COMPARATIVE STUDY OF SALINITY EFFECTS ON IONIC BALANCE AND COMPATIBLE SOLUTES IN NINE IRANIAN TABLE GRAPE (*VITIS VINIFERA* L.) GENOTYPES

Nayer MOHAMMADKHANI*, Reza HEIDARI, Nasser ABBASPOUR and Fatemeh RAHMANI

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

Abstract

Aim: To study the salinity tolerance of own rooted grape cuttings taken from nine table grape (*Vitis vinifera* L.) genotypes growing in the Urmia Salt Lake region (Iran): LaaleBidaneh, Gharashani, Sachagh, Shahroodi, LaaleSefid, Khalili, Chawga, GhezelUzum and Gharagandomeh.

Methods and results: Plants were treated with nutrient solutions containing 0, 25, 50 and 100 mM NaCl for two weeks. Shoot and root growth and dry weights (DW) were significantly (P<0.05) reduced in all treatments. Chloride (Cl^-) and sodium (Na^+) ions highly accumulated in the various parts of the vines with increasing external NaCl concentration. Na^+ accumulation exceeded that of Cl^- in all treatments and all genotypes. However, Gharashani showed a higher ability to restrict Cl^- and Na^+ accumulation in lamina (1.08% of DW and 2.56% of DW, respectively) in comparison to the other genotypes, particularly to GhezelUzum (4.1% of DW and 9.10% of DW, respectively), which showed intensive leaf burn symptoms. Lamina soluble sugar, proline and glycine betaine contents increased with increasing salinity. Correlation analysis (P<0.01) indicated that there was a high positive correlation between Cl^- and Na^+ contents and osmolyte accumulation in lamina.

Conclusion: Taken together, it seems that Gharashani and GhezelUzum, respectively, had a higher and a lower capacity to alleviate salt stress symptoms when compared to the other genotypes.

Significance and impact of the study: Salinity is a serious environmental stress in the regions around Urmia Salt Lake and grapevine is one of the most economically important fruit crops in Urmia. Therefore, screening the native grape genotypes for salt tolerance and using the tolerant genotypes directly as rootstocks or producing hybrids with desirable traits could be one of the improvement programs for viticulture in this area.

Key words: salinity, grapevine, chloride, tolerance, genotype

Résumé

But: Étudier la tolérance à la salinité de boutures racinées provenant de neuf génotypes de raisins de table de la région du lac salé de l'Ourmia (en Iran): LaléBidané, GaréChani, Sajag, Chahroudi, Lalésephid, Khalili, Javka, Gezeluzum et Garégandomé.

Matériels et Méthodes: Les plants ont été traités avec des solutions nutritives contenant 25, 50 et 100 mM NaCl pendant deux semaines. La croissance des sarments et des racines et le poids sec (DV) ont diminué significativement (P<0.05) dans tous les traitements. Le chlorite (Cl^-) et le sodium (Na^+) ions se sont accumulés dans les différentes parties des plantes en augmentant la concentration externe en NaCl. L'accumulation de Na^+ a dépassé celle de Cl^- dans tous les traitements et pour tous les génotypes. Garéchani a montré une plus forte capacité à limiter l'accumulation de Cl^- et de Na^+ (1.08% et 2.56 % de poids sec, respectivement), par comparaison aux autres génotypes, particulièrement Gezeluzum (4.1 % et 9.10 % de poids sec, respectivement), qui a présenté des symptômes importants de brûlure de la feuille. Les sucres solubles, la proline et la glycine betaïne de la feuille ont augmenté selon la salinité. L'analyse de corrélation (P<0.01) indique qu'il y a une corrélation positive élevée entre les teneurs en Cl^- et Na^+ et l'accumulation des osmolytes.

Conclusion: Il semble que Garéchani et Gezeluzum ont respectivement la plus grande et la plus faible capacité pour réduire les symptômes du stress salin par comparaison aux autres génotypes.

Signification et impact de l'étude: La salinité est un stress environnemental très important dans la région du lac salé de l'Ourmia. La vigne est une culture indispensable pour l'économie de cette région. Par conséquent, la sélection de génotypes des raisins indigènes présentant une tolérance au sel et l'utilisation de ces génotypes en tant que porte-greffe ou pour produire des hybrides avec des caractères définis pourraient être l'un des programmes d'amélioration de la viticulture dans cette zone.

Mots clés: salinité, raisin, chlorite, tolérance, génotype

manuscript received 28th January 2012 - revised manuscript received 6th February 2013

*Corresponding author: n.mohammadkhani@urmia.ac.ir

J. Int. Sci. Vigne Vin, 2013, **47**, n°2, 99-114

©Vigne et Vin Publications Internationales (Bordeaux, France)

INTRODUCTION

Over 800 million hectares of land worldwide are affected by salt, causing significant losses in agricultural production. Indeed, it is estimated that at least 20% of the world's arable lands are affected to varying degrees by salinity (Rhoades and Loveday, 1990).

Salinity causes two stresses in plants: (1) a water-deficit stress resulting from high solute concentrations in the soil and (2) ion-specific stress resulting from altered K^+/Na^+ and NO_3^-/Cl^- ratios and Na^+ and Cl^- ion concentrations. The modification of ion ratios in the plant is due to the influx of sodium and chloride through pathways involved in the acquisition of potassium and nitrate, respectively (Blumwald *et al.*, 2000).

Cl^- is an essential plant micronutrient, functioning as an osmotically active solute in plant vacuoles and involved in both turgor and osmoregulation (White and Broadley, 2001). But Cl^- can be toxic to plants at high concentrations, with critical concentrations estimated at 4-7 mg/g of dry weight (DW) for Cl^-sensitive species and 15-50 mg/g of DW for Cl^-tolerant species (Xu *et al.*, 2000). In fact, Na^+ and Cl^- are both toxic to plants at high concentrations, but some species can control Na^+ transport better than Cl^- and vice versa (Munns and Tester, 2008).

"Why focus on Na^+, why not consider Cl^-?" This question relates particularly to species that accumulate high concentrations of Cl^- and not Na^+ in leaves and to species that are routinely grown on Cl^-excluding rootstocks such as grapevines and citrus. For these species, Cl^- toxicity is more important than Na^+ toxicity. However, this does not imply that Cl^- is more toxic than Na^+, but rather that these species are better Na^+- than Cl^-excluders. Indeed, Na^+ may be a more toxic solute, but because Na^+ is transported more efficiently than Cl^-, Cl^- becomes a more toxic component (Munns and Tester, 2008).

Vitis vinifera grapevines are classified as moderately sensitive to salinity (Maas and Hoffman, 1977). Salinity damage is caused by chloride ions (Walker, 1994), as grapevines have only a modest capacity to exclude Cl^- (Christensen *et al.*, 1978).

Grapevine responses to salinity include physiological and systemic disturbances leading to reductions in both growth and yield (Walker *et al.*, 2002). Ion accumulation in leaves decreases growth (Fisarakis *et al.*, 2001). The reduction in growth in response to salinity is usually attributed to ion toxicity and low external osmotic potential (Munns and Termaat, 1986), both of which may affect plant physiological and biochemical processes (Greenway and Munns, 1980). The symptoms of severe salt stress include necrotic areas on leaves: starting at the leaf margins and progressing inwards. Such visible symptoms are often referred to as 'leaf burn' (Walker *et al.*, 2004).

Salt tolerance is related to the ability of genotypes to regulate both Cl^- and Na^+ transport in order to avoid toxicity. Some genotypes are more efficient at regulating Na^+ or Cl^- transport (or both) than others, due to different mechanisms of ion balance (Teakle and Tyerman, 2010). There is general agreement that high Cl^- accumulation by certain genotypes can cause growth reduction (Alexander and Groot, 1971). This suggests that Cl^- content of grapevine is definitely an important factor for vine health under saline conditions. The role played by NO_3^- and its relationship to Cl^- in salinity tolerance should also be considered because NO_3^- is the most common univalent anion in plants under non-saline conditions (Frachisse *et al.*, 1999) and because Cl^- uses NO_3^- channels for entry into plants (Roberts, 2006).

Potassium plays an important role in balancing membrane potential and turgor, activating enzymes, and regulating osmotic pressure, stomatal movement and tropisms (Cherel, 2004). Potassium leakage normally happens as a result of NaCl-induced membrane depolarization under saline conditions (Shabala *et al.*, 2003).

Given that intracellular K^+/Na^+ homeostasis is essential for cell metabolism and is considered a key component of salinity tolerance in plants (Chen *et al.*, 2007), the accumulation of excessive amounts of Na^+ in the cytosol should be prevented, along with retention of physiological concentrations of cytosolic K^+.

Plants have many adaptive strategies in response to abiotic environmental stresses. These adaptive mechanisms include changes in physiological and biochemical processes. Adaptation to all these stresses is associated with metabolic adjustments that lead to the accumulation of several organic solutes like soluble sugars, betaines and proline (Yancey *et al.*, 1982).

Glycine betaine and proline are key osmolytes contributing to osmotic adjustment (Iqbal *et al.*, 2008). Proline accumulation is a widespread plant

©Vigne et Vin Publications Internationales (Bordeaux, France)

response to environmental stresses and has a clear role as an osmoticum and a compatible solute (Yancey *et al.*, 1982). In addition to its osmoregulation role, proline can also serve as a sink for energy to regulate redox potentials, as a hydroxyl radical scavenger, and as a storage compound and nitrogen source for rapid growth after stress (Sairam *et al.*, 2002). It has been demonstrated that under salt stress, glycine betaine prevent damage of PSII complex in light (Allakhverdiev *et al.*, 1996; Sakamoto and Murata, 2002) and enhance the activation of Rubisco (Nomura *et al.*, 1998).

