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Effect of Salinity on Some Growth Characteristics and Concentration of Elements in Two Grape (*Vitis vinifera* L.) Cultivars, 'Rishbaba' and 'Sahebi'

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ABSTRACT

Two grape (*Vitis vinifera* L.) cultivars ('Rishbaba' and 'Sahebi') were subjected to different concentrations of NaCl and some growth characteristics, including shoot length, specific leaf area, leaf number and root dry weight to stem dry weight ratio were determined, as was the concentration of some elements including Na^+, Cl^-, K^+ and Ca^{2+} in roots and leaves. The project was conducted in a factorial experiment based on a complete randomized block design with four replications. Salinity levels were 0 (control), 25, 50, 75, 100, 125 and 150 mM NaCl. Rooted cuttings were cultivated in pots containing perlite and fed with Hoagland's nutrient solution. Plants treated with salinity were kept for 20 days. Results showed that by increasing salinity level, shoot length, specific leaf area and leaf number decreased significantly. Because growth reduction in the shoots was higher than the roots, by increasing salinity level the ratio of root dry weight to stem dry weight increased. Salt stress had a significant effect on Na^+, Cl^-, K^+ and Ca^{2+} concentrations in both roots and leaves. Increasing salinity level caused an increase in Na^+ and Cl^- concentrations in roots and leaves. This increase was observed more in roots than the leaves and was higher in 'Rishbaba' showing low transportation of the above ions to the upper sections of the plants in this cultivar. Increasing salinity levels decreased K^+ and Ca^{2+} concentrations in tissues. This reduction was more in the roots than in the leaves. 'Rishbaba' transported more Ca^{2+} to upper plant sections. Based on the results of this experiment, 'Rishbaba' was more tolerant to salinity stress than 'Sahebi'.

Keywords: element concentration, growth indices, salinity tolerance, salt stress, Ca^{2+} transport

INTRODUCTION

Salinity is one of the major environmental stresses adversely affecting various physiological and biochemical processes in plants which result in reduced biomass production. This adverse effect of salt stress appears at the whole plant level at almost all growth stages including germination, seedling, vegetative and maturity stages (Levitt 1980; Mahajan and Tuteja 2005; Nawaz *et al*. 2010). The main impact of salinity on plants include osmotic effect, ionic toxicity and nutritional imbalance (Paranychianakis and Chartzoulakis 2005; Saied *et al*. 2005; Zhang *et al*. 2006; Neocleus and Vasilakakis 2007). Salinity also leads to oxidative stress in plants due to the production of reactive oxygen species such as the super oxide radical, hydrogen peroxide and hydroxyl radical (Baby and Jini 2010, 2011). Vines are moderately salt-sensitive plants and vulnerable to climate change (Ben-Asher *et al.* 2006; dos Santos *et al.* 2007).

Salt-tolerant plants transport a low amount of Na^+ and Cl^- to their leaves and this is why they differ from salt-sensitive plants (Munns 2002; Paranychianakis and Chartzoulakis 2005). Low water potential of saline soils makes it increasingly difficult for the plant to acquire both water and nutrients, and this first affects vegetative growth and leaf expansion (Mahajan and Tuteja 2005; Saied *et al.* 2005), then leads to reduced water uptake and transpiration as well as stomatal closure (Ben-Asher *et al.* 2006). Salt toxicity appears more in older leaves and makes them turn yellow and die (Munns 2002). Chartzoulakis *et al.* (2002) studied the effect of sodium chloride (NaCl) on the growth characteristics of olives and showed that salinity decreased plant growth more in upper sections of the plants than the roots. Salinity also decreased leaf production in olive twigs. Saied *et al.* (2005) stated that salinity reduced growth up to 44% in 'Korona' and 90% in 'Elsanta' strawberry cultivars. By increasing salinity, leaf senescence, which was accompanied tip burning symptoms, was faster in 'Elsanta' (highly sensitive cultivar to salt stress); leaf area was also more reduced in this cultivar than 'Korona'.

