K⁺. The differences between rootstocks could result from differences in K⁺ uptake and transport from root to shoot, plant vigor and/or interactions between ions (Rühl, 1989). The mechanisms/factors responsible for differential K⁺ uptake, transport and accumulation into the shoot (or shoot parts) of grapevine rootstocks are not fully understood. Plant roots play a predominant role in the absorption and

*Corresponding author

E-mail address: n.mohammadkhani@urmia.ac.ir

Received: February, 2015

Accepted: June, 2015

transport of nutrients including K^+ (Kodur *et al.*, 2009).

The capacity of plants to counteract salinity stress strongly depends on the status of their K^+ nutrition. Conversely, although most plants can cope (i.e. show no symptoms of either deficiency or toxicity), the physiological 'window' of optimum K^+ concentrations narrows in the presence of increasing amounts of Na^+ (Marschner, 1995).

Na^+ competition at transport sites for K^+ entry into the symplast may result in K^+ deficiency. Furthermore, cytoplasmic Na^+ competes for K^+ binding sites and hence inhibits metabolic processes that crucially depend on K^+ . Clearly, Na^+ in the cytosol has to be restricted by limiting Na^+ entry and/or operating an efficient system for Na^+ efflux into the vacuole or the apoplast (Ahn *et al.*, 2004). Under salinity stress excessive Na^+ accumulation in plant tissue leads to K^+ leakage from the cell (Shabala *et al.*, 2003).

Information on major molecular determinants of K^+ accumulation in grapes is strongly required. There are multiple mechanisms for potassium transport into plant cells. Potassium channels mediate passive low affinity potassium transport across plant cell membranes, whereas potassium carriers mediate energized high and low affinity uptake (Very and Sentenac, 2003).

Candidate genes for potassium transporters identified in the *Arabidopsis thaliana* genome revealed three major transporter families, the KT/HAK/KUP and TRK/HKT transporters and the CPA cation proton antiporter families (Maser *et al.*, 2001), in addition to K^+ channels.

The KT/HAK/KUP family has been identified in plants by high homology to high-affinity K^+ transporters (HAKs) in fungi (Banelos *et al.* 1995). Individual KT/HAK/KUP genes have been cloned from some of plant species like grapevine (Davies *et al.*, 2006).

The most studied members of the KT/KUP/HAK family belong to the two largest groups, which were named Clusters I and II (Banelos *et al.*, 2002). Current analysis indicates that all plants have transporters homologous to members of Cluster I or Cluster II. In *Arabidopsis*, the Cluster II genes may be involved in plant responses to salinity because their expression is

affected by increased salt concentrations (Maathuis, 2006). Two KT/HAK/KUPs from grape, *VvKUP1* and *VvKUP2*, were highly expressed in berry skin at the beginning of grape development, suggesting that *VvKUPs* may function in potassium accumulation in grape berries (Davies *et al.*, 2006).

VvK1.1 as a grapevine Shaker K^+ channels mainly expressed in the root cortex, which has been shown to be involved in K^+ uptake from the soil (Hirsch *et al.*, 1998).

In this study ten table grape genotypes commonly grown in the region around Urmia salt lake were evaluated from the view point of salt tolerance parameters (Mohammadkhani *et al.*, 2013; 2014). Also the genotypes were compared by determining ion (K^+ and Na^+) accumulation. The genotype with the highest K^+ accumulation capacity was selected for absorption experiments and molecular analysis. The aim of our molecular study was to characterize grape K^+ transporters (KUPs) and *K1.1* channel expression in root and leaf under salinity.

Materials and Methods

Plant materials and growth conditions

Hardwood cuttings of ten genotypes of grapevine (LaaleBidaneh, Gharashani, Sachagh, Shahroodi, LaaleSefid, Khalili, Chawga, Gharagandomeh, Ghazandayi and Shirazi) were obtained from Kahriz vineyard (Agricultural Research Center, grape genotypes collection). The cuttings were disinfected with benomyl (1% w/v) and then basal parts soaked in Indole-3-butyric acid 0.1% (w/v) for 5-10 s. All cuttings were struck in a mist house (relative humidity 80%) with a heat-bed temperature of 20-30 °C. After two weeks, the rooted cuttings were transferred into 2 L pots containing Hoagland solution. The pots were protected with Aluminium foil to avoid light effects and alga proliferation. Plants with 4-5 fully expanded leaves were treated with NaCl (0, 25, 50, 100 mM in screening experiments and 125, 150, 175, 200, 225, 250 mM in absorption kinetics experiments) in Hoagland solution for 2 weeks. NaCl was added to the nutrient solution by incremental increases until the final desired concentrations were reached. Our experimental design was Complete Randomized Block Design (CRBD). We had three replicates per treatment

and 2 pots per replicate. Plants were harvested after 2 weeks and plant parts were weighed separately and dried at 70 °C for 48 hours.

For RT-PCR experiment, 50 mM NaCl (threshold salinity determined for the local genotypes) treated root and leaf tissues were collected at different time periods, frozen in liquid nitrogen immediately and stored at -80 °C until RNA isolation.

Ion Analysis

100 mg of ground tissues of all treatments were weighed into 15 ml plastic centrifuge tubes containing 10 ml deionized water. The tubes were placed in a boiling water bath for approximately 1 hour. Samples were centrifuged at 5000 rpm. The supernatant was transferred into new tubes and the volume made up to 10 ml by addition of deionized water. Sodium and Potassium concentrations were measured by a flame photometer (Fater 405).

RNA Isolation, cDNA synthesis and RT-PCR conditions

Total RNA was extracted from root and leaf tissues by Louime *et al.* (2008) method with small modification. The RNA concentration was determined by Biophotometer (Eppendorf, Germany). The integrity of RNA was checked on an 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, 56-60 °C for 30 s and 72 °C for 20 s and

final extension at 72 °C for 5 min. The *VvTUB* gene (Tubulin) was used as internal reference. 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 50bp plus (50-1500 bp) was used as DNA ladder (Fermentas). Experiments repeated three times. The intensity of the RT-PCR bands was measured using Image J software 1.43 (Table 1).