Accumulation of sugars in different parts of plants is enhanced in response to the variety of environmental stresses (Prado *et al.*, 2000). Sugars are involved in the synthesis of other compounds, the production of energy and the stabilization of membranes (Hoekstra *et al.*, 2001).

In grapevines, decreased growth rates and the accumulation of toxic concentrations of salt in leaves have led to the conclusion that rootstocks can help to alleviate the inhibitory effects of Cl^- by slowing the rate of Cl^- accumulation (Bernstein *et al.*, 1969). Chloride exclusion has subsequently been seen as an important rootstock character and is commonly used as a screening test for salt tolerance in rootstock improvement programs for grapevines (Sykes, 1985). Screening techniques to determine salt-tolerant grapevine genotypes should therefore take into account both the innate vigor and salt (Cl^- and Na^+) exclusion ability of the rootstock (Walker *et al.*, 2002).

In this study, nine table grape genotypes commonly grown in the region around Urmia Salt Lake (West Azerbaijan, Iran) were evaluated for salt tolerance. To that end, the genotypes were compared for ion accumulation, compatible solute content and growth parameters under various saline conditions. The correlation between all parameters was also statistically analyzed.

MATERIALS AND METHODS

1- Plant materials and growth conditions

Hardwood cuttings of nine genotypes of grapevine (LaaleBidaneh, Gharashani, Sachagh, Shahroodi, LaaleSefid, Khalili, Chawga, GhezelUzum and Gharagandomeh) were obtained from Kahriz vineyard (Agricultural Research Center, Urmia, Iran). The cuttings were disinfected with benomyl (1.5% w/v) and then the basal parts were soaked in indole-3-butyric acid (IBA) 0.1% (w/v) for 5-10 s. All cuttings were placed in a mist chamber (relative humidity 80%) at 25-35ºC. After two weeks, the rooted cuttings were planted in pots filled with perlite and irrigated with 1/8 strength Hoagland's solution containing 0.125 mM KNO_3, 0.125 mM $Ca(NO_3)_2$, 0.05 mM $MgSO_4.7H_2O$, 0.0125 mM KH_2PO_4, 5.75 µM H_3BO_3, 1.34 µM $MnCl_2.4H_2O$, 0.1 µM $ZnSO_4.7H_2O$, 0.038 µM $CuSO_4.5H_2O$, 0.025 µM $Na_2MoO_4.2H_2O$ and 8.88 µM Fe-EDTA. Cuttings with opened leaf bud were transferred to 2-l aerated pots containing first 1/4, then half and finally full strength Hoagland's solution, each for two weeks. Solutions were changed every two days and the nutrient concentrations and pH of the solutions were monitored daily (maintained within the pH range of 6.0-6.5). The pots were protected with aluminum foil to avoid light penetration and algal proliferation. Plants with 2-3 fully expanded leaves were treated with NaCl (0, 25, 50 and 100 mM) in full strength Hoagland's solution for two weeks. NaCl was added to the nutrient solution at the desired concentration or by incremental increases until the final desired concentrations were reached. The experiment was designed as a Randomized Complete Block with three replicates per treatment and two pots per replicate. At the beginning and the end of the salt stress period (two weeks), the root and shoot lengths were determined. Then, the plants were harvested and plant parts including leaves, stems, petioles and roots were weighed separately and dried at 70ºC for 48 h. Dried plants were ground to increase sample homogeneity.

2. Ion analysis

Ground samples (100 mg) of lamina, petiole, stem and root from each of the nine genotypes in each treatment were weighed and transferred to 15-ml plastic centrifuge tubes containing 10 ml of deionized water. The tubes were placed in a boiling water bath for approximately 1 h and then centrifuged at 5000 rpm for 20 min. The supernatants were collected and transferred into new tubes, and the volume was adjusted to 10 ml with deionized water. 0.5- ml aliquots were analyzed for chloride concentration using a Chloride Analyzer (model 926, Corning). Sodium and potassium concentrations were measured by using a flame photometer (Fater 405). Nitrate concentration was measured by nitration of salicylic acid (Cataldo *et al.*, 1975). Briefly, 0.5-ml aliquots were mixed with 0.8 ml of 5% (w/v) salicylic acid in concentrated H_2SO_4. After 20 min at room temperature, 19 ml of 2N NaOH were added slowly to raise the pH above 12. The

©Vigne et Vin Publications Internationales (Bordeaux, France)

samples were cooled to room temperature and absorbance at 410 nm was determined by using a spectrophotometer (UV-visible, WPA S2100).

3. Compatible solute content

Soluble sugars were determined based on the phenol-sulfuric acid method (Dubois *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.

Proline was determined following 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 content was determined using the method of Grieve and Grattan (1983). Briefly, ground material of lamina (0.5 g) was mechanically shaken with 20 ml of deionized water for 24 h at 25ºC. The samples were then filtered using Filter Paper S&S 604 and the filtrates were diluted (1:1) with 2N H_2SO_4. Aliquots (0.5 ml) were placed in centrifuge tubes and cooled in ice for 1 h. Cold (0.2 ml) KI-I_2 reagent (20% KI–15.7% I_2) was added and the reactants were stirred. The tubes were stored at 4ºC for 16 h and then centrifuged at 10,000 rpm for 15 min at 0ºC. The supernatant was carefully aspirated with a fine tipped glass pipette and discarded. The periodide crystals were dissolved in 6.0 ml of 1,2-dichloroethane and mixed vigorously. After 2 h, the

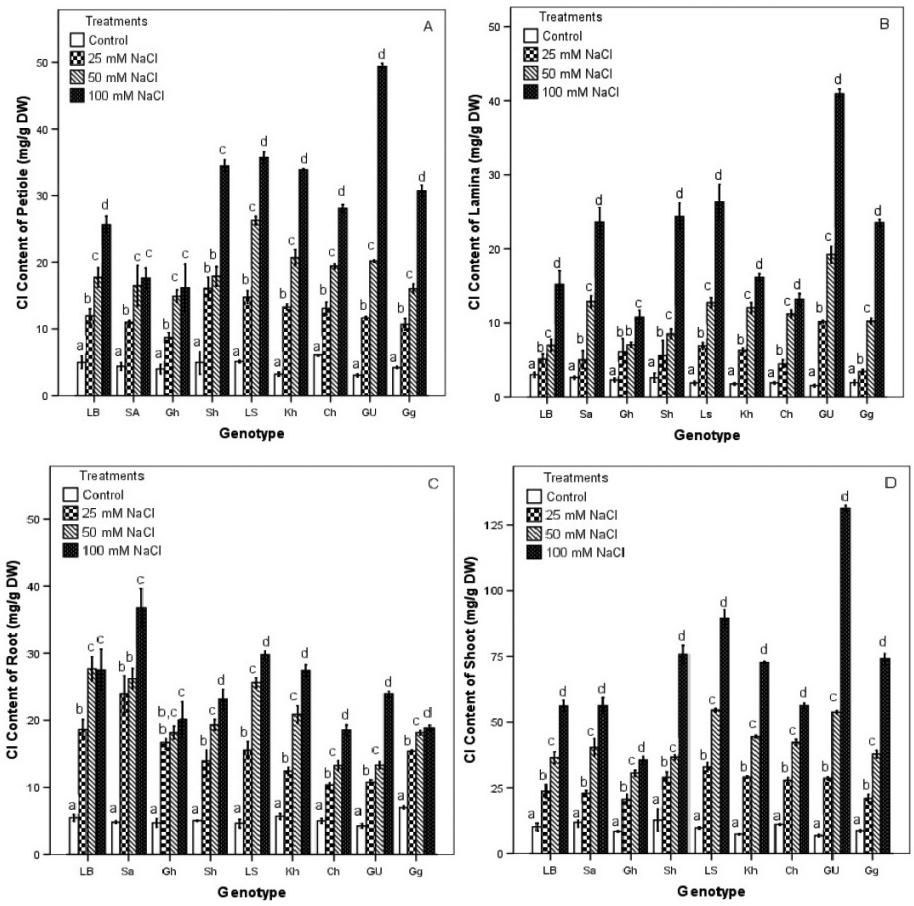


Figure 1 - Cl$^-$ concentrations (mg/g DW) in petioles (A), laminas (B), roots (C) and shoots (D) of nine table grape (*Vitis vinifera L.*) genotypes (LB: LaaleBidaneh, Sa: Sachagh, Gh: Gharashani, Sh: Shahroodi, LS: LaaleSefid, Kh: Khalili, Ch: Chawga, GU: GhezelUzum, Gg: Gharagandomeh) at three different salinity levels (25, 50 and 100 mM NaCl). Bars are the means ± standard error (n=3, one-way ANOVA). Different letters indicate significant differences (*P*<0.05) between treatments according to Duncan's multiple range test.

©Vigne et Vin Publications Internationales (Bordeaux, France)

Table 1 - Mean values for root and shoot length (cm) and dry weights of root, shoot and Lamina (g) in nine table grape (*Vitis vinifera* L.) genotypes at three different salinity levels (25, 50 and 100 mM NaCl).