Increasing soil salinity can affect the absorption of some cations such as Ca^{2+}, Mg^{2+} and K^+ by plants. Soils containing high concentrations of Na^+ and Cl^- reduce the uptake of essential elements significantly (García-Sánchez *et al.* 2002). Plants can deal with this deleterious ionic effect in two ways. They either isolate Na^+ and Cl^- from the cytosol to avoid their toxicities, or maintain appropriate cellular levels of K^+ and Ca^{2+} necessary for metabolic activities (Mansour *et al.* 2003). Storey (1995), in a study on citrus trees, stated that higher tolerance to salinity in *Citrus reticulata* compared to *C. medica* was due to less Cl^- uptake and transport to leaves and more accumulation of this ion in roots of *C. reticulata*. Singh *et al.* (2000) reported that Cl^- content in leaf petioles of grape cuttings increased with increasing NaCl concentration, but K^+, Ca^{2+} and Mg^{2+} contents in stem decreased. Shani and Ben-Gal (2005) exposed grape cv. 'Sugrone' to six salinity levels and showed that by increasing salt concentration, Na^+ and Cl^- contents increased in leaves, while K^+ concentration decreased. In recent years, the response of grapes to salt and water deficit stresses with particular reference to the whole plant and molecular responses have been studied by several groups. They demonstrated that some molecular activity, including metabolism, transcription and protein synthesis are involved to regulate

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important physiological responses such as stomatal conductance, photoprotection and growth (Grimplet *et al*. 2009; Deluc *et al*. 2009; Cramer 2010). Chartzoulakis *et al*. (2006) also reported that by increasing salinity level, Na^+ and Cl^- concentration was increased in olive leaves and this was higher in 'Mastoidis' (a salt-sensitive cultivar) than in 'Koronieki'.

In this study, two popular Iranian grape cultivars, 'Rishbaba' and 'Sahebi', were subjected to different levels of salt stress and some growth characteristics together with the concentration of some elements in their leaves and roots were evaluated.

MATERIALS AND METHODS

Plant materials

One-year old rooted cuttings of two grape (*Vitis vinifera* L.) cultivars, 'Rish-Baba' and 'Sahebi' were transferred into plastic pots containing perlite in March and kept under greenhouse conditions to accelerate growth. Roots of cuttings were washed with deionized water and their branches cut back to one shoot before transplanting. Plants were irrigated daily with half-strength Hoagland's nutrient solution for 45 days before salt treatments, which were imposed in May when the shoots were 15-20 cm in length.

Salinity treatments

A factorial experiment based on a complete randomized block design was conducted in this study with four replications. Salinity was imposed by adding 0 (control), 25, 50, 75, 100, 125 and 150 mM NaCl to half-strength Hoagland's nutrient solution (Hoagland and Arnon 1950) with increments of 25 mM per day to avoid salt shock. Electrical conductivity (EC) of solutions was 1.6, 3.4, 5.1, 6.7, 8.1, 10.1 and 11.4 dS/m, respectively. Plants were irrigated every single day with 350 ml Hoagland's solution for 30 sec. Three rooted cuttings were considered for each treatment (experimental unit). The solution of each tank was brought to its initial volume by the addition of de-ionized water, while a fresh solution was supplied every 10 days, in order to avoid a change in solution concentration greater than 5% (Chartzoulakis *et al*. 2002). Salinity treatments were continued for 20 days.

Growth characteristics measurement

Shoot length and leaf number of plants were measured before the salt treatments began, and the measurements were conducted again at the end of stress period. Leaf, stem and root dry weights were finally determined by detaching and drying them in an oven at 65°C for 48 h (Chartzoulakis *et al*. 2002). In order to remove perlite particles from the surface of the roots, roots were submerged in water and rinsed with distilled water.

The growth rate of shoots and the number of new leaves that appeared during the 20 days of salt stress imposition were calculated by deduction of the first from the second measurements. Specific leaf area (SLA) was also determined at the end of salinity treatments. To achieve this, the surface area of middle leaves selected from new grown shoots of each plant was measured using a plaid paper and SLA was calculated by dividing leaf area (cm^2) by leaf dry weight (g).

Element concentration measurements

Roots and leaves attached to the forth to sixth nodes from the base of shoots were considered for the measurements of element concentration. They were dried in an oven as described above, ground and extracted with 0.1 N HCl and finally analyzed for chloride, sodium, potassium and calcium concentrations. Three plants of any experimental unit were analyzed for these elements. Sodium and potassium concentrations were determined using a flame photometer (JENWAY, PEP-7), chloride was measured using a colorimetric method with $AgNO_3$ (Fisarakis *et al*. 2001) and calcium was evaluated by a spectrophotometer (UV-Vis VARIAN, Carry 100) (Kalra 1998).

Table 1 Shoot length, leaf number, SLA and root to stem dry weight ratio of two grape cultivars in response to salinity, 20 days after salt imposition.