Statistical analysis

All statistical analyses were done using the Statistical Package for Social Sciences (SPSS) for Windows (Version 14.0). The mean values of three replicates and the "Standard Error" of the means was 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 ($P < 0.05$) were performed. 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

K^+/Na^+ selectivity in screening experiments

Sodium has been significantly ($P < 0.05$) increased in different parts of all the genotypes with increasing salinity. At 50 mM NaCl, the roots of Chawga showed a lower Na^+ concentration than other genotypes (Table 2). Unlike sodium, potassium concentration decreased with increasing salinity treatments in roots and shoots, except in Chawga that showed a significant ($P < 0.05$) K^+ concentration increase under salinity in root and especially in shoot (Table 2).

Table 1
Forward and revers primers used in RT-PCR experiment.

Genes	Forward Primer (5'→3')	Reverse Primer (5'→3')
VvKUP1	TGAGCTTTGAAACATGGGAAGACT	TTCTTGTTACCAAGCCTTCCGG
VvKUP2	ATGCTTCCTGCCATTTCCACATA	GGTTGGCATGGTTTATATCGTCTG
VvK1.1	TTGTTGAAACGTGGTCTGGA	GCCCTGCCCATATCTAGT
VvEF1	TCTGCCTTCTCCTGGGTA	GCACCTCGATCAAAGAGGA

Table 2

Mean values for root and shoot Na⁺ concentrations (mg/g DW) and K⁺ concentrations (mg/g DW) in ten table grapes (*Vitis vinifera* L.) at different salinity levels (0, 25, 50 and 100 mM NaCl). Data are the means \pm standard Deviation (n=3, One Way ANOVA). Different letters within a column indicate significant differences ($P<0.05$) according to Tukey's test.

Genotype & Salinity (mM NaCl)	Na ⁺ Content of Root (mg/g DW)	Na ⁺ Content of Shoot (mg/g DW)	K ⁺ Content of Root (mg/g DW)	K ⁺ Content of Shoot (mg/g DW)
LaaleBidaneh				
0	5.66 \pm 0.31 a	10.47 \pm 0.94 a	86.43 \pm 1.85 c	226.85 \pm 2.58 d
25	89.17 \pm 2.08 b	125.28 \pm 3.18 b	59.68 \pm 1.61 b	210.00 \pm 2.45 c
50	100.01 \pm 1.61 c	189.87 \pm 1.84 c	37.75 \pm 0.93 a	184.32 \pm 3.71 a
100	167.41 \pm 2.01 d	245.78 \pm 7.28 d	38.28 \pm 0.93 a	196.63 \pm 2.45 b
Sachagh				
0	10.07 \pm 0.12 a	15.75 \pm 0.20 a	90.18 \pm 1.60 d	245.84 \pm 3.21 d
25	61.48 \pm 2.78 b	88.24 \pm 0.93 b	60.75 \pm 0.93 c	199.30 \pm 1.61 c
50	81.28 \pm 2.45 c	164.20 \pm 2.78 c	38.82 \pm 1.60 b	158.65 \pm 2.45 b
100	85.56 \pm 1.60 c	200.04 \pm 3.34 d	25.98 \pm 1.61 a	86.43 \pm 2.45 a
Gharashani				
0	4.12 \pm 0.20 a	8.60 \pm 0.12 a	53.27 \pm 1.61 d	139.92 \pm 3.21 d
25	37.95 \pm 0.93 b	85.56 \pm 1.60 b	33.47 \pm 0.93 c	118.53 \pm 3.34 c
50	41.70 \pm 0.93 c	101.54 \pm 3.31 c	28.66 \pm 0.93 b	104.62 \pm 1.61 b
100	58.28 \pm 1.61 d	121.40 \pm 1.85 d	20.10 \pm 0.93 a	93.92 \pm 1.85 a
Shahroodi				
0	5.79 \pm 0.12 a	11.34 \pm 0.20 a	38.29 \pm 0.93 d	135.65 \pm 1.85 c
25	28.32 \pm 0.93 b	77.00 \pm 0.93 b	27.05 \pm 0.93 c	114.25 \pm 1.61 b
50	36.88 \pm 0.93 c	99.47 \pm 1.85 c	23.86 \pm 0.93 b	109.43 \pm 2.78 b
100	62.02 \pm 0.93 d	164.20 \pm 1.60 d	18.49 \pm 0.93 a	85.90 \pm 0.93 a
LaaleSefid				
0	6.99 \pm 0.12 a	14.56 \pm 0.53 a	39.62 \pm 1.20 d	177.05 \pm 0.69 d
25	36.21 \pm 1.20 b	71.73 \pm 3.03 b	31.20 \pm 1.20 c	137.33 \pm 1.84 c
50	41.43 \pm 0.69 c	122.28 \pm 0.69 c	20.37 \pm 1.20 b	119.27 \pm 2.51 b
100	45.84 \pm 1.20 d	175.24 \pm 1.39 d	3.92 \pm 0.69 a	86.37 \pm 1.20 a
Khalili				
0	9.20 \pm 0.12 a	11.15 \pm 0.20 a	57.68 \pm 1.20 d	141.74 \pm 1.20 d
25	49.85 \pm 1.39 b	96.60 \pm 1.20 b	32.40 \pm 1.20 c	120.88 \pm 1.39 c
50	53.47 \pm 0.69 c	126.29 \pm 1.39 c	26.38 \pm 0.00 b	95.60 \pm 2.51 b
100	59.88 \pm 1.39 d	147.56 \pm 3.03 d	15.15 \pm 0.69 a	78.35 \pm 1.84 a
Chawga				
0	7.80 \pm 0.12 a	23.45 \pm 0.12 a	7.80 \pm 0.31 a	19.84 \pm 0.00 a
25	24.97 \pm 1.84 b	73.74 \pm 1.20 b	42.83 \pm 0.69 c	122.48 \pm 1.34 b
50	30.20 \pm 1.20 c	90.59 \pm 2.41 c	48.05 \pm 1.20 d	155.78 \pm 0.84 d
100	40.23 \pm 0.69 d	121.08 \pm 1.39 d	33.61 \pm 1.20 b	140.94 \pm 1.02 c
Gharagandomeh				
0	2.68 \pm 0.12 a	10.66 \pm 0.92 a	70.42 \pm 1.40 d	235.14 \pm 1.40 c
25	32.91 \pm 1.40 b	78.13 \pm 1.62 b	54.51 \pm 0.81 c	202.24 \pm 3.72 b
50	39.93 \pm 1.40 c	129.62 \pm 1.40 c	45.15 \pm 1.40 b	162.12 \pm 2.43 a
100	53.97 \pm 1.40 d	214.34 \pm 1.62 d	36.72 \pm 1.40 a	161.18 \pm 2.92 a
Ghazandayi				
0	9.93 \pm 0.20 a	12.28 \pm 0.00 a	54.87 \pm 2.01 d	153.50 \pm 4.74 c
25	57.41 \pm 1.53 b	92.52 \pm 0.57 b	34.48 \pm 2.52 c	133.10 \pm 4.01 a
50	75.13 \pm 1.74 c	132.30 \pm 1.05 c	25.78 \pm 1.00 b	143.13 \pm 2.65 b
100	87.84 \pm 2.90 d	187.13 \pm 0.55 d	19.10 \pm 1.53 a	129.09 \pm 1.74 a
Shirazi				
0	10.00 \pm 0.64 a	15.82 \pm 0.95 a	57.21 \pm 4.05 b	156.17 \pm 1.00 c
25	38.69 \pm 2.52 b	94.52 \pm 1.53 b	34.81 \pm 2.01 a	135.44 \pm 4.18 ab
50	59.75 \pm 1.53 c	142.00 \pm 5.15 c	32.47 \pm 2.09 a	136.45 \pm 2.52 b
100	61.76 \pm 3.22 c	198.83 \pm 6.45 d	28.12 \pm 2.09 a	126.75 \pm 5.05 a