Genotype and Salinity (mM NaCl)	Root Length (cm)	Shoot Length (cm)	Root Dry Weight (g)	Shoot Dry Weight (g)	Lamina Dry Weight (g)
LaaleBidaneh					
0	70.00±5.77 c	55.11±1.78 d	0.86±0.01 c	3.23±0.20 c	1.79±0.11 c
25	53.33±4.41 b	46.67±1.33 c	0.83±0.03 c	2.67±0.08 b	1.57±0.07 c
50	40.00±2.89 a,b	34.67±0.77 b	0.62±0.03 b	2.26±0.14 b	1.15±0.11 b
100	30.00±2.89 a	24.44±0.44 a	0.44±0.04 a	1.57±0.04 a	0.84±0.02 a
Sachagh					
0	55.00±5.00 c	57.33±3.53 c	0.98±0.02 d	4.07±0.11 b	2.07±0.10 b
25	43.33±3.33 b	44.00±2.31 b	0.85±0.03 c	3.57±0.21 b	1.85±0.17 b
50	33.33±3.33 b	28.67±1.76 a	0.59±0.04 b	2.47±0.17 a	1.26±0.16 a
100	21.67±1.67 a	25.33±1.33 a	0.44±0.03 a	1.94±0.38 a	1.03±0.16 a
Gharashani					
0	58.33±4.41 c	51.56±2.35 c	0.85±0.03 d	3.71±0.13 d	2.06±0.12 c
25	39.00±3.79 b	38.67±2.04 b	0.71±0.02 c	2.67±0.03 c	1.66±0.05 b
50	35.00±2.89 a,b	25.78±1.78 a	0.57±0.03 b	1.97±0.11 b	0.93±0.06 a
100	25.00±2.89 a	22.22±0.89 a	0.43±0.03 a	1.37±0.05 a	0.69±0.06 a
Shahroodi					
0	55.00±5.00 c	52.00±0.00 d	0.83±0.04 b	3.65±0.34 c	2.23±0.27 c
25	41.00±4.00 b	39.33±0.67 c	0.78±0.03 b	3.04±0.12 b	1.89±0.09 b,c
50	35.00±2.50 a,b	28.67±0.67 b	0.77±0.05 b	2.49±0.03 a,b	1.48±0.05 a,b
100	27.50±2.50 a	22.67±0.67 a	0.58±0.04 a	2.09±0.09 a	1.19±0.05 a
LaaleSefid					
0	70.67±0.33 d	50.78±0.29 c	0.95±0.02 d	3.83±0.05 d	2.28±0.05 d
25	53.67±1.86 c	53.33±0.39 d	0.77±0.01 c	3.36±0.01 c	1.95±0.01 c
50	36.67±1.20 b	30.44±0.22 b	0.43±0.00 b	2.27±0.01 b	1.26±0.02 b
100	24.67±0.33 a	21.78±0.22 a	0.34±0.02 a	1.55±0.03 a	0.86±0.02 a
Khalili					
0	65.00±1.00 d	48.33±0.88 c	0.90±0.02 d	3.83±0.16 d	2.51±0.01 d
25	55.75±1.10 c	50.33±0.88 c	0.77±0.02 c	2.87±0.02 c	1.97±0.01 c
50	48.00±0.87 b	32.83±1.01 b	0.62±0.02 b	2.36±0.02 b	1.62±0.01 b
100	37.50±0.87 a	26.00±0.29 a	0.45±0.00 a	1.83±0.02 a	1.39±0.01 a
Chawga					
0	63.00±1.00 d	52.67±1.20 d	0.86±0.01 c	3.60±0.06 d	2.34±0.05 d
25	55.67±0.67 c	45.00±0.58 c	0.77±0.01 b	2.98±0.02 c	1.85±0.01 c
50	44.00±0.58 b	36.67±0.33 b	0.75±0.00 a,b	2.52±0.02 b	1.69±0.01 b
100	33.00±0.58 a	31.33±0.33 a	0.73±0.01 a	2.18±0.01 a	1.51±0.01 a
GhezelUzum					
0	67.00±1.32 c	46.67±0.33 d	0.95±0.01 d	4.05±0.05 d	2.55±0.04 d
25	69.00±0.87 c	31.67±0.33 c	0.74±0.01 c	3.08±0.02 c	1.83±0.01 c
50	34.50±0.87 b	22.67±0.33 b	0.46±0.01 b	2.79±0.02 b	1.69±0.01 b
100	22.50±0.43 a	19.67±0.33 a	0.36±0.00 a	2.23±0.01 a	1.45±0.01 a
Gharagandomeh					
0	54.50±0.29 c	48.33±0.44 d	0.88±0.01 d	3.42±0.01 d	1.99±0.01 d
25	55.67±0.33 c	41.17±0.17 c	0.73±0.01 c	3.01±0.02 c	1.69±0.01 c
50	40.00±0.58 b	34.67±0.33 b	0.60±0.00 b	2.47±0.02 b	1.45±0.01 b
100	34.17±0.60 a	29.00±0.00 a	0.45±0.01 a	2.05±0.01 a	1.27±0.01 a

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

©Vigne et Vin Publications Internationales (Bordeaux, France)

Table 2 - Mean values for root, shoot and lamina nitrate concentrations (mg/g DW) in nine table grape (*Vitis vinifera* L.) genotypes at three different salinity levels (25, 50 and 100 mM NaCl).

Genotype and Salinity (mM NaCl)	NO_3^- Content of root (mg/g DW)	NO_3^- Content of shoot (mg/g DW)	NO_3^- Content of Lamina (mg/g DW)
LaaleBidaneh			
0	4.10±0.06 a	4.89±0.12 a	0.86±0.06 a
25	4.80±0.04 b	8.80±0.08 b	0.95±0.01 a
50	4.01±0.04 a	9.18±0.29 b	1.14±0.03 b
100	8.20±0.23 c	11.76±0.18 c	1.45±0.12 c
Sachagh			
0	1.13±0.05 a	2.10±0.01 a	0.39±0.04 a
25	3.17±0.10 b	2.16±0.04 a	0.76±0.01 c
50	5.35±0.08 c	3.60±0.04 b	0.65±0.03 b
100	1.33±0.05 a	4.30±0.12 c	0.81±0.03 c
Gharashani			
0	2.07±0.03 c	5.47±0.10 c	0.70±0.04 a
25	1.77±0.05 b	3.73±0.14 b	0.64±0.01 a
50	0.77±0.03 a	3.15±0.01 a	0.63±0.01 a
100	0.72±0.01 a	3.37±0.02 a	0.68±0.02 a
Shahroodi			
0	1.88±0.01 d	3.20±0.08 c	0.77±0.02 d
25	1.21±0.02 c	2.12±0.07 b	0.68±0.03 c
50	0.91±0.01 b	2.06±0.04 b	0.57±0.03 b
100	0.69±0.05 a	1.56±0.04 a	0.29±0.02 a
LaaleSefid			
0	4.31±0.05 d	4.12±0.04 a	1.50±0.03 c
25	3.08±0.02 c	7.36±0.04 d	2.00±0.05 d
50	2.52±0.02 b	5.42±0.06 c	1.37±0.03 b
100	1.98±0.05 a	4.60±0.03 b	0.92±0.02 a
Khalili			
0	4.75±0.02 d	7.18±0.04 d	1.53±0.02 d
25	4.32±0.04 c	6.61±0.02 c	1.20±0.01 c
50	3.25±0.01 b	3.44±0.03 b	0.73±0.01 b
100	1.83±0.02 a	1.16±0.01 a	0.57±0.01 a
Chawga			
0	2.16±0.02 d	3.47±0.03 b	1.77±0.02 c
25	1.37±0.01 c	4.59±0.03 d	1.45±0.02 b
50	1.16±0.01 b	4.32±0.02 c	1.84±0.01 d
100	0.45±0.02 a	2.67±0.03 a	1.32±0.00 a
GhezelUzum			
0	2.13±0.03 d	1.59±0.02 a	0.53±0.01 a
25	1.52±0.02 c	1.85±0.02 c	0.79±0.01 c
50	1.12±0.02 b	1.74±0.03 b	0.88±0.02 d
100	0.52±0.01 a	1.59±0.01 a	0.68±0.02 b
Gharagandomeh			
0	0.41±0.02 a	3.88±0.02 d	0.50±0.01 a
25	1.02±0.01 c	3.75±0.06 c	0.74±0.01 b
50	0.89±0.01 b	2.28±0.01 b	0.53±0.01 a
100	1.85±0.03 d	1.81±0.03 a	0.53±0.02 a

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

absorbance was measured at 365 nm by using a spectrophotometer (UV-visible, WPA S2100).

4. 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 Duncan's multiple range tests (P<0.05) were performed. A GLM (General Linear Model)

©Vigne et Vin Publications Internationales (Bordeaux, France)

analysis was used to determine differences among genotypes and interactions between genotypes and salinity treatments. Correlations (P<0.01) of ion concentration with growth and compatible solutes were determined.

RESULTS

1. Growth response to salt treatments

Increasing salinity resulted in decreased root and shoot growth in grape plants, except at 25 mM NaCl for the shoot length of LaaleSefid and Khalili and the root length of GhezelUzum and Gharagandomeh, which increased (Table 1). High salt treatments were deleterious for the roots. GhezelUzum had higher shoot and root length decrease (57.85% and 66.42%, respectively) than the others.

Dry weights of all plant parts were significantly reduced at all salinity levels (Table 1). Gharashani and LaaleSefid had higher reduction in shoot and root dry weight (63.07% and 64.21%, respectively, over control levels) than the others.