Treatments	Shoot length (cm)	Leaf number	SLA (cm^2/g leaf dry weight)	Root dry weight/ Stem dry weight
V1	10.09 a	4.19 a	4.19 a	3.95 a
V2	8.36 b	3.73 b	3.73 b	3.66 b
T1	17.00 a	7.70 a	7.70 a	3.30 f
T2	15.41 b	7.33 a	7.33 a	3.35 f
T3	11.95 c	4.58 b	4.58 b	3.63 e
T4	9.20 d	3.04 c	3.04 c	3.81 d
T5	5.79 e	2.62 c	2.62 c	4.01 c
T6	3.58 f	1.45 d	1.45 d	4.20 b
T7	1.66 g	1.00 d	1.00 d	4.32 a
V1T1	17.16 a	7.75 a	7.75 a	3.49 d
V1T2	16.00 b	7.41 a	7.41 a	3.39 d
V1T3	14.33 c	5.25 a	5.25 a	3.72 c
V1T4	10.16 d	3.25 a	3.25 a	4.00 b
V1T5	6.58 e	2.91 a	2.91 a	4.14 b
V1T6	4.50 f	1.58 a	1.58 a	4.40 a
V1T7	1.91 g	1.16 a	1.16 a	4.49 a
V2T1	16.83 a	7.66 a	7.66 a	3.11 g
V2T2	14.83 b	7.25 a	7.25 a	3.31 f
V2T3	9.58 c	3.91 a	3.91 a	3.55 e
V2T4	8.25 c	2.83 a	2.83 a	3.63 d
V2T5	5.00 d	2.33 a	2.33 a	3.88 c
V2T6	2.66 e	1.33 a	1.33 a	4.01 b
V2T7	1.41 e	0.83 a	0.83 a	4.14 a

Different letters within a column indicate significant differences according to Duncan's multiple range test ($P < 0.05$). V1: 'Rishbaba' cultivar, V2: 'Sahebi' cultivar, T1 to T7: 0 (Control), 25, 50, 75, 100, 125 and 150 mM NaCl, respectively.

Data analysis

Data analysis was conducted to obtain the main effects and interactions between cultivars and salinity levels using SAS package version 6.12 (SAS Institute Inc., USA). Means were compared by Duncan's multiple range test at $P < 0.01$ or $P < 0.05$.

RESULTS

By increasing salinity level, plants' shoot length decreased, so that the shortest shoots were associated with the 150 mM NaCl treatment. Shoot length was reduced more in 'Sahebi' than in 'Rishbaba'. Symptoms of necrosis appeared on buds of plants treated with salt, especially at higher concentrations (100-150 mM), more so in 'Sahebi'. The number of leaves and SLA decreased with increasing salt stress and the leaves grown during salinity treatments were smaller than those of the control.

The root to stem dry weight ratio in 'Rishbaba' was more than in 'Sahebi' and the maximum value was obtained in the 150 mM NaCl treatment. Salinity stress affected shoots more than roots in both cultivars; thus, the ratio of root to stem dry weight was increased by increasing the NaCl concentration (**Table 1**).

Na^+ and Cl^- concentrations in leaves and roots of both cultivars increased by increasing salinity stress, more in 'Sahebi' than in 'Rishbaba', Na^+ and Cl^- concentrations increasing more in roots than in leaves (**Table 2**).

The effect of salinity stress on K^+ and Ca^{2+} accumulation in the roots and leaves was opposite to that observed for Na^+ and Cl^- since their concentrations decreased with increasing salt stress; this was observed more in roots than in leaves. A reduction in the Ca^{2+} concentration in both roots and leaves occurred more in 'Rishbaba' than in 'Sahebi' (**Table 2**).

DISCUSSION

In this experiment, decreasing shoot length and leaf production, especially at higher levels of salinity, can be related to the reduction of cellular turgor followed by decreasing cell

Table 2 Element concentrations (%) in roots and leaves of two grape cultivars in response to salinity, 20 days after salt imposition.