Based on these preliminary observations, Chawga was selected for further analyses. Chawga is a wild genotype that grows in the region around Urmia salt lake (West Azarbaijan). We did absorption experiments at different salinity levels and molecular experiments at 50 mM NaCl and different time points in Chawga genotype.

Sodium uptake

Sodium has been significantly ($P < 0.05$) increased in different parts (petiole, lamina, shoot and root) of Chawga with increasing salinity in medium (Fig. I). In petiole and lamina, sodium increased until 225 mM NaCl and then decreased at 250 mM NaCl, whereas in roots it increased in all treatments. In all treatments sodium concentration in root was higher than in lamina, but in shoot was higher than in root.

Potassium uptake

Like sodium, and unlike many other grape genotypes, potassium was significantly ($P < 0.05$) increased in different parts (petiole, lamina, shoot and root) of Chawga genotype with increasing salinity (Fig. II). Chawga accumulated higher K^+ in lamina with increasing salinity up to 150 mM NaCl and then it increased gradually. However, in roots K^+ accumulation was highly increased with higher NaCl than 150 mM.

Like sodium, potassium concentration in shoots was higher than that of roots. Interestingly potassium concentration increased even at high salinity in Chawga genotype. Therefore, Chawga transporters can transport potassium as well as sodium effectively at high salinity stress.

Nonlinear regression curves for Na^+ and K^+ uptake rate

Fig. III showed nonlinear regression curves for sodium and potassium uptake rate in Chawga genotype after 14 days treated by different salinity levels (0-150 mM NaCl). In roots of Chawga K^+ accumulated with $K_m = 33.55$ compare to $K_m = 36.78$ for Na^+ , but in shoots K^+ accumulated with $K_m = 27.82$ compare to $K_m = 134.4$ for Na^+ .

Expression profile of K^+ transporter genes

In roots and leaves of Chawga, expression of three K^+ genes (*KUP1*, *KUP2* transporters and *K1.1* channel) increased under salinity (Fig. IV). This increase was higher in roots than in leaves, perhaps because roots were in exposure to salt solutions and there was no opportunity for salt avoidance. Moreover, Na^+ and K^+ accumulation in roots was higher than in lamina at 50 mM NaCl. Expression of all these genes increased with time passing in roots. It means that the gene expression was higher after 14 days than 24 hours in 50mM salinity. In leaves the expression of KUP genes decreased at the beginning and then increased by increasing salinity. The expression of *VvK1.1* in leaves increased by increasing salinity, although it was lower than that of roots (Fig. IV).

Discussion

Salt tolerance in glycophytes is associated with the ability to limit uptake or transport of ions (mainly Na^+ and Cl^-) from the root zone to aerial parts (Greenway and Munns, 1980).

The results showed significant differences between genotypes in accumulation of Na^+ and K^+ into the root and shoot. Similar differences between grapevines rootstocks in accumulation of K^+ have been reported (Rühl, 1989). Under low and moderate saline conditions; the K^+ concentration was reduced in all grape tissues (Greenway and Munns, 1980), as in glycophytes (Troncoso et al., 1999). The decrease in root K^+ resulted may provide a mechanism for ionic balance following uptake of high Na^+ concentrations in roots. Thus, sodium exclusion from the plant combined with the ability to maintain high K^+ concentrations in plant may result in create tolerant vines in salinity conditions (West, 1986).