2. Effects of salinity on mineral contents

a. Chloride

All genotypes showed high Cl^- accumulation in different plant parts under salt treatments when compared to control plants (Figure 1). The Cl^- content in petiole increased with increasing salinity in all genotypes, with the highest increase in GhezelUzum and LaaleSefid at 100 and 50 mM NaCl, respectively. Gharashani showed the lowest shoot and lamina Cl^- accumulation at 50 and 100 mM, while GhezelUzum showed the highest. Serious damages such as leaf chlorosis and burning were observed in GhezelUzum at high external

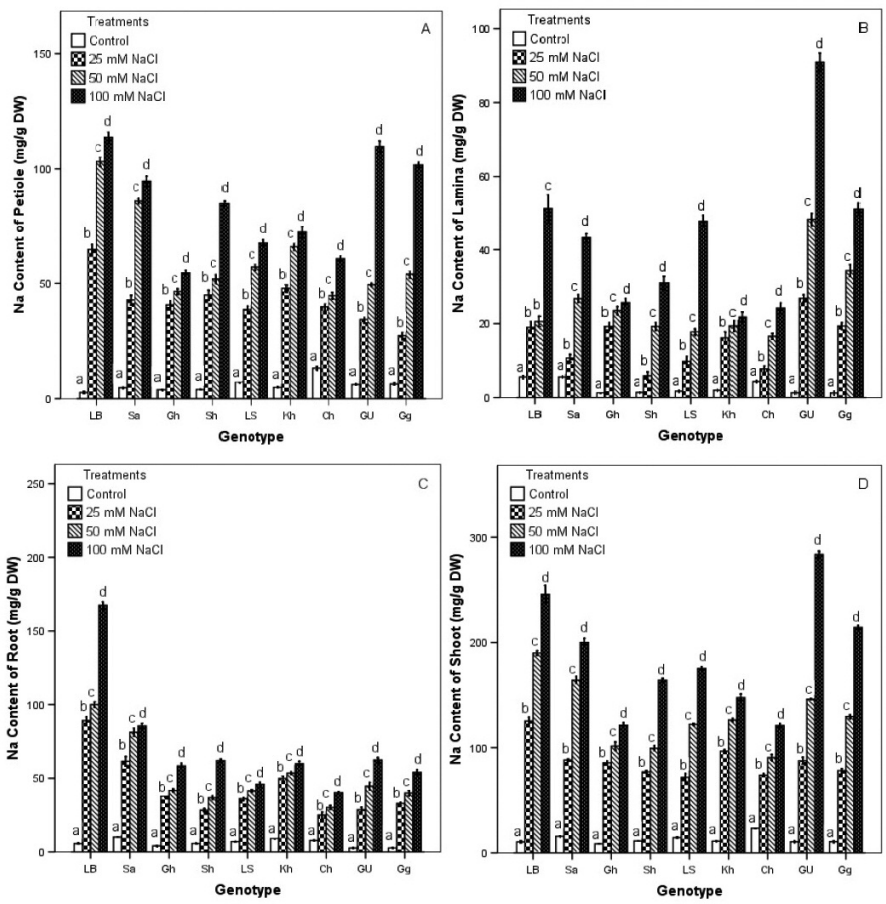


Figure 2 - Na^+ concentrations (mg/g DW) in petioles (A), laminas (B), roots (C) and shoots (D) of nine table grape (*Vitis vinifera* L.) genotypes (LB: LaaleBidaneh, Sa: Sachagh, Gh: Gharashani, Sh: Shahroodi, LS: LaaleSefid, Kh: Khalili, Ch: Chawga, GU: GhezelUzum, Gg: Gharagandomeh) at three different salinity levels (25, 50 and 100 mM NaCl). Bars are the means ± standard error (n=3, one-way ANOVA). Different letters indicate significant differences (P<0.05) between treatments according to Duncan's multiple range test.

©Vigne et Vin Publications Internationales (Bordeaux, France)

Table 3 - Mean values for root, shoot and lamina potassium concentrations (mg/g DW) in nine table grape (*Vitis vinifera* L.) genotypes at three different salinity levels (25, 50 and 100 mM NaCl).

Genotype and salinity (mM NaCl)	K^+ Content of root (mg/g DW)	K^+ Content of shoot (mg/g DW)	K^+ Content of Lamina (mg/g DW)
LaaleBidaneh			
0	86.43±1.07 c	226.85±1.49 d	43.64±0.93 b
25	59.68±0.93 b	210.00±1.42 c	39.89±0.54 a
50	37.75±0.53 a	184.32±2.14 a	37.75±0.53 a
100	38.29±0.53 a	196.63±1.42 b	45.24±0.93 b
Sachagh			
0	90.18±0.93 d	245.84±1.85 d	54.34±0.54 d
25	60.75±0.54 c	199.30±0.93 c	42.03±0.93 c
50	38.82±0.93 b	158.65±1.42 b	34.01±0.93 b
100	25.98±0.93 a	86.43±1.42 a	27.59±0.00 a
Gharashani			
0	53.27±0.93 d	139.92±1.85 d	27.59±0.93 b
25	33.47±0.54 c	118.53±1.93 c	27.05±0.54 a,b
50	28.66±0.54 b	104.62±0.93 b	26.52±0.54 a,b
100	20.10±0.54 a	93.92±1.07 a	24.91±0.54 a
Shahroodi			
0	38.29±0.53 d	135.65±1.07 c	30.80±0.93 b
25	27.05±0.54 c	114.25±0.93 b	28.12±0.54 a
50	23.84±0.54 b	109.43±1.60 b	27.59±0.93 a
100	18.49±0.54 a	85.90±0.54 a	27.05±0.54 a
LaaleSefid			
0	39.62±0.69 d	177.05±0.40 d	30.40±0.40 d
25	31.20 ±0.69 c	137.33±1.06 c	28.39±0.40 c
50	20.37±0.69 b	119.27±1.45 b	26.38±0.69 b
100	3.92±0.40 a	86.37±0.69 a	20.37±0.69 a
Khalili			
0	57.68±0.69 d	141.74±0.70 d	34.81±0.69 d
25	32.40±0.69 c	120.88±0.80 c	32.00±0.40 c
50	26.38±0.00 b	95.60±1.45 b	29.19±0.40 b
100	15.15±0.40 a	78.35±1.06 a	27.19±0.40 a
Chawga			
0	7.80±0.18 a	19.84±0.29 a	3.31±0.12 a
25	42.83±0.40 c	122.48±0.69 b	32.00±0.40 b
50	48.05±0.69 d	155.78±0.40 d	53.26±0.40 d
100	33.61±0.69 b	140.94±1.06 c	36.82±0.40 c
GhezelUzum			
0	61.99±0.82 d	151.35±1.69 a,b	36.72±0.81 c
25	53.57±0.81 c	157.44±0.94 c	33.91±0.81 a,b
50	44.68±0.47 b	154.63±0.47 b,c	32.98±0.47 a
100	28.29±0.81 a	149.95±1.69 a	35.79±0.47 b,c
Gharagandomeh			
0	70.42±0.81 d	235.14±0.81 c	47.95±0.81 d
25	54.51±0.47 c	204.24±2.14 b	40.93±0.81 b
50	45.15±0.81 b	162.12±1.40 a	38.59±0.47 a
100	36.72±0.81 a	161.18±1.69 a	43.74±0.81 c

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

NaCl concentration. GhezelUzum and Chawga showed lower Cl^- contents in root at 50 mM NaCl than the other genotypes.

b. Nitrate

The results showed some fluctuations in NO_3^- concentration among genotypes (Table 2). However, different salinity levels induced a decrease in root and shoot NO_3^- content in most genotypes.

c. Sodium

Sodium as a counterpart cation for Cl^- significantly (P<0.05) increased in the different plant parts of all genotypes with increasing salinity (Figure 2).

©Vigne et Vin Publications Internationales (Bordeaux, France)

Table 4 - Mean values for $NO3^-/Cl^-$ and K^+/Na^+ ratio in roots and shoots of nine table grape (*Vitis vinifera* L.) genotypes at three different salinity levels (25, 50 and 100 mM NaCl).

