ION AND MINERAL CONCENTRATIONS IN ROOTS AND LEAVES OF TWO GRAPEVINE CULTIVARS AS AFFECTED BY NITRIC OXIDE FOLIAR APPLICATION UNDER NACL STRESS

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Abstract

Aim: The present study was conducted to investigate the effects of SNP (sodium nitroprusside, as nitric oxide donor) on mineral concentration in two grapevine (*Vitis vinifera* L.) cultivars, Qarah Shani and Thompson Seedless, under different levels of NaCl stress.

Methods and results: The plants were exposed to NaCl at the rate of 0, 25, 50, 75 and 100 mM in nutrient solution and foliar spray of SNP at 0, 0.5, 1 and 1.5 mM under an open hydroponic system. Results indicated that with increasing salinity levels, the Cl^- and Na^+ concentrations increased and the K^+, Ca^{2+}, Mg^{2+}, NO_3-N, Zn^{2+}, Fe^{2+} concentrations and K^+/Na^+ ratio decreased in both cultivars. However, application of SNP mitigated the Cl^- and Na^+ concentrations and improved the K^+, Ca^{2+}, Mg^{2+} and NO_3-N concentrations in leaves and roots of both cultivars. The application of SNP did not significantly affect Zn^{2+} and Fe^{2+} concentrations under 100 mM NaCl.

Conclusion: The adverse effects of NaCl stress in nutrient elements uptake were ameliorated by the exogenous application of SNP in grapevine.

Significance and impact of the study: Salinity of soil and water sources is one of the most serious environmental threats in Iran. Iran ranks tenth among grape-producing countries in the world. Therefore, the application of SNP can serve as an important component to reduce the adverse effects of salinity stress in nutrient elements uptake in grapevine.

Key words: grapevine, nutrient concentration, salinity, SNP

Résumé

Objectif: La présente étude a été effectuée afin de déterminer les effets du SNP (nitroprussiate de sodium, comme donneur d'oxyde nitrique) sur la concentration en minéraux de deux cultivars de vigne (*Vitis vinifera* L.), Qarah Shani et Thompson Seedless, sous différents niveaux de stress NaCl.

Méthodes et résultats: Les plantes ont été exposées à des niveaux de NaCl de 0, 25, 50, 75 et 100 mM dans une solution nutritive et des pulvérisations foliaires de SNP de 0, 0.5, 1 et 1.5 mM dans un système hydroponique ouvert. Les résultats indiquent que l'augmentation de la salinité entraîne une augmentation de la concentration de Cl^- et Na^+ et une diminution de la concentration de K^+, Ca^{2+}, Mg^{2+}, NO_3-N, Zn^{2+}, Fe^{2+} et du rapport K^+/Na^+ dans les deux cultivars. Cependant, l'application de SNP a atténué la concentration de Cl^- et Na^+ et amélioré la concentration de K^+, Ca^{2+}, Mg^{2+} et NO_3-N dans les feuilles et les racines des deux cultivars. L'application de SNP n'a pas eu d'effet notable sur la concentration de Zn^{2+} et Fe^{2+} dans les conditions de stress NaCl de 100 mM.

Conclusion: L'application exogène de SNP a atténué les effets négatifs du stress NaCl sur l'absorption d'éléments nutritifs chez la vigne.

Signification et impact de l'étude: La salinité des sols et des cours d'eau est l'une des menaces environnementales les plus importantes en Iran. L'Iran se classe au dixième rang des pays producteurs de raisin dans le monde. Par conséquent, l'application de SNP peut constituer un élément important dans la diminution des effets négatifs du stress salin sur l'absorption d'éléments nutritifs chez la vigne.

Mots clés: vigne, concentration d'éléments nutritifs, salinité, SNP

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INTRODUCTION

The total global area of salt-affected soils has recently been estimated to be approximately 830 million ha (Martinez-Beltran and Manzur, 2005). The salinity of soil and water sources is a serious threat worldwide and in many parts of our country, Iran. Estimated land area affected by salinity varies between 16 and 23 million ha (Siadat *et al.*, 1997). Salinity decreases water potential and causes osmotic effects, specific ion toxicity and/or nutritional disorders (Läuchli and Grattan, 2007). Many reports have shown that salinity reduces the absorption of some nutrients in plants (Garcia and Charbaji, 1993 ; Rogers *et al.*, 2003 ; Hu and Schmidhalter, 2005).

Grapevine (*Vitis vinifera* L.) is usually grown in semi-arid areas where drought and salinity are the most prevalent problems (Cramer *et al.*, 2007). Grapevines are considered as moderately sensitive to salinity and the damage is primarily caused by Cl^- ions (Fisarakis *et al.*, 2001). It has been shown that the addition of NaCl to nutrient solution increases Na^+ ions in the vegetative organs of grapevine (Garcia and Charbaji, 1993). Physiological disturbances such as reduction in stomatal conductance and photosynthesis, reduction in both growth and vegetative biomass, and reduction in yield were reported in grapevine cultivars under saline conditions. In severe cases, salt stress symptoms develop into necrotic areas on leaves, starting at leaf margins and progressing inwards (Walker *et al.*, 2004).