In our study, it was shown that increased salinity levels caused a considerable reduction of K^+ concentrations in all parts of the genotypes, except Chawga. The general decline in potassium concentrations with increasing salinity can be due to replacement by sodium ion which increased with increasing sodium in nutrient solutions (Stevens et al., 1996). At high external salinity the accumulation of Na^+ in petiole of Chawga may indicate the existence of an inhibition of Na^+ entry to lamina. The preferential accumulation of Na^+ in the root system previously has been reported by

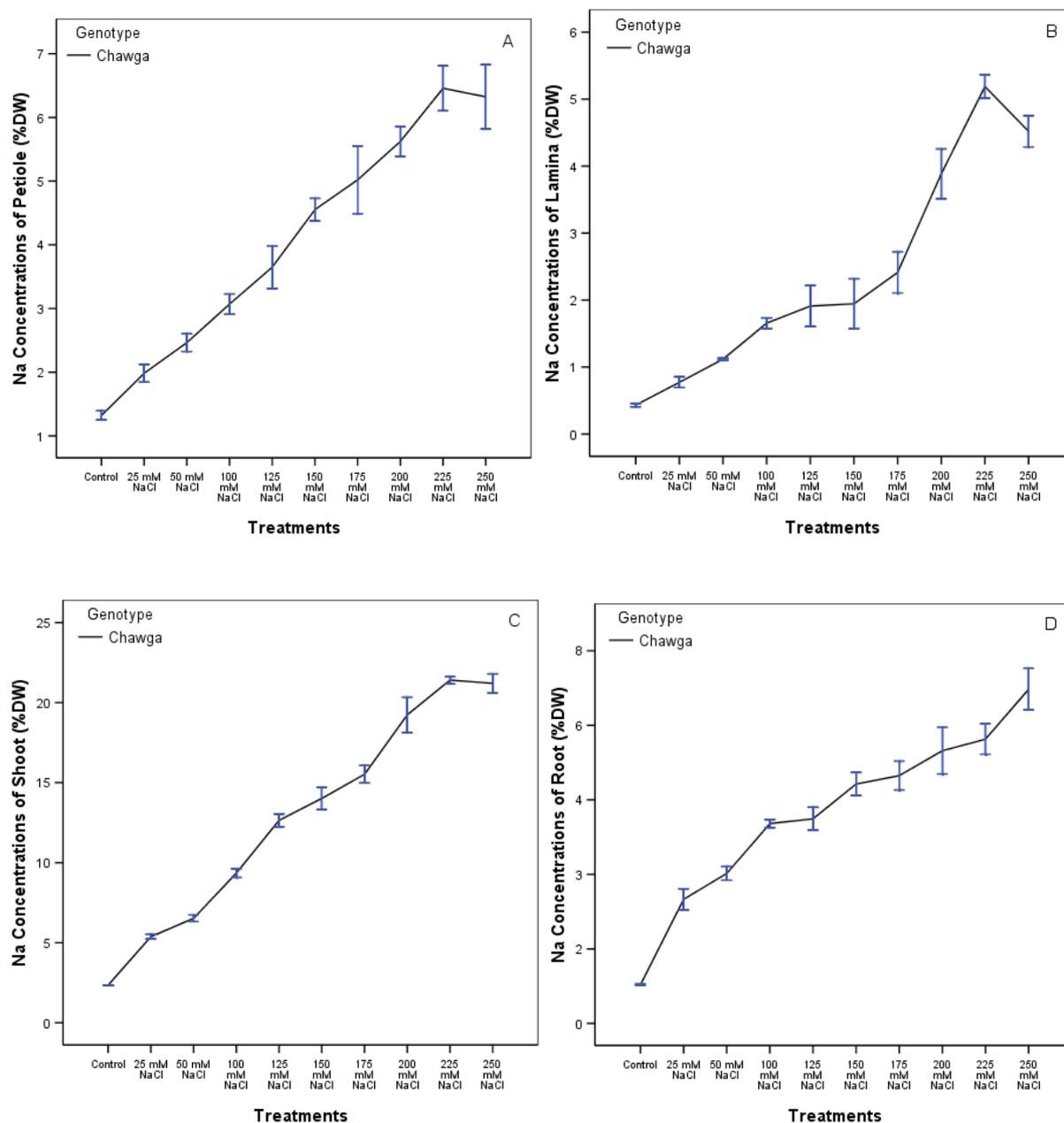


Fig. 1. Na⁺ concentrations (%DW) in petioles (A), lamina (B), shoots (C) and roots (D) of Chawga genotype (*Vitis vinifera* L.) at nine different salinity levels (0, 25, 50, 100, 125, 150, 200, 225 and 250 mM NaCl) after 14 days treatment. Bars are the means ± SE (n=3, One Way ANOVA, Tukey's test).

Downton (1977) in grapevine. The differences in behavior of plant species towards Na⁺ accumulation within their various organs are related to their resistance to salinity (Downton, 1977). In all genotypes studied here, sodium concentration increased with increasing salinity,

but Chawga accumulated low sodium concentration in shoot and root when compared to others.