Genotype and salinity (mM NaCl)	(NO_3^-/Cl^-) Ratio in root	(NO_3^-/Cl^-) Ratio in shoot	(K^+/Na^+) Ratio in root	(K^+/Na^+) Ratio in shoot
LaaleBidaneh				
0	0.75±0.04 c	0.49±0.04 c	15.37±0.37 b	21.85±0.96 b
25	0.26±0.01 b	0.38±0.02 b	0.67±0.02 a	1.68±0.01 a
50	0.15±0.01 a	0.25±0.01 a	0.38±0.01 a	0.97±0.02 a
100	0.30±0.01 b	0.21±0.00 a	0.23±0.00 a	0.80±0.02 a
Sachagh				
0	0.26±0.01 d	0.18±0.01 b	8.99±0.05 d	15.56±0.23 d
25	0.13±0.01 b	0.09±0.00 a	0.99±0.02 c	2.26±0.02 c
50	0.20±0.01 c	0.08±0.00 a	0.48±0.01 b	0.97±0.01 b
100	0.04±0.00 a	0.08±0.00 a	0.30±0.01 a	0.43±0.01 a
Gharashani				
0	0.44±0.02 c	0.65±0.01 c	13.03±0.59 b	16.33±0.25 c
25	0.11±0.00 b	0.18±0.01 b	0.88±0.01 a	1.39±0.02 b
50	0.04±0.00 a	0.10±0.00 a	0.69±0.00 a	1.03±0.02 a,b
100	0.04±0.00 a	0.10±0.00 a	0.35±0.01 a	0.77±0.01 a
Shahroodi				
0	0.38±0.00 d	0.26±0.04 b	6.64±0.09 d	12.00±0.06 d
25	0.09±0.01 c	0.07±0.00 a	0.96±0.02 c	1.48±0.02 c
50	0.05±0.00 b	0.06±0.00 a	0.65±0.01 b	1.09±0.01 b
100	0.03±0.00 a	0.02±0.00 a	0.30±0.01 a	0.52±0.01 a
LaaleSefid				
0	0.94±0.06 c	0.43±0.01 d	5.69±0.15 d	12.13±0.24 d
25	0.20±0.01 b	0.22±0.01 c	0.86 ±0.02 c	1.92±0.04 c
50	0.10±0.00 a	0.10±0.00 b	0.49±0.01 b	0.98±0.01 b
100	0.07±0.00 a	0.05±0.00 a	0.09±0.01 a	0.49±0.00 a
Khalili				
0	0.84±0.03 d	0.97±0.02 c	6.30±0.12 c	12.77±0.17 c
25	0.35±0.01 c	0.23±0.00 c	0.65±0.02 b	1.25±0.02 b
50	0.16±0.01 b	0.08±0.00 b	0.49±0.00 b	0.76±0.02 a
100	0.07±0.00 a	0.02±0.00 a	0.25±0.01 a	0.53±0.01 a
Chawga				
0	0.44±1.02 d	0.31±0.01 d	1.00±0.01 a	0.85±0.01 a
25	0.13±0.01 c	0.16±0.00 c	1.72±0.09 b	1.66±0.02 c
50	0.09±0.00 b	0.10±0.00 b	1.59±0.06 b	1.72±0.03 c
100	0.02±0.00 a	0.05±0.00 a	0.84±0.02 a	1.16±0.01 b
GhezelUzum				
0	0.50±0.01 d	0.23±0.01 d	23.93±0.93 b	14.35±0.45 c
25	0.14±0.00 c	0.07±0.00 c	1.87±0.07 a	1.80±0.02 b
50	0.08±0.00 b	0.03±0.00 b	1.00±0.04 a	1.06±0.00 a
100	0.02±0.00 a	0.01±0.00 a	0.45±0.01 a	0.53±0.01 a
Gharagandomeh				
0	0.06±0.01 b	0.45±0.01 d	26.43±0.72 b	22.09±1.20 b
25	0.07±0.00 b	0.18±0.01 c	1.66±0.05 a	2.61±0.05 a
50	0.05±0.00 a	0.06±0.00 b	1.13±0.04 a	1.25±0.02 a
100	0.10±0.00 c	0.02±0.00 a	0.68±0.00 a	0.75±0.01 a

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

At 50 mM NaCl, LaaleBidaneh and Chawga showed higher and lower root Na^+ content than the other genotypes, respectively. At the same salinity level (50 mM), GhezelUzum and Gharagandomeh accumulated higher Na^+ in lamina than the other genotypes (as was shown for lamina Cl^- content). Unexpectedly, Na^+ content was higher (2-5 times) than Cl^- content in all plant parts and all salinity treatments.

d. Potassium

Unlike for sodium, the potassium concentration of roots, laminas and shoots decreased with increasing salinity treatments in all genotypes, except in

©Vigne et Vin Publications Internationales (Bordeaux, France)

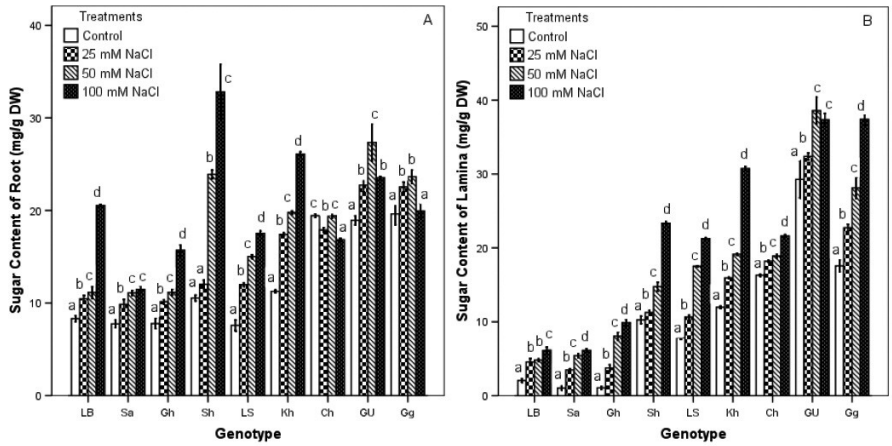


Figure 3 - Soluble sugar concentrations (mg/g DW) in roots (A) and laminas (B) of nine table grape (*Vitis vinifera* L.) genotypes (LB: LaaleBidaneh, Sa: Sachagh, Gh: Gharashani, Sh: Shahroodi, LS: LaaleSefid, Kh: Khalili, Ch: Chawga, GU: GhezelUzum, Gg: Gharagandomeh) at three different salinity levels (25, 50 and 100 mM NaCl). Bars are the means ± standard error (n=3, one-way ANOVA). Different letters indicate significant differences (*P*<0.05) between treatments according to Duncan's multiple range test.

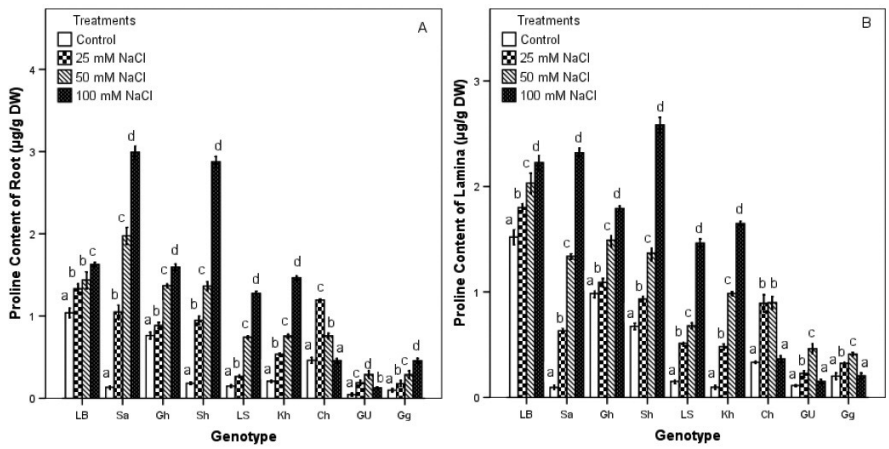


Figure 4- Proline concentrations (µg/g DW) in roots (A) and laminas (B) of nine table grape (*Vitis vinifera* L.) genotypes (LB: LaaleBidaneh, Sa: Sachagh, Gh: Gharashani, Sh: Shahroodi, LS: LaaleSefid, Kh: Khalili, Ch: Chawga, GU: GhezelUzum, Gg: Gharagandomeh) at three different salinity levels (25, 50 and 100 mM NaCl). Bars are the means ± standard error (n=3, one-way ANOVA). Different letters indicate significant differences (*P*<0.05) between treatments according to Duncan's multiple range test.

Chawga, which showed a significant ($P<0.05$) increase in the K^+ content of root and, particularly, shoot under salinity (Table 3).

The K^+ content of roots was highly reduced in LaaleSefid and Khalili with increasing salinity, whereas Shahroodi and Gharagandomeh showed the lowest reduction (51.74% and 47.86% compared to control, respectively). LaaleBidaneh and GhezelUzum could retain higher shoot K^+ content than the other genotypes under salinity stress.

e. NO_3^-/Cl^- and K^+/Na^+ ratios

Under high salinity conditions, LaaleBidaneh had higher NO_3^-/Cl^- ratio and higher NO_3^-/Cl^- selectivity than the other genotypes, whereas Chawga had higher K^+/Na^+ ratio and better K^+/Na^+ selectivity (Table 4).

3. Salinity effects on compatible solutes

a. Soluble sugars

The soluble sugar content in roots and laminas generally increased with increasing salinity

(Figure 3). However, in some genotypes the root sugar content increased and then decreased at 100 mM NaCl (GhezelUzum and Gharagandomeh) or varied across treatments reaching lower levels than in control plants at 100 mM NaCl (Chawga).

b. Proline

Proline content in laminas and roots of all genotypes increased with increasing salt treatments (Figure 4). In comparison to the other genotypes, Sachagh and Shahroodi accumulated higher proline content in lamina and root.

c. Glycine betaine

Glycine betaine as an important osmoticum increased in all genotypes under salinity stress (Figure 5). Gharashani showed a higher lamina glycine betaine content than the others for all treatments. GhezelUzum accumulated lower levels of glycine betaine in lamina compared to the other genotypes.

DISCUSSION

Grapevines are classified as moderately sensitive to salinity (Maas and Hoffman, 1977). Comparison of the effects of different salts on grapevine revealed that chloride salts cause more leaf damage than sulfate or carbonate salts at the same concentrations (Kishore *et al.*, 1985).

Growth reductions are observed even at low salinities, often before the appearance of visible symptoms. In the present study, increased root zone salinity led to a progressive growth decline in all genotypes. GhezelUzum showed the most important root and shoot length decrease. Salinity reduced shoot dry weight by reducing both shoot length and total leaf area. The decline in leaf growth is the earliest response of glycophytes exposed to salt stress (Munns and Termaat, 1986).

High salinity causes the appearance of foliar (leaf curling and necrosis) and root (fishbone face and absence of rootlets) symptoms (Walker *et al.*, 1981). In our study, GhezelUzum and LaaleSefid showed more leaf necrosis and root damage symptoms than the other genotypes.