Treatments	Na^+		Cl^-		K^+		Ca^{2+}	
	Root	Leaf	Root	Leaf	Root	Leaf	Root	Leaf
V1	0.32 b	0.78 b	0.35 b	1.01 b	0.07 a	0.07 a	1.34 a	2.28 a
V2	0.48 a	0.98 a	0.47 a	1.20 a	0.05 b	0.07 a	1.26 b	2.18 b
T1	0.08 g	0.33 g	0.17 g	0.47 g	0.12 a	0.15 a	1.54 a	2.47 a
T2	0.15 f	0.68 f	0.28 f	0.90 f	0.10 ab	0.11 b	1.50 a	2.43 b
T3	0.23 e	0.78 e	0.32 e	1.02 e	0.08 bc	0.09 bc	1.44 b	2.35 c
T4	0.39 d	0.91 d	0.42 d	1.15 d	0.05 cd	0.07 cd	1.37 c	2.52 d
T5	0.53 c	1.00 c	0.47 c	1.26 c	0.03 de	0.05 de	1.24 d	2.19 e
T6	0.65 b	1.17 b	0.56 b	1.43 b	0.02 e	0.03 ef	1.09 e	1.06 f
T7	0.79 a	1.30 a	0.68 a	1.53 a	0.01 e	0.02 f	0.91 f	1.86 g
V1T1	0.06 f	0.31 g	0.15 g	0.45 e	0.13 a	0.14 a	1.57 a	2.49 a
V1T2	0.10 ef	0.55 f	0.21 f	0.75 d	0.12 a	0.12 a	1.55 a	2.47 a
V1T3	0.16 de	0.66 e	0.28 e	0.89 d	0.09 b	0.09 a	1.46 b	2.39 a
V1T4	0.25 d	0.76 d	0.35 d	1.00 c	0.08 b	0.07 a	1.41 b	2.32 a
V1T5	0.43 c	0.90 c	0.40 c	1.16 b	0.04 c	0.05 a	1.29 c	2.24 a
V1T6	0.57 b	1.07 b	0.50 b	1.38 a	0.02 cd	0.03 a	1.13 d	2.12 a
V1T7	0.70 a	1.23 a	0.60 a	1.48 a	0.02 d	0.02 a	0.96 e	1.93 a
V2T1	0.10 f	0.36 e	0.20 d	0.49 f	0.11 a	0.16 a	1.50 a	1.14 a
V2T2	0.21 ef	0.81 d	0.34 d	1.05 e	0.08 b	0.10 a	1.46 a	1.16 a
V2T3	0.31 de	0.89 d	0.37 cd	1.15 d	0.07 b	0.08 a	1.41 ab	1.16 a
V2T4	0.53 d	1.07 c	0.48 c	1.30 c	0.02 c	0.07 a	1.33 bc	1.09 ab
V2T5	0.63 c	1.11 c	0.50 c	1.35 c	0.02 c	0.05 a	1.20 c	0.83 c
V2T6	0.73 b	1.27 b	0.63 b	1.49 b	0.02 c	0.03 a	1.04 d	0.60 d
V2T7	0.88 a	1.38 a	0.77 a	1.58 a	0.01 c	0.02 a	0.85 e	0.48 e

Different letters within a column indicate significant differences according to Duncan's multiple range test ($P < 0.05$). V1: 'Rishbaba' cultivar, V2: 'Sahebi' cultivar, T1 to T7: 0 (Control), 25, 50, 75, 100, 125 and 150 mM NaCl, respectively.

expansion and velocity resulting in the reduction of plant final size (Hsiao 1973; Munns 2002; Bacelar *et al.* 2006; Neocleus and Vasilakakis 2007). On the other hand, reducing water uptake by roots due to the reduction of soil osmotic potential causes stomatal closure, limits photosynthesis and decreases the assimilation of photosynthetic components and growth (Ben-Asher *et al.* 2006; Grimplet *et al.* 2009; Nawaz *et al.* 2010). The other impact of salinity stress on plants is leaf abscission which in turn decreases the availability of carbohydrates or growth regulators in meristematic regions, thereby suppressing growth (Kozlowski 1997). Low water potential in saline soils also reduces leaf production and expansion and finally decreases the photosynthesis rate (Boyar 1976).

In this study, by increasing the salinity level, leaf production and expansion decreased as well. Blum and Pnuel (1990) reported that the reason why SLA decreases under salinity stress is because dry matter accumulates in plants. Decreasing salinity stress reduces water loss through evaporation and transpiration resulting in reducing stress injury and preserving photosynthesis in plants (Dichio *et al.* 2002; Mahajan and Tuteja 2005; Rouhi *et al.* 2007; Grimplet *et al.* 2009; Cramer 2010). Chartzoulakis *et al.* (2002) proposed that by increasing salinity level, leaf area decreased in olives, but this reduction was less in more salt-tolerant cultivars. They also reported that salinity affected leaf area more than height and dry weight of plants.