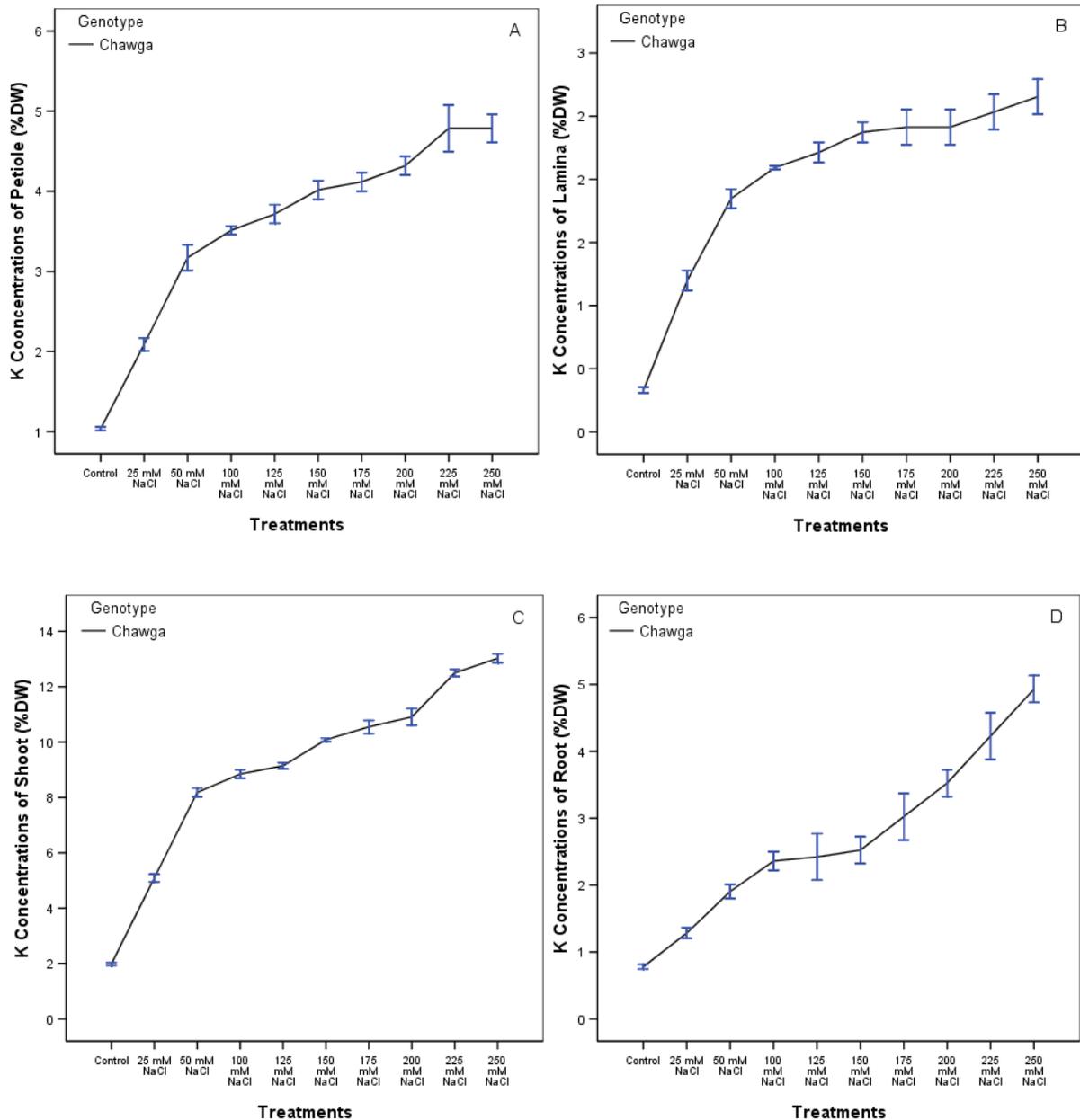


Fig. II. K⁺ concentrations (%DW) in petioles (A), lamina (B), shoots (C) and roots (D) of Chawga genotype (*Vitis vinifera* L.) at nine different salinity levels (0, 25, 50, 100, 125, 150, 200, 225 and 250 mM NaCl) after 14 days treatment. Bars are the means ± SE (n=3, One Way ANOVA, Tukey's test).

The correlation between Na⁺ concentration with K⁺ in roots and lamina was negative ($P < 0.05$, $r^2 = -0.841$) in all genotypes, whereas Chawga showed significant positive correlation ($P < 0.01$, $r^2 = 0.998$) between Na⁺ concentrations and K⁺. Chawga had higher K⁺ and

lower Na⁺ accumulations in lamina than that of other genotypes. It showed that Chawga is able to transport K⁺ as well as Na⁺ to shoot when faced to high salinity.