It has been documented that some bioregulators ameliorate the deleterious effects of environmental stresses on plants. Under environmental stress conditions (of both abiotic and biotic origins), elevated generation of nitric oxide (NO) occurs in various organs of the plant (Wu *et al.*, 2011). NO is an endogenous signaling molecule in animals and plants, which mediates responses to abiotic and biotic stresses (Zhang *et al.*, 2007). Research findings have indicated that application of exogenous NO increases tolerance of plants to salt, heavy metals, chilling and ultraviolet-B radiation (Tan *et al.*, 2008). The involvement of NO in salt tolerance has drawn much attention in the past few years. The NO function in salt tolerance has been demonstrated in some plant species (Uchida *et al.*, 2002).

Pretreatment with NO donor, SNP (sodium nitroprusside), protected young rice seedlings, resulting in better plant growth and viability (Uchida *et al.*, 2002), promoted seed germination and root growth of yellow lupine seedlings (Kopyra and Gwozdz, 2003), and increased the growth and dry weight of maize seedlings (Zhang *et al.*, 2007) under saline conditions. Zhao *et al.* (2004) in *Phragmites communis* and Zhang *et al.* (2007) in *Populus euphratica* reported that NO enhances salt tolerance of calluses under salinity through increasing K^+/Na^+ ratio. Strong evidence that NO regulates cytosolic Ca^{2+} homeostasis in plant cells was provided by Lamotte *et al.* (2006).

So, there is a strong potential for NO to reduce ion toxicity and improve mineral nutrient uptake by grapevine roots under salinity. However, there is no report on grapevine in relation to the effect of NO on plant responses in saline conditions. Therefore, the main objective of this research was the evaluation of the effects of SNP, as a NO donor, on mineral composition in two grapevine (*Vitis vinifera* L.) cultivars which differ in tolerance under salt stress.

MATERIALS AND METHODS

1. Plant materials and growth conditions

Hardwood cuttings (with two nodes) of *Vitis vinifera* L. cvs. Qarah Shani and Thompson Seedless were collected (winter 2011) and planted in perlite to root. Well rooted cuttings were transplanted to pots filled with perlite and cocopeat (1:1 v/v) in an open hydroponic system. The pots were kept in a greenhouse with a photoperiod of 16:8, a relative humidity of 60±5 % and night and day temperatures of 19±3 and 27±3 °C, respectively.

Modified ½ strength Hoagland nutrient solution (Hoagland and Arnon, 1950) containing 2.5 mM Ca $(NO_3)_2$, 1 mM $MgSO_4$, 2.5 mM KNO_3, 0.5 mM KH_2PO_4, 23 µM H_3BO_3, 6 µM $MnSO_4$, 0.7 µM $ZnSO_4$, 0.3 µM $CuSO_4$, 0.1 µM H_2MoO_4 and 32 µM Fe-EDTA was used. The nutrient solution pH was adjusted to 6.3.

At the beginning of the experiment, the plants were supplied with 200 mL nutrient solution three times a week. Each time, about 20 % of the nutrient solution was drained from the bottom of the pots. Culture medium was weekly leached by tap water to prevent ion accumulation. The nutrient solution was replaced weekly. All vines were trimmed to a single shoot and axillary buds were removed as they appeared.

2. NaCl application

Salt stress was implemented to the 100-day-old grapevine plants fertilized with ½ strength Hoagland nutrient solutions. Final concentrations were 25, 50, 75, and 100 mM of NaCl salinity. The control plants only received nutrient solution. The electrical

conductivity (EC) of the salt-treated solutions at 25 °C was 1.4, 3.6, 7.5, and 11.8 ds/m, respectively. To avoid salinity shock, NaCl was gradually added to the nutrient solutions at the rate of 25 mM per day to reach the final salinity level. The trial lasted for seven weeks.

3. NO foliar spray

SNP ($Na_2[Fe(CN)_5NO]$), as a NO donor, was applied to the foliage at the rate of 0, 0.5, 1 and 1.5 mM. A control group of plants was grown without NaCl and sprayed with deionized water. Tween-20 (0.1 %) was added to all solutions as surfactant. The plants were sprayed with SNP solutions at two-week intervals during the experimental period.

4. Mineral content in leaves and roots

At the end of the experiment, mature leaves on mid stem and fibrous roots were sampled and rinsed with deionized water, then oven-dried at 70°C for 48 hours. The dried tissues were analyzed for their Na^+, Cl^-, NO_3-N, K^+, Ca^{2+}, Mg^{2+}, Zn^{2+}, and Fe^{2+} contents.

The Na^+ and K^+ contents of tissues were analyzed by flame photometry (Fater 405 model, Iran), the Cl^- content was analyzed by chloride analyzer (Model 926, Sherwood Scientific Ltd.), and nitrate (NO_3-N) was colorimetrically analyzed by nitration of salicylic acid (Cataldo *et al.*, 1975). Another group of samples (leaves and roots) was used to determine Ca^{2+}, Mg^{2+}, Fe^{2+}, and Zn^{2+} concentrations. Dried samples (0.4 g) were ground and ashed at 550 °C in a porcelain crucible for 5 hours, separately. The white ash was digested in 10 mL HCl (2M), filtered into a 50-mL volumetric flask, and brought to a final volume of 50 mL with distilled water. The concentration of Ca^{2+}, Mg^{2+}, Fe^{2+}, and Zn^{2+} was determined by an atomic absorption spectrophotometer (Shimadzu AA-6300, Japan).

5. Statistical analysis

The experiment was a CRD-based factorial with four replicates. Data were analyzed by SAS 9.1 Statistical Software (2002) and means were compared using Duncan's Multiple Range Test at $P < 0.01$.