There were significant correlations ($P<0.01$) between growth factors (root and shoot length and dry weights) and plant ion content (Cl^-, Na^+, K^+ and NO_3^-). Growth factors showed negative correlations with sodium, chloride and compatible solute contents in plant parts, while positive correlations

Table A1 - Mean values and analysis of variance for Cl^-, Na^+, K^+ and NO_3^- concentrations (mg/g DW) in nine table grape (*Vitis vinifera* L.) genotypes at three different salinity levels (0, 25, 50 and 100 mM NaCl). Data are the means ± standard error (GLM analysis).

	Cl^- Content of root (mg/g DW)	Cl^- Content of shoot (mg/g DW)	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)	NO_3^- Content of root (mg/g DW)	NO_3^- Content of shoot (mg/g DW)
Salinity (mM NaCl)								
0	5.17±0.22 a	9.63±0.29 a	6.10±0.25 a	12.95±0.42 a	56.19±0.23 d	163.71±0.43 d	2.55±0.02 d	3.99±0.03 c
25	15.29±0.22 b	26.17±0.29 b	43.29±0.25 b	87.09±0.42 b	43.94±0.23 c	153.83±0.43 c	2.48±0.02 c	4.55±0.03 d
50	20.29±0.22 c	41.90±0.29 c	52.17±0.25 c	130.00±0.42 c	34.86±0.23 b	138.27±0.43 b	2.22±0.02 b	3.85±0.03 b
100	25.13±0.22 d	71.97±0.29 d	70.62±0.25 d	185.92±0.42 d	24.50±0.23 a	119.96±0.43 a	1.96±0.02 a	3.64±0.03 a
Genotypes								
LaaleBidaneh	19.81±0.32	31.62±0.44	90.56±0.38	142.85±0.63	55.54±0.34	204.45±0.65	5.29±0.03	8.66±0.04
Sachagh	22.93±0.32	32.83±0.44	59.60±0.38	117.06±0.63	53.93±0.34	172.56±0.65	2.75±0.03	2.89±0.04
Gharashani	14.93±0.32	23.79±0.44	35.51±0.38	79.28±0.63	33.87±0.34	114.25±0.65	1.33±0.03	3.93±0.04
Shahroodi	15.37±0.32	38.53±0.44	33.26±0.38	88.00±0.63	26.92±0.34	111.31±0.65	1.17±0.03	2.33±0.04
LaaleSefid	18.90±0.32	46.68±0.44	32.62±0.38	95.95±0.63	23.78±0.34	130.01±0.65	2.97±0.03	5.38±0.04
Khalili	16.62±0.32	38.40±0.44	43.10±0.38	95.40±0.63	32.90±0.34	109.14±0.65	3.54±0.03	4.60±0.04
Chawga	11.79±0.32	34.39±0.44	25.80±0.38	77.21±0.63	33.07±0.34	109.76±0.65	1.29±0.03	3.76±0.04
GhezelUzum	13.06±0.32	55.13±0.44	34.58±0.38	131.94±0.63	47.14±0.34	153.34±0.65	1.32±0.03	1.69±0.04
Gharagandomeh	14.85±0.32	35.40±0.44	32.37±0.38	108.19±0.63	51.70±0.34	190.67±0.65	1.042±0.03	2.93±0.04
Analysis of Variance (F-Values)								
Genotype	118.02*	422.88*	2858.97*	1335.77*	1296.07*	3378.52*	3070.00*	2871.24*
Salinity	1568.98*	8176.42*	11564.15*	30450.55*	3544.77*	1970.25*	237.59*	224.73*
Genotype × Salinity	26.09*	214.29*	472.13*	488.75*	339.72*	726.92*	540.93*	435.15*

Salinity means calculated for all genotypes and genotype means calculated for all salinity treatments.
Different letters within columns indicate significant differences at $P<0.05$ according to the Duncan's test. *Indicate significance level at $P<0.05$ according to the Duncan's test.

©Vigne et Vin Publications Internationales (Bordeaux, France)

were determined between growth factors and nitrate and potassium contents.

Salt tolerance in glycophytes is associated with the ability to limit the uptake or transport of ions (mainly Na^+ and Cl^-) from the root zone to aerial parts (Greenway and Munns, 1980). Clearly, higher chloride or sodium accumulation in leaves leads to greater leaf physiological disturbance.

The physiological and biochemical basis of the growth reduction are not fully understood. The visible symptoms (marginal chlorosis and necrosis on leaves) are, in most cases, due to the accumulation of toxic concentrations of chloride in leaves. In this study, the high accumulation of chloride in lamina may have inhibited growth. GhezelUzum had higher Cl^- accumulation than the other genotypes and showed leaf burn symptoms. This genotype also had the highest growth inhibition. Ehlig (1960) reported salt burn symptoms in grapevine leaves when lamina Cl^- concentrations were in the range of 1.24-1.9% DW. Nevertheless, uninjured leaves may sometimes have higher Cl^- accumulation than injured leaves of the same species. In the present study, the Cl^- concentration of lamina in GhezelUzum was 1.9% of DW at 50 mM NaCl and 4% of DW at 100 mM and salt burn symptoms were evident.

Cl^- concentrations lower than 1% of DW is regarded as normal for vines under salts tress, while concentrations higher than 1.5% of DW are considered as excessive (Reuter and Robinson, 1997). Lamina Cl^- concentrations of the genotypes studied here were higher than 1.5% at 100 mM NaCl, except Gharashani.

Petiole analysis provides a good evaluation of whether vines are suffering from salt stress (Christensen *et al.*, 1978). But high petiole Cl^- concentrations does not necessarily mean that the vines are «stressed». Still, petiole Cl^- analysis is a good screening method for assessing genotypes for chloride exclusion ability, i.e., the ability to restrict the uptake and root-to-shoot transport of Cl^-. The higher Cl^- concentrations in petioles and laminas reflect the poor capacity of *V. vinifera* vines for Cl^- exclusion (Downton, 1977b). Cl^- accumulation in shoots is an important criterion to survey root control capability. In our study, Gharashani and GhezelUzum had, respectively, the lowest and highest Cl^- accumulation in petioles and laminas at 50 and 100 mM NaCl. It can be attributed to a better capacity in Gharashani to exclude Cl^- or to a better root control of Cl^- transport from root to shoot.

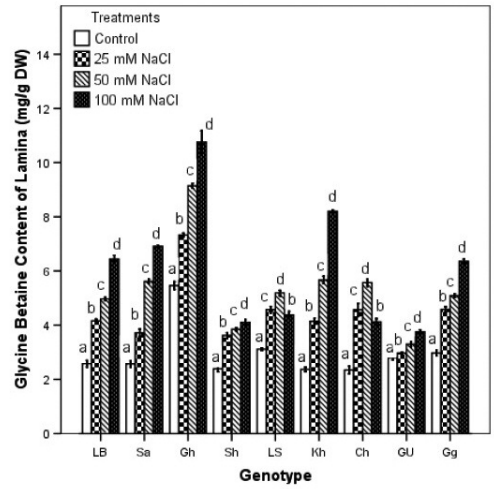


Figure 5 - Glycine betaine concentrations (mg/g DW) in laminas of nine table grape (Vitis vinifera L.) genotypes (LB: LaaleBidaneh, Sa: Sachagh, Gh: Gharashani, Sh: Shahroodi, LS: LaaleSefid, Kh: Khalili, Ch: Chawga, GU: GhezelUzum, Gg: Gharagandomeh) at three different salinity levels (25, 50 and 100 mM NaCl). Bars are the means ± standard error (n=3, one-way ANOVA). Different letters indicate significant differences (P<0.05) between treatments according to Duncan's multiple range test.

In all genotypes, Cl^- accumulation in shoots was nearly two-fold higher than in roots. This suggests that there is no strong control of Cl^- transport from root to shoot in all the genotypes, although Gharashani showed a better status regarding ion transport control.

Increasing salinity levels results in decreased nitrate concentrations in all vine parts, probably due to $NO_3^-Cl^-$ antagonism (McClure *et al.*, 1986). Accordingly, in most of the genotypes studied here, a decrease in nitrate accumulation was observed with increasing salinity. Furthermore, a significant negative correlation (P<0.01, r^2=-0.926) was found between NO_3^- and Cl^- in all parts of the plants.

A strong antagonistic effect between NO_3^- and Cl^- has been reported by several authors (Bar *et al.*, 1997; Gunes *et al.*, 1996; McClure *et al.*, 1986). The NO_3^-/Cl^- ratio is an important factor and salt-sensitive genotypes maintain lower NO_3^- content under conditions of high salinity. Among our genotypes, LaaleBidaneh and GhezelUzum showed, respectively, higher and lower NO_3^-/Cl^- selectivity compared to the others.

©Vigne et Vin Publications Internationales (Bordeaux, France)

Sodium as well as chloride was accumulated in all vine parts of the nine genotypes under salinity. The distribution of sodium differed markedly from that of chloride (Downton, 1977a; Downton and Hawker, 1980). In laminas of Khalili and roots of Shahroodi, there were high significant positive correlations ($P<0.01$) at the value 1 between Cl^- and Na^+ at control and 50 mM NaCl.

Previous studies (Downton, 1985; Fisarakis *et al.*, 2001; Fisarakis *et al.*, 2005; Walker *et al.*, 2004) showed that grape petioles and laminas accumulated higher levels of Cl^- than Na^+ under salinity.