In the present experiment, by increasing salinity stress, the fresh and dry weigh of plants decreased which could be due to a reduction of photosynthesis capacity. Chartzoulakis *et al.* (2002) stated that reduced photosynthesis under salinity stress is the main factor reducing biomass in olive. Neocleus and Vasilakakis (2007) also obtained similar results from red raspberries. In fact, increasing the root to shoot fresh and/or dry weight ratios are generally recognized to be a morphological adaptation to water potential reduction (Chartzoulakis *et al.* 2002; Neocleus and Vasiakakis 2007). This is because salinity affects the aerial parts of plants more than roots, so the ratio of roots to shoots increases under stress (Mahajan and Tuteja 2005). Shani and Ben-Gal (2005) reported a linear relation between the amount of transpiration and biomass production in 'Sugrone' grape.

By increasing salinity stress, Na^+ and Cl^- concentrations increased significantly in the leaves and roots of grape. This increase was observed more in roots than in leaves and more in 'Sahebi' than in 'Rishbaba'. This is an important mechanism of tolerant plants preventing toxic ions to be transported to the aerial organs. By using this mechanism, 'Rishbaba' transported less Na^+ and Cl^- from roots to leaves under salinity stress. The Na^+ concentration in leaves of salt-sensitive rootstocks has been reported to be higher compared to tolerant ones. This shows that Na^+ transportation from roots to aerial parts of the plant occurs more in sensitive rootstocks (Storey and Walker 1999). A high concentration of Na^+ in leaves causes cell growth to be reduced and inhibits cell division and expansion. Cells could prevent toxicity of the above ions by isolating them from the cytoplasm by compartmentalizing the ions in vacuoles. Salt injury occurs when Na^+ and Cl^- over-accumulate in leaves and cells cannot compartmentalize these ions in their own vacuoles (Munns 2002). Inhibition of Na^+ transport from root to aerial parts in tolerant cultivars is an important mechanism to avoid toxic effects of this ion (Paranychianakis and Chartzoulakis 2005). Although by increasing salinity level, K^+ concentration decreased in roots and leaves of both cultivars, no significant difference was observed between the cultivars in terms of K^+ concentration in their leaves. In salt-sensitive plants, decreasing K^+ concentration in leaves causes osmotic imbalance, limits photosynthesis through stomatal closure and finally reduces total plant growth (Mahajan and Tuteja 2005). Salt stress affects plant physiology at the cellular level and at the entire plant level through osmotic and ionic adjustments resulting in the reduction of biomass production. Despite causing osmotic and ionic stress, salinity can induce ionic imbalances that induce potassium deficiency and impair the selectivity of root membranes (Nawaz *et al.* 2010).

In this study, relations were also observed between K^+ concentration in leaves, shoot length and SLA. By increasing salinity stress, Na^+ and Cl^- concentrations increased and caused toxicity in plants. This in turn could affect the uptake and transportation of some essential elements such as K^+, Ca^{2+} and Mg^{2+} creating a nutritional imbalance (García-Sánchez *et al.* 2002). Less transportation of Cl^- in plants than Na^+ is the main reason why the ratio of Na^+ to Cl^- increases in tissues under salinity stress (Tattini *et al.* 1992). Storey and Walker (1999) stated that the reason for salinity resistance in some citrus cultivars since these cultivars transport more K^+ and less Na^+ and Cl^- from roots to the shoots.

There was a significant difference between the cultivars in this study in terms of Ca^{2+} concentration. Ca^{2+} decreased by increasing salinity level and this was observed more in 'Sahebi' than 'Rishbaba' and in roots more than in leaves. Salinity increases the Na^+ to Ca^{2+} ratio in plants (Parida and Das 2005), reduces available water under salt stress and decreases Ca^{2+} transport in xylem and phloem resulting in a reduction of Ca^{2+} concentration in aerial parts of plants (Jones and Taridiue 1998.

CONCLUSION

Salinity decreased growth in both studied grape cultivars, more in plant aerial parts than roots. In this study, grapes used a mechanism of salt tolerance by lowering Na^+ and Cl^- transport from roots to aerial parts, similar to glycophytes under stress conditions. By increasing salinity stress level in plant tissues, K^+ and Ca^{2+} concentrations decreased and this occurred more in roots than in leaves. The K^+ to Na^+ and Na^+ to Cl^- ratios can be the good characters for measuring the amount of plant tolerance to salinity stress. Based on the results of the present study, 'Rishbaba' was recognized as being more tolerant to salinity than 'Sahebi'.

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