RESULTS

1. Na^+ and Cl^- concentrations

The presence of NaCl, especially at higher level, resulted in an increase in total Na^+ content in the leaves and roots of both Thompson Seedless and Qarah Shani cultivars; however, in the leaves of Qarah Shani Na^+ content was 10.5 % higher than in the leaves of Thompson Seedless. Foliar application of SNP significantly decreased Na^+ concentration, as compared to salt-exposed plants, in both leaves and roots (Figure 1).

At the end of the salinity stress period, leaf and root Cl^- concentration increased with increasing NaCl

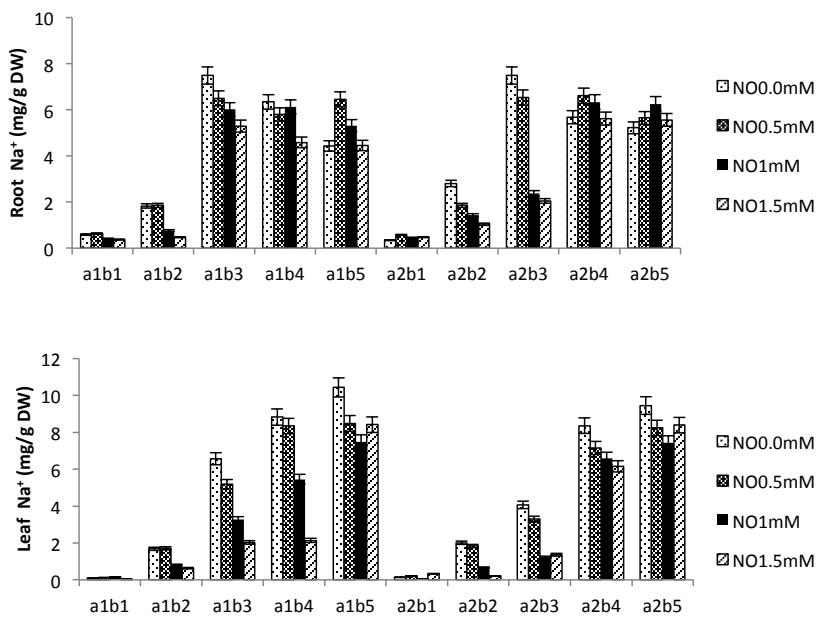

Figure 1 - Interaction of cultivar, salinity and SNP on Na^+ contents in roots (upper graph) and leaves (lower graph) of two grapevine cultivars. a : cultivar (a1 : Qarah Shani, a2 : Thompson Seedless), b : salinity (b1 : 0 (control), b2 : 25, b3 : 50, b4 : 75 and b5 : 100 mM NaCl).

concentration in nutrient solution. In the presence of 100 mM NaCl in the medium (without SNP), Cl^- concentration in the leaves of Thompson Seedless and QarahShani was almost 8.4- and 4.6-fold higher, respectively, compared to the control (without NaCl) and in the roots almost 4- and 6-fold higher, respectively, compared to control plants. The application of SNP mitigated Cl^- concentration in roots of both cultivars compared to salt stress-exposed plants without SNP (Figure 2).

2. Ca^{2+} and Mg^{2+} concentrations

NaCl treatments decreased Ca^{2+} concentration in the leaves and roots of both cultivars. In the 100 mM NaCl treatment, Ca^{2+} content in the leaves of both cultivars was not significantly different, but was 39 % higher in the roots of Qarah Shani compared to Thompson Seedless. Application of SNP increased Ca^{2+} concentration in the roots compared to salinity treatments without SNP application (Table 1).

Salinity significantly decreased Mg^{2+} concentration in the leaves of Thompson Seedless but not in leaves of Qarah Shani. The application of SNP did not significantly change Mg^{2+} concentration in the leaves of Qarah Shani but increased Mg^{2+} concentration in the leaves of Thompson Seedless, compared to NaCl-exposed plants, when salinity in nutrient solution increased (Table 1).

3. K^+ and NO_3-N concentrations

K^+ concentration decreased markedly when NaCl concentration increased; this decrement was more pronounced in the roots than in the leaves. In the 100 mM NaCl treatment, K^+ content in the leaves of Qarah Shani and Thompson Seedless decreased by 71.4 and 70.1 % when compared to control plants, respectively. The application of SNP resulted in an increase of K^+ content compared to control in saline conditions (Figure 3).

NO_3-N concentrations in the roots and leaves decreased considerably when the NaCl concentration in the nutrient solution increased. The application of SNP substantially increased the content of NO_3-N in the salt-treated leaves and roots of both cultivars, when compared to salt-treated plants without SNP (Figure 4).

4. Fe^{2+} and Zn^{2+} concentrations

Fe^{2+} content in the leaves and roots of NaCl-exposed plants was significantly decreased in both cultivars. In the 100 mM NaCl treatment, Fe^{2+} content in the leaves of Thompson Seedless and Qarah Shani was almost 2.3- and 2.6-fold lower compared to control (without NaCl) plants, respectively; however, this amount was almost 3-fold lower in the roots of both cultivars in comparison with control plants. The Fe^{2+}