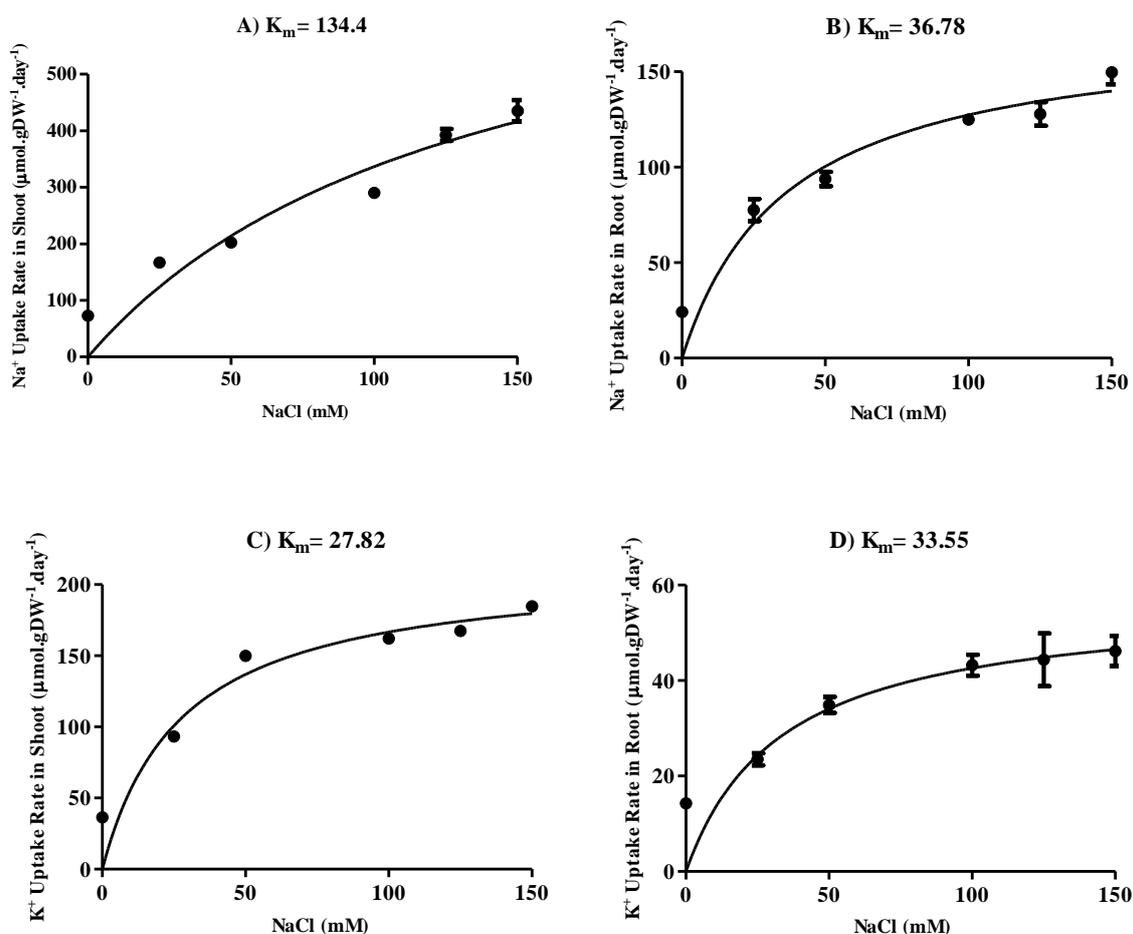


Fig. III. nonlinear regression curve fit (Michaelis-Menten) for Na⁺ uptake rate ($\mu\text{mol.gDW}^{-1}.\text{day}^{-1}$) in shoot (A) and root (B) and K⁺ uptake rate ($\mu\text{mol.gDW}^{-1}.\text{day}^{-1}$) in shoot (C) and root (D) of Chawga genotype at different salinity levels (0- 150 mM NaCl). Bars are the means \pm SE (n=3, Graphpad Prism 5).

Establishment of ion homeostasis is an essential requirement for plants to survive under salt stress conditions. Salt tolerance requires not only adaptation to Na⁺ toxicity but also the acquisition of K⁺. K⁺ transport systems involving good selectivity of K⁺ over Na⁺ is considered as an important salt tolerant determinant (Amini and Ehsanpour, 2005). Maintenance of high K⁺ concentrations in salt-tolerant genotypes may be one of the mechanisms underlying their superior salt tolerance (Tester and Davenport, 2003).

In our absorption experiments in lamina of Chawga Na⁺ was gradually accumulated until 150 mM NaCl and then highly increased, but in roots accumulation was gradual in all treatments. It means that defense demand in Chawga (roots,

stems and petioles) can prevent Na⁺ transport to lamina until 150 mM NaCl, but in severe salinity lamina accumulated high sodium concentration. On the other hand in petiole and lamina, sodium increased until 225 mM NaCl and then decreased at 250 mM NaCl, whereas in roots it increased in all treatments. It seems that roots of Chawga can control sodium transport to shoot in salinity higher than 225 mM NaCl. In all treatments sodium concentration in root was higher than in lamina, but in shoot was higher than in root. These observations indicate that Chawga is sensitive and accumulates sodium in shoots, but petioles and stems can control Na⁺ transport to lamina.

Unlike sodium, in lamina of Chawga genotype potassium was highly accumulated until 150 mM NaCl and then accumulation was low. In

roots of Chawga K^+ accumulation was inverse. In all treatments K^+ accumulation in roots was higher than in lamina and in shoots was higher than in roots. Sodium and potassium use the same channels and transporters for influx to the plant. In lamina of Chawga when sodium transport was low (0-150 mM NaCl), potassium transport was high and inverse.

present data revealed that Chawga can be an exceptional genotype with a high K^+ uptake rate in spite of higher Na^+ concentration under salinity. Cuin *et al.* (2008) reported similar results in some wheat lines.

Sodium interference under conditions of salt stress affects K^+ acquisition and the activity of potassium-dependent enzymes, including

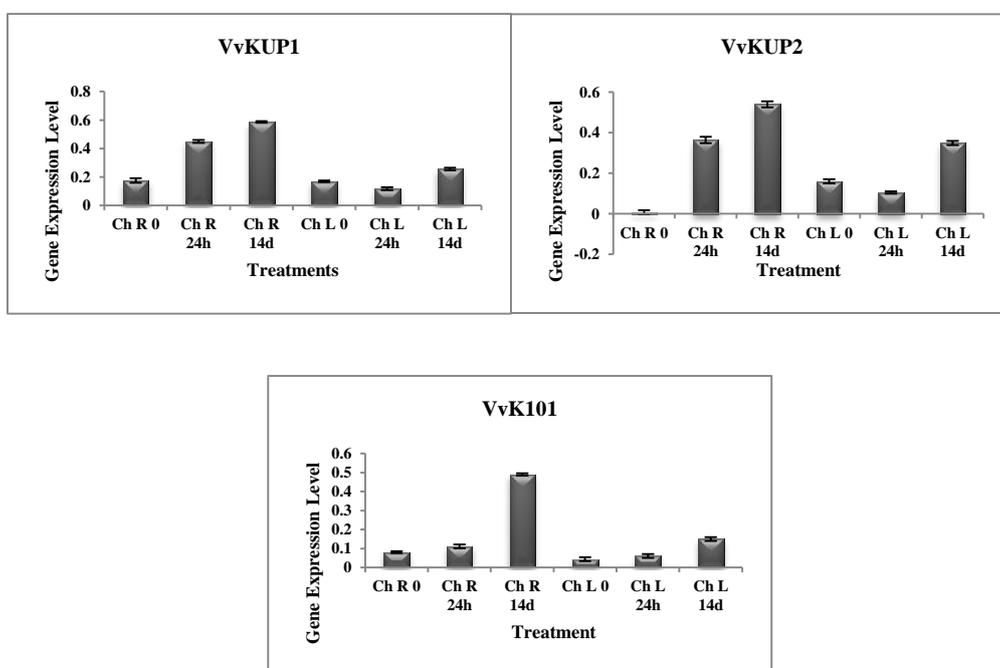
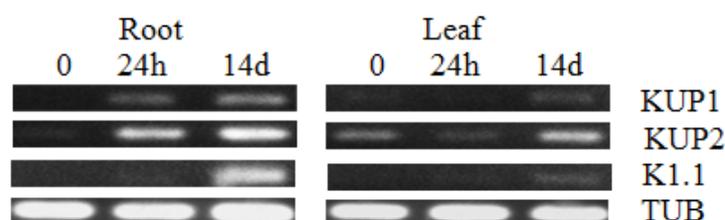


Fig. IV. Expression profile of K^+ transporter genes in root and leaf of Chawga (*Vitis vinifera* L.) after 0, 24 hours and 14 days treated by 50 mM NaCl. Graphs show relative expression of K^+ transporter genes in root (R) and leaf (L) of Chawga (*Vitis vinifera* L.). Bars are the means \pm Standard Deviation (n=3, One Way ANOVA, Tukey's test).