While some works showed that grape genotypes had higher Na^+ than Cl^- content in salt conditions, Arbabzadeh and Dutt (1987) showed that rootstocks 41B, 110R and Ramsey accumulated higher levels of Na^+ than Cl^- at low salinity. Walker *et al.* (2004) reported that Na^+ concentrations exceeded those of Cl^- in the juice of Sultana grapes on R3, R1, J17-69, 1103 Paulsen and Ramsey rootstocks. The present study confirms these results, as Na^+ accumulation exceeded that of Cl^- in all vine parts and all treatments. It seems that all the genotypes studied here may adapt to salinity because of their ecological conditions and can tolerate high concentrations of sodium ions. Indeed, they have been grown in the areas around Urmia Salt Lake for several centuries.

However, the fact that less Cl^- is accumulated in the leaves than Na^+ does not necessarily means that Na^+ is responsible for the toxicity symptoms. Leaf burn is more likely due to Cl^-, even if it occurs at lower concentration than Na^+ (Fisarakis *et al.*, 2001), as observed in GhezelUzum.

Under low and moderate saline conditions, the K^+ concentration is reduced in all grape tissues, like in many glycophytes (Greenway and Munns, 1980). The results obtained here showed that increased salinity levels resulted in a considerable reduction in K^+ concentrations in all vine parts, except for Chawga. The general decline in K^+ concentrations with increasing salinity is a consequence of K^+ replacement by higher sodium content under saline conditions in nutrient solutions (Stevens *et al.*, 1996). Establishment of ion homeostasis is an essential requirement for plants to survive under salt stress conditions. Maintenance of high K^+ concentrations in salt-tolerant genotypes may be one of the mechanisms underlying their superior salt tolerance (Tester and Davenport, 2003). Among our genotypes, Chawga exhibited the opposite pattern: the K^+ content in roots and shoots increased with increasing salinity. Chawga had lower Na^+ and higher K^+ accumulation in roots and shoots compared to the other genotypes under salinity.

The correlation between Cl^- and Na^+ contents with K^+ in roots and laminas was negative ($P<0.05$, $r^2=-0.841$) in all genotypes except Chawga, which showed a significant positive correlation ($P<0.01$, $r^2=0.998$).

The Na^+:K^+ antagonism is quite strong in grapevine under salinity (Garcia and Charbaji, 1993), even at low external NaCl concentrations. Chawga showed a better K^+/Na^+ ratio than the other genotypes. Chawga had considerable K^+ content in root and shoot (nearly 2-fold higher than Na^+). It also had higher K^+/Na^+ selectivity compared to the others.

During adaptation, plants may respond to environmental stresses by accumulating some small organic solutes known as compatible materials (Bohnert *et al.*, 1995).

Osmotic adjustment is a mechanism used for maintaining turgor and reducing the deleterious effects of salt stress. Changes in soluble sugar content have already been reported for a number of plant species in salt-stress condition, but information regarding the role of sugars in adaptation of plants to salinity is insufficient to conclude that they are universally associated with salt tolerance (Ashraf and Harris, 2004). The accumulation of compatible materials in mature source leaves may be due to a stress-induced inhibition of phloem loading (Daie, 1989) or to a stimulation of sucrose–phosphate synthase activities (Huber and Huber, 1996). Some authors reported a strong correlation between sugar accumulation and osmotic stress tolerance (Taji *et al.*, 2002). Although the soluble sugar content in roots and laminas increased with increasing salinity in all of our genotypes, it seems that the increase was not associated with salt tolerance. Some genotypes that had lower toxic ion accumulation and are assumed to be salt tolerant did not necessarily show higher sugar content than others.

The major compatible osmoprotectant solutes in plants are betaine, proline, and polyols (Rontein *et al.*, 2002). In response to salt stress, a large group of plants accumulates high contents of organic solutes, particularly glycine betaine and proline in their tissues (Yancey *et al.*, 1982).

©Vigne et Vin Publications Internationales (Bordeaux, France)

Proline content of laminas and roots of all the genotypes increased with increasing salt treatments (Figure 4) but was barely detectable under control conditions (Ueda *et al.*, 2007). Proline accumulation was one of the earliest biochemical processes to be observed in higher plants under salt stress conditions and has now been documented for a large number of species, but its role as an adaptive process is still a matter of debate. Some authors reported a positive correlation between proline accumulation and salt tolerance (Kumar *et al.*, 2003). On the other hand, it has also been reported that salt-sensitive cultivars accumulated significantly higher levels of proline compared to the tolerant ones (Lutts *et al.*, 1999). In this study, there was no correlation between proline content and salt tolerance, but positive correlations (P<0.01, r^2=0.98) were observed between proline and toxic ion contents in roots and laminas of all genotypes.

The involvement of glycine betaine as a protectant against abiotic stress in plants is well known. Glycine betaine has been shown to be highly effective as a compatible solute (Le Rudulier *et al.*, 1984) and provides protection against osmotic stress by adjusting the osmotic balance between cell and environment at high salinity (Hassine *et al.*, 2008). Here the glycine betaine content increased in the lamina of all nine 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 lead to increased stress tolerance (Martinez *et al.*, 2005). Our results confirm this observation. Gharashani and GhezelUzum had, respectively, higher and lower glycine betaine content than the other genotypes. Gharashani accumulated lower levels of toxic ions and seems to be a salt-tolerant genotype.

In the present study, there were positive correlations (P<0.01, r^2=0.987) between lamina and root Na^+ and Cl^- contents and compatible solutes. Conversely, there were significant negative correlations (P<0.01, r^2=-0.941) between growth factors (shoot and root lengths and dry weights) and compatible solutes (soluble sugars, proline and glycine betaine) in all genotypes.

GLM analysis was done to evaluate differences among genotypes. Differences within genotypes were significant (P<0.05) for all measured factors, especially for ion concentrations (Appendix Table A1). Interactions between genotypes and salinity treatments (analysis of variance) are also given in Table A1.

CONCLUSION

Salinity decreased plant growth because of high accumulation of Na^+ and Cl^- in all plant parts. The higher accumulation of Na^+ than Cl^-, particularly in shoots, indicated that all genotypes studied here were poor Na^+ excluders. There was a high positive correlation between Cl^- and Na^+ contents with glycine betaine, proline and soluble sugar accumulation in lamina. Among the genotypes, Gharashani showed a higher glycine betaine content in lamina when compared to the others. Taken together, Gharashani and GhezelUzum showed, respectively, higher and lower ability to inhibit excessive Na^+ and Cl^- transport to shoot.

Acknowledgements: The authors would like to thank the Urmia Agricultural Research Center (Kahriz vineyard, Urmia, Iran) for providing grapevine cuttings.

REFERENCES

Alexander D.McE. and Obbink J.G., 1971. Effect of chloride in solution culture on growth and chloride uptake of Sultana and Salt Creek grape vines. *Aust J Exp Agric Anim Husb*, **11**, 357-361.

Allakhverdiev S.I., Feyziev Ya.M., Ahmed A., Hayashi H., Aliev Ja.A., Klimov V.V., Murata N. and Carpentier R., 1996. Stabilization of oxygen evolution and primary electron transport reactions in photosystem II against heat stress with glycinebetaine and sucrose. *J Photochem Photobiol B*, **34**,149-157.

Arbabzadeh F. and Dutt G., 1987. Salt tolerance of grape rootstocks under greenhouse conditions. *Am J Enol Vitic*, **38**, 95-99.

Ashraf M. and Harris P.J.C., 2004. Potential biochemical indicators of salinity tolerance in plants. *Plant Sci*, **166**, 3-16.

Bar Y., Apelbaum A., Kafkafi U. and Goren R., 1997. Relationship between chloride and nitrate and its effect on growth and mineral composition of avocado and citrus plants. *J Plant Nutr*, **20**, 715-731.

Bates L.S., Waldren R.P. and Teare I.D., 1973. Rapid determination of free proline for water-stress studies. *Plant Soil*, **39**, 205-207.

Bernstein L., Ehlig C.F. and Clark R.A., 1969. Effect of grape rootstocks on chloride accumulation in leaves. *J Am Soc Hortic Sci*, **94**, 584-590.

©Vigne et Vin Publications Internationales (Bordeaux, France)

Blumwald E., Aharon G.S. and Apse M.P., 2000. Sodium transport in plant cells. *Biochim Biophys Acta*, **1465**, 140-151.

Bohnert H.J., Nelson D.E. and Jensen R.G., 1995. Adaptations to environmental stresses. *Plant Cell*, **7**, 1099-1111.

Cataldo D.A., Haroon M., Schrader L.E. and Youngs V.L., 1975. Rapid colorimetric determination of nitrate in plant tissue by nitration of salicylic acid. *Commun Soil Sci Plant Anal*, **6**, 71-80.

Chen Z., Zhou M., Newman I.A., Mendham N.J., Zhang G. and Shabala S., 2007. Potassium and sodium relations in salinised barley tissues as a basis of differential salt tolerance. *Funct Plant Biol*, **34**, 150-162.

Cherel I., 2004. Regulation of K^+ channel activities in plants: from physiological to molecular aspects. *J Exp Bot*, **55**, 337-351.

Christensen L.P., Kasimatis A.N. and Jensen F.L., 1978. *Grapevine Nutrition and Fertilization in the San Joaquin Valley*. University of California, Berkeley, Agriculture & Natural Resources, Publication No. 4087.

Daie J., 1989. Phloem loading of sucrose: update and opportunities in molecular biology. *Plant Mol Biol Rep*, **7**, 106-115.

Downton W.J.S., 1977a. Influence of rootstocks on the accumulation of chloride, sodium and potassium in grapevines. *Aust J Agric Res*, **28**, 879-889.

Downton W.J.S., 1977b. Chloride accumulation in different species of grapevine. *Sci Hortic*, **7**, 249-253.