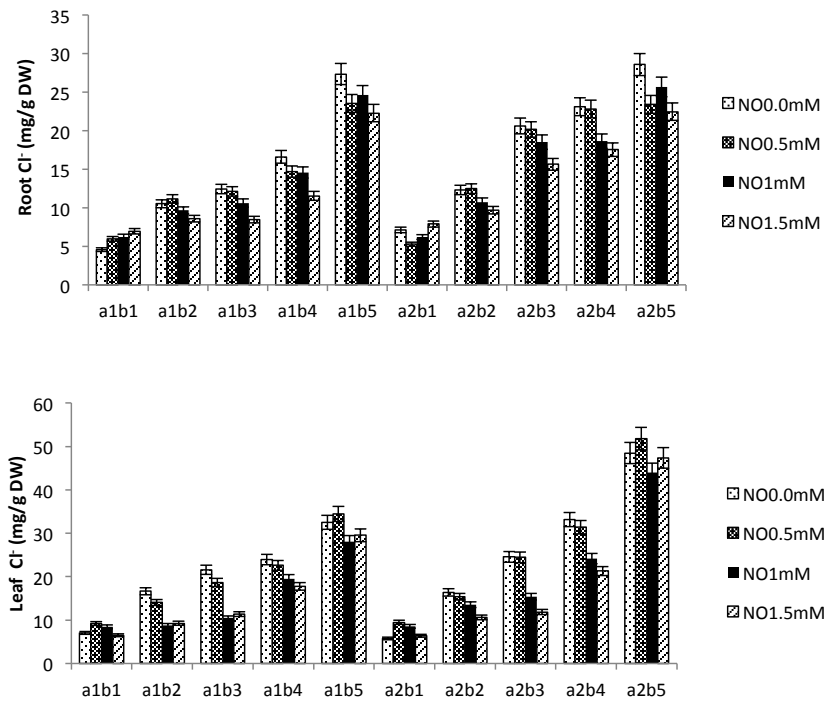

Figure 2 - Interaction of cultivar, salinity and SNP on Cl^- contents in roots (upper graph) and leaves (lower graph) of two grapevine cultivars. a: cultivar (a1: Qarah Shani, a2: Thompson Seedless), b: salinity (b1: 0 (control), b2: 25, b3: 50, b4: 75 and b5: 100 mM NaCl).

concentration of roots was significantly decreased from 172.93 to 56.83 mg/kg DW in Thompson Seedless and from 179.13 to 61.27 mg/kg DW in Qarah Shani with increasing salinity (Table 1).

Salt treatment significantly decreased Zn^{2+} content in the leaves and roots when compared to control plants. The decrease in Zn^{2+} concentration was alleviated by the application of SNP under low levels of salinity (25 and 50 mM). SNP application did not significantly affect Zn^{2+} content in the leaves and roots under the 75 and 100 mM NaCl treatments (Table 1).

DISCUSSION

The present study focused on the possible role of NO in improving tolerance of two grapevine cultivars, Thompson Seedless and Qarah Shani, to salt stress. We assessed the effects of NO on counteracting Na^+ and Cl^- toxicity on mineral nutrition absorption. To date, there are limited reports about NO influences on nutrient absorption under salinity stress. The findings of this research showed that Na^+ content increased in leaves and roots of both cultivars as NaCl level increased. The Na^+ concentration in the leaves of Qarah Shani, especially in salinity levels higher than 50 mM, was higher than in Thompson Seedless.

Our results are consistent with previous studies conducted on salt stress in grapevine (Stevens *et al.*, 1996; Singh *et al.*, 2000; Fisarakis *et al.*, 2005) and pistachio (Picchioni *et al.*, 1991). The difference in Na^+ content in roots showed that the roots have limited ability for Na^+ accumulation and, thus, Na^+ is most probably transported to aerial parts. It is reported that Na^+ is initially retained in the roots of woody plants and then transported to the leaves, causing leaf burn (Tester and Davenport, 2003).

The similarity of the hydrated ionic radii of Na^+ and K^+ makes it difficult for cell membrane transport system to discriminate between these two ions and it seems that this is the basis of Na^+ toxicity under high salinity (Blumwald, 2000). Na^+ ions can be transported into cells by K^+ transporters (Parida and Das, 2005). This factor could be the reason for the toxicity of Na^+ ions, especially under salinity levels higher than 50 mM, in both grapevine cultivars. In grapevine, it has been indicated that Na^+ and K^+ ions show strong competition even with addition of small amounts of NaCl in nutrient solution (Troncoso *et al.*, 1999).

Table 1 - Interaction of cultivar, salinity and SNP on Ca^{2+}, Mg^{2+}, Fe^{2+} and Zn^{2+} contents in roots and leaves of two grapevine cultivars