The results showed that K^+ was accumulated in root with a rate nearly similar to Na^+ . Also, it seems that K^+ has been easily released to long distance transport ion stream and rapidly accumulated and transported from root to shoot when compared to Na^+ .

Potassium accumulation continued with increasing salinity unlike other studies (Greenway and Munns, 1980; Troncoso *et al.*, 1999). The

transporters (Banuelos *et al.*, 1995). Some KT/KUP/HAK transporters have been found to play important roles in plant development. The various functions of plant KT/KUP/HAK transporter genes range from mineral nutrition to the regulation of cell growth and development. In growing grapevine fruits (*Vitis vinifera* L.) expression of *VvKUP1* and *VvKUP2* potassium transporter genes is strongly dependent on developmental stage. It

is likely that these transporters are required for the potassium-driven cell expansion in young grape berries (Davies *et al.*, 2006). Both *VvKUP1* and *VvKUP2* were most highly expressed in reproductive tissues (berries, flowers, and seeds) in Shiraz variety (Davies *et al.*, 2006).

To better understand how potassium is accumulated in grape, two potassium transporter genes from the KUP/KT/HAK family -*VvKUP1* and *VvKUP2*- were studied from *Vitis vinifera* (Chawga genotype) leaves and roots. Our results showed that under salinity stress the expression of *VvKUP1* and *VvKUP2* increased in roots and leaves of Chawga, but leaves were not affected as much as roots. The expression of *VvKUP1* increased 3 fold in roots after 14 days exposure to 50 mM NaCl, but *VvKUP2* increased seven fold. In leaves the increase in expression of *KUP2* was higher than (2.1 fold) *KUP1* (1.56 fold). Based on the expression levels of these two *VvKUP* transporter genes (especially *KUP2*) and the accumulation of potassium, it seems that these two transporters are involved in uptake of potassium, especially in roots. Other authors reported that *KUP1* expression is dependent on the membrane potential (Fu and Luan, 1998) and the expression of *KUP2* transporter could be important for developmental and physiological responses to salt stress (Elumalai *et al.*, 2002). These studies supported our results about the increase in KUP transporters expression under salinity.

Many authors reported different results of salinity effects on *VvK1.1* channel in plants. *VvK1.1* can be considered as the grapevine orthologue of *Arabidopsis* *AKT1* (71% sequence identity) (Cue'llar *et al.*, 2010). In *Arabidopsis*, *AKT1* is mainly expressed in roots, where it gives rise to inward K^+ channel activity involved in K^+ uptake from the soil (Hirsch *et al.*, 1998). According to Cue'llar *et al.* report (2010) checking the sensitivity of *VvK1.1* expression to grapevine watering with 50 mM NaCl or KCl revealed no significant change in transcript accumulation, either in roots or in leaves. *VvK1.1* transcript accumulation is strongly sensitive to drought stress, with the leaves and berries displaying an increase in transcript accumulation, whereas the roots display a decrease.

A second set of our experiments aimed to investigate the effects of salt stress on *VvK1.1*

transcript accumulation in roots and leaves. Our results were not confirmed Cue'llar *et al.* report (2010) about any change in expression of *VvK1.1* under salinity. In our study, salt stress was found to have the same effects on *VvK1.1* transcript levels in roots and leaves: transcript accumulation was highly increased in roots; the expression of *VvK1.1* increased six fold in roots after 14 days treated by salinity. In leaves this transcript increased three fold after 14 days under salinity. We can conclude roots were highly affected by salinity.

In conclusion, salinity resulted in increased Na^+ concentrations in all organs. Accumulation of high Na^+ concentration particularly in shoots indicated that all ten genotypes studied here are poor Na^+ excluders, perhaps due to adaptation caused by ecological conditions. Among the genotypes, the rate of Na^+ accumulation in Chawga was lower than others. Considering significantly higher root and shoot K^+ accumulation in Chawga, we thought that studying kinetics of ion uptake may be useful to understand the regulation mechanisms of sodium and potassium transport. In our absorption experiments in lamina of Chawga when sodium transport was low (0-150 mM NaCl), potassium transport was high and inverse. K_m for K^+ uptake to root in the range of 0-150 mM NaCl was nearly similar to K_m calculated for Na^+ , so K^+ concentration of root was near to Na^+ . It showed that potassium competes with sodium for entering to plant through root system. Under salinity stress the expression of *VvKUP1* and *VvKUP2* transporter profiles and *VvK1.1* channel increased significantly ($P < 0.05$) in roots and leaves of Chawga, but that decrease in roots was higher than in leaves. Chawga was interest our because of high K^+ accumulation even in high salinity and we suggest more physiological and molecular experiments on Chawga genotype.

References

- Ahn, S.J., R. Shin and D. P. Schachtman. 2004. 'Expression of KT/KUP genes in Arabidopsis and the role of root hairs in K^+ uptake'. *Plant Physiology*, 134: 1135–1145.
- Amini, F. and A. A. Ehsanpour. 2005. 'Soluble proteins, proline, carbohydrates and Na^+/K^+

changes in two tomato (*Lycopersicon esculentum* Mill.) cultivars under *in vitro* salt stress'. *American Journal of Biochemistry and Biotechnology*, 1: 212-216.