Downton W.J.S. and Hawker J.S., 1980. Interaction of boron and chloride on growth and mineral composition of Cabernet Sauvignon vines. *Am J Enol Vitic*, **31**, 277-282.

Downton W.J.S., 1985. Growth and mineral composition of the Sultana grapevine as influenced by salinity and rootstock. *Aust J Agric Res*, **36**, 425-434.

Dubois M., Gilles K.A., Hamilton J.K., Rebers P.A. and Smith F., 1956. Colorimetric method for determination of sugars and related substances. *Anal Chem*, **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.

Fisarakis I., Chartzoulakis K. and Stavrakas D., 2001. Response of Sultana vines (*V. vinifera* L.) on six rootstocks to NaCl salinity exposure and recovery. *Agric Water Manage*, **51**, 13-27.

Fisarakis I., Nikolaou N., Tsikalas P., Therios I. and Stavrakas D., 2005. Effect of salinity and rootstock on concentration of potassium, calcium, magnesium, phosphorus and nitrate-nitrogen in Thompson seedless grapevine. *J Plant Nutr*, **27**, 2117-2134.

Frachisse J.M., Thomine S., Colcombet J., Guern J. and Barbier-Brygoo H., 1999. Sulfate is both a substrate and an activator of the voltage-dependent anion channel of Arabidopsis hypocotyl cells. *Plant Physiol*, **121**, 253-261.

Garcia M. and Charbaji T., 1993. Effect of sodium chloride salinity on cation equilibria in grapevine. *J Plant Nutr*, **16**, 2225-2237.

Greenway H. and Munns R., 1980. Mechanisms of salt tolerance in nonhalophytes. *Annu Rev Plant Physiol*, **31**, 149-190.

Grieve C.M. and Grattan S.R., 1983. Rapid assay for determination of water soluble quaternary ammonium compounds. *Plant Soil*, **70**, 303-307.

Gunes A., Inal A. and Alpaslan M., 1996. Effect of salinity on stomatal resistance, proline, and mineral composition of pepper. *J Plant Nutr*, **19**, 389-396.

Hassine A.B., Ghanem M.E., Bouzid S. and Lutts S., 2008. An inland and a coastal population of the Mediterranean xero-halophyte species *Atriplex halimus* L. differ in their ability to accumulate proline and glycinebetaine in response to salinity and water stress. *J Exp Bot*, **59**, 1315-1326.

Hoekstra F.A., Golovina E.A. and Buitink J., 2001. Mechanisms of plant desiccation tolerance. *Trends Plant Sci*, **6**, 431-438.

Huber S.C. and Huber J.L., 1996. Role and regulation of sucrose–phosphate synthase in higher plants. *Annu Rev Plant Physiol Plant Mol Biol*, **47**, 431-444.

Iqbal N., Ashraf M. and Ashraf M.Y., 2008. Glycinebetaine, an osmolyte of interest to improve water stress tolerance in sunflower (*Helianthus annuus* L.): water relations and yield. *S Afr J Bot*, **74**, 274-281.

Kishore D.K., Pandey R.M. and Singh R., 1985. Effect of salt stress on growth characteristics of Perlette grapevines. *Prog Hortic*, **17**, 289-297.

Kumar S.G., Reddy A.M. and Sudhakar C., 2003. NaCl effects on proline metabolism in two high yielding genotypes of mulberry (*Morus alba* L.) with contrasting salt tolerance. *Plant Sci*, **165**, 1245-1251.

Le Rudulier D., Strom A.R., Dandekar A.M., Smith L.T. and Valentine R.C., 1984. Molecular biology of osmoregulation. *Science*, **224**, 1064-1068.

Lutts S., Majerus V. and Kinet J.M., 1999. NaCl effects on proline metabolism in rice (*Oryza sativa*) seedlings. *Physiol Plant*, **105**, 450-458.

Maas E.V. and Hoffman G.J., 1977. Salt crop tolerance – Current assessment. *J Irrig Drain Div*, **103**, 115-134.

©Vigne et Vin Publications Internationales (Bordeaux, France)

Martinez J.P., Kinet J.M., Bajji M. and Lutts S., 2005. NaCl alleviates polyethylene glycol-induced water stress in the halophyte species *Atriplex halimus* L. *J. Exp. Bot.*, **56**, 2421-2431.

McClure P.E., Omholt T.E. and Pace G.M., 1986. Anion uptake in maize roots: interactions between chlorate and nitrate. *Physiol. Plant*, **68**, 107-112.

Munns R. and Termaat A., 1986. Whole-plant responses to salinity. *Aust J Plant Physiol*, **13**, 143-160.

Munns R. and Tester M., 2008. Mechanisms of salinity tolerance. *Ann. Rev. Plant Biol.*, **59**, 651-681.

Nomura M., Hibino T., Takabe T., Sugiyama T., Yokota A., Miyake H. and Takabe T., 1998. Transgenically produced glycinebetaine protects ribulose 1,5-bisphosphate carboxylase/oxygenase from inactivation in *Synechococcus* sp. PCC7942 under salt stress. *Plant Cell Physiol*, **39**, 425-432.

Prado F.E., Boero C., Gallardo M. and Gonzalez J.A., 2000. Effect of NaCl on germination, growth, and soluble sugar content in *Chenopodium quinoa* Willd. seeds. *Bot Bull Acad Sin*, **41**, 27-34.

Reuter D.J. and Robinson J.B., 1997. *Plant Analysis: An Interpretation Manual*, 2nd ed, CSIRO Publishing: Collingwood, Australia.

Rhoades J.D. and Loveday J., 1990. Salinity in irrigated agriculture. In: *Irrigation of Agricultural Crops*, American Society of Civil Engineers, B.A. Stewart and D.R. Nielsen (ed), American Society of Agronomists, Monograph 30, pp. 1089-1142.

Roberts S.K., 2006. Plasma membrane anion channels in higher plants and their putative functions in roots. *New Phytol*, **169**, 647-666.

Rontein D., Basset G. and Hanson A.D., 2002. Metabolic engineering of osmoprotectant accumulation in plants. *Metab Eng*, **4**, 49-56.

Sairam R.K., Rao K.V. and Srivastava G.C., 2002. Differential response of wheat genotypes to long term salinity stress in relation to oxidative stress, antioxidant activity and osmolyte concentration. *Plant Sci*, **163**, 1037-1046.

Sakamoto A. and Murata N., 2002. The role of glycine betaine in the protection of plants from stress: clues from transgenic plants. *Plant Cell Environ*, **25**, 163-171.

Shabala S.N., Shabala L. and Van Volkenburgh E., 2003. Effect of calcium on root development and root ion fluxes in salinised barley seedlings. *Funct Plant Biol*, **30**, 507-514.

Stevens R.M., Harvey G. and Davies G., 1996. Separating the effects of foliar and root salt uptake on growth and mineral composition of four grapevine cultivars on their own roots and on 'Ramsey' rootstock. *J. Am. Soc. Hortic. Sci.*, **121**, 569-575.

Sykes S.R., 1985. Variation in chloride accumulation by hybrid vines from crosses involving the cultivars Ramsey, Villard Blanc, and Sultana. *Am J Enol Vitic*, **36**, 30-37.

Taji T., Ohsumi C., Iuchi S., Seki M., Kasuga M., Kobayashi M., Yamaguchi-Shinozaki K. and Shinozaki K., 2002. Important roles of drought- and cold-inducible genes for galactinol synthase in stress tolerance in *Arabidopsis thaliana*. *Plant J*, **29**, 417-426.

Teakle N.L. and Tyerman S.D., 2010. Mechanisms of Cl^- transport contributing to salt tolerance. *Plant Cell Environ*, **33**, 566-589.

Tester M. and Davenport R., 2003. Na^+ tolerance and Na^+ transport in higher plants. *Ann Bot*, **91**, 503-527.

Ueda A., Yamamoto-Yamane Y. and Takabe T., 2007. Salt stress enhances proline utilization in the apical region of barley roots. *Biochem Biophys Res Commun*, **355**, 61-66.

Walker R.R., Torokfalvy E., Scott N.S. and Kriedemann P.E., 1981. An analysis of photosynthetic response to salt treatment in *Vitis vinifera*. *Aust J Plant Physiol*, **8**, 359-374.

Walker R.R., 1994. Grapevine responses to salinity (Réaction de la vigne à la salinité). *Bull OIV (Organisation Internationale de la Vigne et du Vin)*, **67**, 634-661.

Walker R.R., Blackmore D.H., Clingeleffer P.R. and Correll R.L., 2002. Rootstock effects on salt tolerance of irrigated field-grown grapevines (*Vitis vinifera* L. cv. Sultana) 1. Yield and vigour inter-relationships. *Aust J Grape Wine Res*, **8**, 3-14.

Walker R.R., Blackmore D.H., Clingeleffer P.R. and Correll R.L., 2004. Rootstock effects on salt tolerance of irrigated field-grown grapevines (*Vitis vinifera* L. cv. Sultana) 2. Ion concentrations in leaves and juice. *Aust J Grape Wine Res*, **10**, 90-99.

White P.J. and Broadley M.R., 2001. Chloride in soils and its uptake and movement within the plant. *Ann Bot*, **88**, 967-988.

Xu G., Magen H., Tarchitzky J. and Kafkafi U., 2000. Advances in chloride nutrition. *Adv Agron*, **68**, 97-150.

Yancey P.H., Clark M.E., Hand S.C., Bowlus R.D. and Somero G.N., 1982. Living with water stress: evolution of osmolyte systems. *Science*, **217**, 1214-1222.

©Vigne et Vin Publications Internationales (Bordeaux, France)