Cultivar of *V. vinifera* L.	NaCl (mM)	SNP (mM)	Ca^{2+} (%)		Mg^{2+} (%)		Fe^{2+} (mg/kg DW)		Zn^{2+} (mg/kg DW)	
			leaves	roots	leaves	roots	leaves	roots	leaves	Roots
Qarah Shani	0	0	0.86 a	0.94 a	1.99 a-c	0.56 ab	312.4 a	179.13 ab	51.56 a-c	21.77 b-e
		0.5	0.83 a	0.92 ab	1.98 a-d	0.55 ab	300.84 ab	180.23 ab	53.04 ab	21.57 b-f
		1	0.85 a	0.95 a	2.09 a	0.59 a	308.08 a	183.27 a	49.34 a-c	18.99 d-f
		1.5	0.81 a	0.96 a	2.04 ab	0.57 ab	307.19 a	177.90 ab	47.65 a-c	19.41 d-f
	25	0	0.81 a	0.70 d-g	1.79 b-i	0.56 ab	277.11 a-e	150.43 c-g	43.68 a-c	16.01 f-j
		0.5	0.77 a-d	0.73 d-f	1.92 a-f	0.53 a-c	263.36 b-f	156.69 b-f	46.66 a-c	17.91 e-h
		1	0.76 a-d	0.75 c-e	1.97 a-d	0.57 ab	280.36 a-e	162.26 a-d	48.25 a-c	18.42 d-g
		1.5	0.8 ab	0.88 a-c	1.91 a-f	0.58 a	287.85 a-d	163.08 a-d	50.14 a-c	19.79 c-f
	50	0	0.64 e-h	0.56 g-k	1.74 c-i	0.37 f-k	232.34 f	133.49 f-i	31.48 d-f	12.91 h-l
		0.5	0.67 c-g	0.59 f-j	1.78 b-i	0.39 f-j	247.01 d-f	136.65 e-i	27.99 e-g	13.40 g-k
		1	0.66 d-g	0.72 d-f	1.79 b-i	0.37 f-k	256.42 c-f	143.89 d-h	39.08 c-e	17.76 e-i
		1.5	0.69 b-f	0.79 b-d	1.87 a-g	0.46 c-f	272.97 a-f	161.10 a-e	49.62 a-c	18.87 d-f
	75	0	0.62 f-i	0.47 i-n	1.67 e-k	0.32 i-n	146.24 h-m	95.45 k-n	17.63 g-i	9.18 k-o
		0.5	0.61 f-j	0.54 h-l	1.71 d-j	0.30 j-p	171.03 g-k	94.53 k-n	19.99 f-i	10.67 j-n
		1	0.67 c-g	0.59 f-j	1.65 f-k	0.34 i-m	166.32 g-l	116.51 i-k	14.68 hi	9.59 k-o
		1.5	0.65 e-h	0.64 e-h	1.75 c-i	0.28 k-r	177.89 g-i	127.53 g-j	18.63 g-i	11.59 j-m
	100	0	0.48 kl	0.41 l-o	1.74 c-i	0.25 m-s	121.23 m	61.27 o-q	18.64 g-i	5.68 n-p
		0.5	0.56 g-l	0.39 m-p	1.56 i-l	0.26 l-s	123.78 lm	56.70 o-q	15.55 hi	4.39 op
		1	0.46 l	0.46 j-n	1.59 h-k	0.18 s	128.47 k-m	67.69 o-q	21.30 f-i	6.45 m-p
		1.5	0.58 g-k	0.52 h-m	1.89 a-g	0.19 rs	137.81 i-m	72.97 n-p	20.54 f-i	3.45 p
Thompson Seedless	0	0	0.85 a	0.72 d-f	2.01 a-c	0.56 ab	298.97 a-c	172.93 a-c	54.99 a	26.62 ab
		0.5	0.87 a	0.75 c-e	1.95 a-e	0.52 a-d	297.32 a-c	169.97 a-c	51.23 a-c	25.21 a-c
		1	0.82 a	0.71 d-f	2.00 a-c	0.48 b-e	311.51 a	176.37 a-c	47.94 a-c	27.65 a
		1.5	0.83 a	0.75 c-e	1.91 a-f	0.46 c-f	301.31 ab	175.58 a-c	54.83 a	25.73 ab
	25	0	0.79 ab	0.60 e-i	1.95 a-e	0.39 e-i	247.56 d-f	163.93 a-d	44.45 a-c	19.18 d-f
		0.5	0.76 a-d	0.62 e-I	2.02 a-c	0.44 d-g	251.24 d-f	155.59 b-f	53.28 ab	17.70 e-i
		1	0.78 a-c	0.71 d-f	2.01 a-c	0.43 d-h	263.39 b-f	163.42 a-d	47.58 a-c	23.24 a-e
		1.5	0.81 a	0.75 c-e	2.04 ab	0.40 e-i	273.95 a-f	158.34 a-f	53.91 a	23.92 a-d
	50	0	0.60 f-j	0.42 k-o	1.59 h-k	0.31 i-o	184.28 gh	104.99 j-l	25.03 f-h	11.42 j-m
		0.5	0.61 f-j	0.39 l-p	1.53 i-l	0.29 k-q	191.23 g	99.38 k-m	47.54 a-c	12.43 i-l
		1	0.75 a-e	0.44 k-o	1.61 g-k	0.35 g-l	244.14 ef	116.79 i-k	40.87 b-d	22.13 b-e
		1.5	0.77 a-d	0.61 e-i	1.86 a-h	0.35 g-l	258.44 b-f	125.67 h-j	44.78 a-c	21.91 b-e
	75	0	0.57 g-l	0.35 n-p	1.45 j-l	0.24 n-s	133.78 j-m	63.85 o-q	15.19 hi	7.60 l-p
		0.5	0.52 i-l	0.37 m-p	1.51 i-l	0.23 n-s	160.34 g-m	79.35 m-o	11.65 i	6.69 m-p
		1	0.63 f-i	0.41 l-o	1.53 i-l	0.24 n-s	174.52 g-j	75.56 m-p	13.64 hi	8.48 k-p
		1.5	0.65 e-h	0.49 h-n	1.96 a-d	0.26 l-s	179.45 g-i	81.19 l-o	17.70 hi	7.45 l-p
	100	0	0.51 i-l	0.25 p	1.32 l	0.21 p-r	131.11 k-m	56.83 o-q	13.48 hi	4.27 op
		0.5	0.49 j-l	0.29 op	1.40 kl	0.19 q-s	129.9 k-m	44.65 q	15.28 hi	5.64 n-p
		1	0.58 g-k	0.38 m-p	1.56 i-l	0.23 n-s	149.02 h-m	51.53 pq	17.39 g-i	5.20 n-p
		1.5	0.54 h-l	0.43 k-o	1.79 b-i	0.22 o-s	135.59 j-m	67.45 o-q	12.62 hi	6.39 m-p

Mean values followed by different letters are significantly different ($P < 0.01$).