- Banuelos, M.A., B. Garcideblas, B. Cubero and A. Rodriguez- Navarro.** 2002. 'Inventory and functional characterization of the HAK potassium transporters of rice'. *Plant Physiology*, 130: 784–795.
- Banuelos, M.A., R. D. Klein, S. J. Alexander-Bowman and A. Rodriguez- Navarro.** 1995. 'A potassium transporter of the yeast *Schwanniomyces occidentalis* homologous to the Kup system of *Escherichia coli* has a high concentrative capacity'. *EMBO Journal*, 14: 3021–3027.
- Cuellar, T., F. Pascaud, J. L. Verdeil, L. Torregrosa, A. F. Adam-Blondon, J. B. Thibaud, H. Sentence and I. Gaillard.** 2010. 'A grapevine Shaker inward K⁺ channel activated by the calcineurin B-like calcium sensor 1–protein kinase CIPK23 network is expressed in grape berries under drought stress conditions'. *Plant Journal*, 61: 58–69.
- Cuin, T. A., S. A. Betts, R. Chalmandrier and S. Shabala.** 2008. 'A roots ability to retain K⁺ correlates with salt tolerance in wheat'. *Journal of Experimental Botany*, 59: 2697–2706.
- Davies, C., R. Shin, W. Liu, M. R. Thomas and D. P. Schachtman.** 2006. 'Transporters expressed during grape berry (*Vitis vinifera* L.) development are associated with an increase in berry size and berry potassium accumulation'. *Journal of Experimental Botany*, 57: 3209–3216.
- Downton, W.J.S.** 1977. 'Chloride accumulation in different species of grapevines'. *Scientia Horticulturea*, 7: 249-253.
- Elumalai, R. P., P. Nagpal and J. W. Reed.** 2002. 'A mutation in the Arabidopsis KT2/KUP2 potassium transporter gene affects shoots cell expansion'. *Plant Cell*, 14: 119–131.
- FAO,** 2000. 'Production year book'. Food and Agriculture Organization of the United Nations, Rome.
- Fu, H.H. and S. Luan.** 1998. 'AtKuP1: a dual-affinity K⁺ transporter from Arabidopsis'. *Plant Cell*, 10: 63–73.
- Greenway, H. and R. Munns.** 1980. 'Mechanisms of salt tolerance in non-halophytes'. *Annals Review of Plant Physiology*, 31: 149–190.
- Hirsch, R.E., B. D. Lewis, E. P. Spalding and M. R. Sussman.** 1998. 'A role for the AKT1 potassium channel in plant nutrition'. *Science*, 280: 918–921.
- Kodur, S., J. M. Tisdall, C. Tang and R.R. Walker.** 2010. 'Accumulation of potassium in grapevine rootstocks (*Vitis*) as affected by dry matter partitioning, root traits and transpiration'. *Australian Journal of Grape Wine and Research*, 16: 273–282.
- Lang, A.** 1983. 'Turgor-related translocation'. *Plant, Cell and Environment*, 6: 683–689.
- Louime, C., H. Vasanthaiah, Y. Jittayasothorn, J. Lu, S. M. Basha, P. Thipyapong and N. Boonkerd.** 2008. 'A simple and efficient protocol for high quality RNA extraction and cloning of chalcone synthase partial cds from muscadine grape cultivars (*Vitis Rotundifolia* Michx.)'. *European Journal of Scientific Research*, 22: 232-240.
- Maathuis, F.J.M.** 2006. 'The role of monovalent cation transporters in plant responses to salinity'. *Journal of Experimental Botany*, 57: 1137–1147.
- Marschner, H.** 1995. 'Mineral nutrition in higher plants'. Academic Press, London.
- Maser, P., S. Thomine, J. I. Schroeder, J.M. Ward, K. Hirschi and H. SZE.** 2001. 'Phylogenetic relationships within cation transporter families of Arabidopsis'. *Plant Physiology*, 126: 1646–1667.
- Mohammadkhani N, R. Heidari, N. Abbaspour and F. Rahmani.** 2014. 'Evaluation of salinity effects on ionic balance and compatible solute contents in nine Grape (*Vitis* L.) genotypes'. *J Plant Nutr.*37: 1817-1836.
- Mohammadkhani N, R. Heidari, N. Abbaspour and F. Rahmani .**2013. 'Comparative study of salinity effects on ionic balance and compatible solutes in nine iranian table grape (*Vitis vinifera* L.) genotypes'. *J Int Sci Vigne Vin* 47 (2): 99–114.
- Ruhl, E.H.** 1989. 'Uptake and distribution of potassium by grapevine rootstocks and its implication for grape juice pH of scion varieties'. *Australian Journal of Experimental Agriculture*, 29: 707–712.

- Shabala, S.N., L. Shabala and E. Van Volkenburgh.** 2003. 'Effect of calcium on root development and root ion fluxes in salinized barley seedlings'. *Functional Plant Biology*, 30: 507–514.
- Stenens, R. M., G. Harvey and G. Davies.** 1996. 'Separating the effects of foliar and root salt uptake on growth and mineral composition of four grapevine cultivars on their own roots and Ramsey rootstocks'. *Journal of the American Society for Horticultural Science*, 121: 569–575.
- Tester, M. and R. Davenport.** 2003. 'Na⁺ tolerance and Na⁺ transport in higher plants'. *Annals of Botany*, 91: 503–527.
- Troncoso, A., C. Matte, M. Cantos and S. Lavee** 1999. 'Evaluation of salt tolerance in vitro-grown grapevine rootstock varieties'. *Vitis*, 38: 55-60.
- Very, A.A. and H. Sentenc.** 2003. 'Molecular mechanisms and regulation of K⁺ transport in higher plants'. *Annals Review of Plant Biology*, 54: 575–603.
- West, D.W.** 1986. 'Stress physiology in trees-salinity'. *Acta Horticulturae*, 175: 321-332.