Our results indicate that SNP application could reduce the absorption of Na^+ ions in both leaves and roots, especially in salinity levels less than 50 mM. There are few reports about the effect of NO on decreasing Na^+ ion uptake under salinity stress. Zhang *et al.* (2004) reported that both NO and NaCl treatments stimulated vacuolar H^+-ATPase and H^+-PPase activities, resulting in increased H^+ translocation and Na^+/H^+ exchange. NaCl-induced H^+-ATPase and H^+-PPase activities were diminished by NO scavenger MB-1 (Zhang *et al.*, 2007).

The higher Cl^- concentrations in petioles and laminas reflect the poor capacity of *Vitis vinifera* L. vines for Cl^- exclusion (Downton, 1977). The concentration of Cl^- in leaves of salinized grapevines was higher than in roots. The lower Cl^- concentrations in leaves of Qarah Shani compared to Thompson Seedless at high salinity indicate a greater ability of Qarah Shani to restrict uptake and/or root-to-shoot transport of Cl^- and a possible dilution effect due to distribution of accumulated Cl^- throughout plant biomass. However, our study showed that Qarah Shani is a good Cl^- excluder and Thompson Seedless a poor Cl^- excluder. In woody perennial species, enhanced Cl^- exclusion from leaves/shoots is associated with increased salt tolerance rather than Na^+ exclusion (Storey *et al.*, 2003). Control of Cl^- transport and Cl^- exclusion from shoots is correlated with salt tolerance in many species, for example, *Citrus* and *Vitis* (Sykes, 1992; Romero-Aranda *et al.*, 1998; Moya *et al.*, 2003) and *Pinus banksiana* (Franklin and Zwiazek, 2004).

In the present study, NO_3-N content was significantly reduced in salt-stressed grapevines. It has been reported that an increase in salinity levels in root medium leads to a decrease of nitrogen uptake (Neumann, 1997). Competition between Cl^- and NO^-_3 uptakes can occur in plants grown under saline stress (Melgar *et al.*, 2008). The increase in uptake and accumulation of Cl^- ions in plant tissue generally results in the decrease of NO^-_3 accumulation in the plant's aerial parts (Lara *et al.*, 2003). Banuls *et al.* (1990) indicated that N accumulation in Navel orange scions grafted onto Cleopatra mandarin and Troyer citrange was negatively correlated with Cl^- accumulation during salinity stress.

It could be concluded that there is a strong relationship between decreased NO^-_3 uptake and increased Cl^- content under salt stress in both cultivars. Measurement of NO^-_3 under high salinity showed that with increasing Cl^- concentration in roots and leaves, NO^-_3 content was reduced. Based on our findings, SNP significantly increased NO^-_3 concentration in the roots and leaves in both cultivars when compared to NaCl-exposed plants. The use of SNP, especially in salinity levels lower than 50 mM,

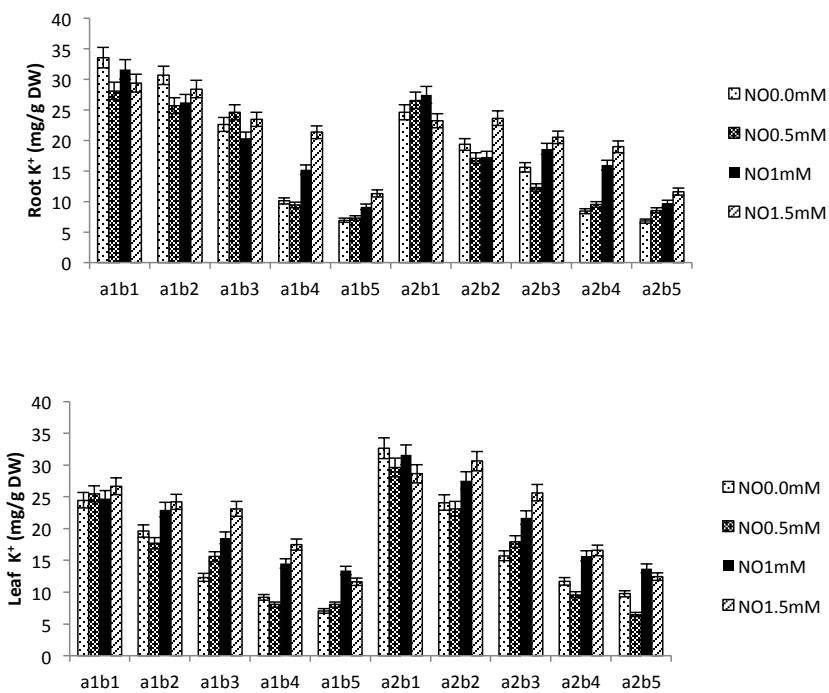

Figure 3. Interaction of cultivar, salinity and SNP on K^+ contents in roots (upper graph) and leaves (lower graph) of two grapevine cultivars. a : cultivar (a1 : Qarah Shani, a2 : Thompson Seedless), b : salinity (b1 : 0 (control), b2 : 25, b3 : 50, b4 : 75 and b5 : 100 mM NaCl).

had a positive impact on increasing the amount of nitrate.

Increased NaCl salinity caused a significant decrease in the K^+ content of leaves and roots in both cultivars. It is now clear that K^+ ions can enter cells through channels which are often more permeable to Na^+ under saline conditions (Parida and Das, 2005). Because of the physicochemical similarity between Na^+ and K^+ (i.e., ionic radius and ion hydration energy), the former competes with K^+ for major binding sites in key metabolic processes in the cytoplasm (Shabala and Cuin, 2008).

The results of the present study showed that the leaf and root K^+/Na^+ ratio of Thompson Seedless and Qarah Shani tended to decrease progressively with increasing salinity. The decrease of K^+ recorded in roots, which resulted in a decrease in the K^+/Na^+ ratio, may also provide a mechanism by which grapevines achieve ionic balance following uptake of high Na^+ concentrations in root. As a result, K^+/Na^+ ratio may be a useful criterion for screening salt-tolerant plants under saline conditions (Munns *et al.*, 2006). In untreated control plants, Qarah Shani had much greater K^+/Na^+ ratio than Thompson Seedless. This implies a competition between Na^+ and K^+ in grapevines, resulting in K^+/Na^+ antagonism. The reduction in K^+ uptake caused by Na^+ is likely to be the result of the competitive intracellular influx of both ions (Cerda *et al.*, 1995).

The results presented here indicated that application of SNP increased K^+/Na^+ ratio in the roots and leaves of both cultivars under salinity conditions. NO may enhance salt tolerance in plants via increasing the expression of plasma membrane Na^+/H^+ antiporter gene and H^+-ATPase gene, which are required for Na^+ homeostasis and K^+ acquisition (Qiao and Fan, 2008). In another research, Zhang *et al.* (2004) reported that NO enhanced salt tolerance in maize seedlings through increasing K^+ accumulation in roots, leaves and sheathes, while decreasing Na^+ accumulation. NO induced salt tolerance of *Populus euphratica* calluses under salt stress via increasing the K^+/Na^+ ratio and was dependent on the increased plasma membrane H^+-ATPase activity (Zhang *et al.*, 2007).

Ca^{2+} and Mg^{2+} concentration in both Thompson Seedless and Qarah Shani cultivars was decreased under the influence of salt stress. Given that Na^+ readily displaces Ca^{2+} from its extracellular binding sites, Ca^{2+} availability could be seriously reduced under salinity, especially at low Ca^{2+}/Na^+ ratios. Calcium deficiency, in general, can impair the selectivity and the integrity of cell membrane and

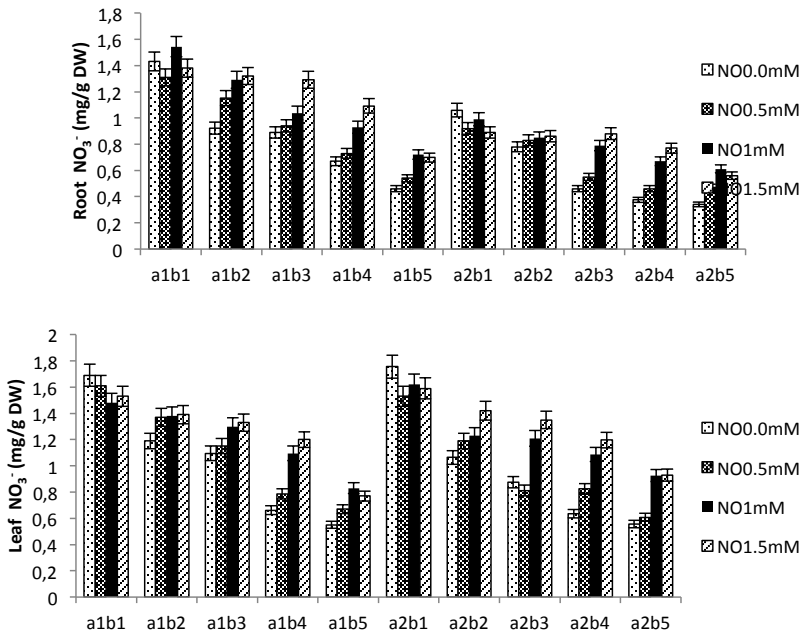

Figure 4. Interaction of cultivar, salinity and SNP on NO_3^- contents in roots (upper graph) and leaves (lower graph) of two grapevine cultivars. a : cultivar (a1 : Qarah Shani, a2 : Thompson Seedless), b : salinity (b1 : 0 (control), b2 : 25, b3 : 50, b4 : 75 and b5 : 100 mM NaCl).

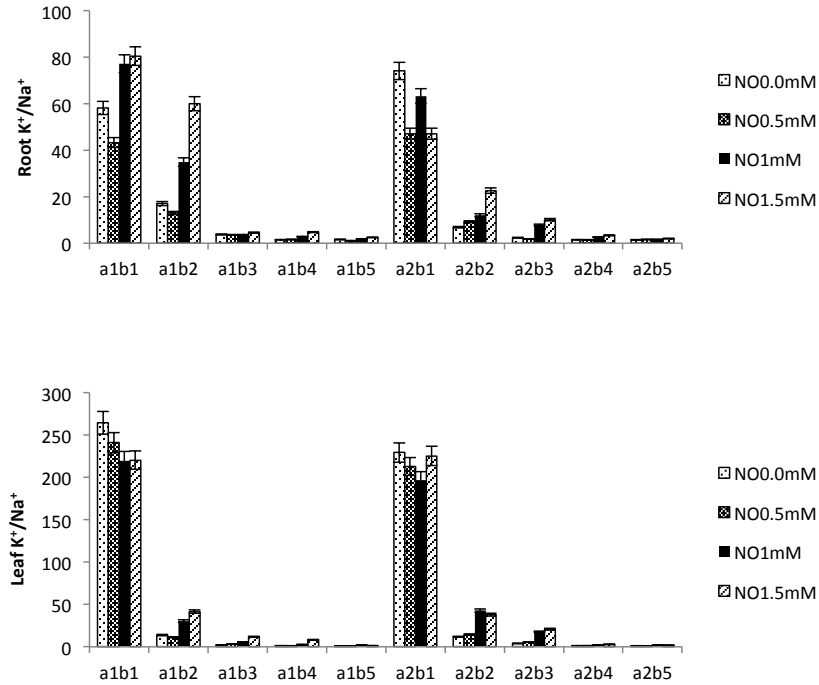

Figure 5. Interaction of cultivar, salinity and SNP on K^+/Na^+ contents in roots (upper graph) and leaves (lower graph) of two grapevine cultivars. a : cultivar (a1 : Qarah Shani, a2 : Thompson Seedless), b : salinity (b1 : 0 (control), b2 : 25, b3 : 50, b4 : 75 and b5 : 100 mM NaCl).

permit the passive accumulation of Na^+ in plant tissue (Hu and Schmidhalter, 2005).

As NaCl concentration in nutrient solution was increased, Mg^{2+} content in the roots and leaves of both grape cultivars decreased. These results are in agreement with those of Sivritepe *et al.* (2010), who reported that NaCl salinity led to decreased Mg^{2+} concentration in the leaves and roots of 'Muskule' grafted vines. Mg^{2+} concentration of leaves was increased with increasing salinity in own-rooted and 'Ramsey' rootstock-grafted 'Sultana' vines (Walker *et al.*, 1997). Salinity significantly reduced Mg^{2+} content in *Pistacia vera* L. leaves on UCB-1 and *P. atlantica* (Ferguson *et al.*, 2002) and leaf Mg^{2+} concentration in Citrus (Ruiz *et al.*, 1997). It is well known that H^+-ATPase in plasma membrane plays an important role in the transport of multiple ions (Shi and Zhu, 2008) and there are investigations indicating that NO could induce H^+-ATPase activity (Hayat *et al.*, 2010), which might be responsible for increasing absorption of Ca^{2+} and Mg^{2+} under salinity stress. Zn^{2+} and Fe^{2+} concentrations were also gradually decreased in both leaves and roots, depending upon the salinity levels. Changes of Fe^{2+}, Zn^{2+}, Mg^{2+} and Ca^{2+} concentration in grapevine indicate that NaCl stress disturbs ionic homeostasis and application of SNP stimulates their maintenance.

CONCLUSION

Salinity disturbs the mineral nutrient contents in plants through its effects on nutrient availability, transport and partitioning. The results of our study indicated that salinity stress reduced the uptake of nutrient elements (NO^-_3, K^+, Ca^{2+}, Mg^{2+}, Fe^{2+} and Zn^{2+}), whereas Na^+ and Cl^- uptake increased under NaCl treatments. On the other hand, Cl^- inhibited NO^-_3 uptake in both cultivars under salinity stress. Also, K^+/Na^+ ratio was decreased progressively with increasing salinity in both cultivars. The reduction in K^+ uptake caused by Na^+ is likely to be the result of the competitive feature of both ions. Excessive influx of Na^+ accompanied by efflux of K^+ can disturb cellular function in plant. The application of SNP significantly reduced Na^+ and Cl^- concentrations in both roots and leaves, with a stronger effect on leaves. Meanwhile, SNP treatments increased the concentrations of NO^-_3, K^+, Mg^{2+} and Ca^{2+} in roots and leaves of both cultivars in saline conditions. At high levels of NaCl treatments (75 and 100 mM NaCl), application of 1.5 mM SNP did not significantly influence the Fe^{2+} and Zn^{2+} contents in the leaves and roots of both cultivars. The results of this study highlight the role of SNP in nutrient elements uptake under salinity stress.

REFERENCES

Banuls J., Legaz F. and Primo-Millo E., 1990. Effect of salinity on uptake and distribution of chloride and sodium in some citrus scion-rootstock combinations. *J. Hortic. Sci. Biotechnol.*, **65**, 715-724.

Blumwald E., 2000. Sodium transport and salt tolerance in plants. *Curr. Opin. Cell Biol.*, **12**, 431-434.

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.

Cerda A., Pardines J., Botella M.A. and Martinez V., 1995. Effect of potassium on growth, water relations, and the inorganic and organic solute contents for two maize cultivars grown under saline conditions. *J. Plant Nutr.*, **18**, 839-851.

Cramer G.R., Ergul A., Grimplet J., Tillett R.L., Tattersall E.A.R., Bohlam M.C., Vincent D., Sonderegger J., Evans J., Osborne C., Quilici D., Schlauch K.A., Schooley D.A. and Cushman J.-C., 2007. Water and salinity stress in grapevines: early and late changes in transcript and metabolite profiles. *Funct. Integr. Genomics*, **7**, 111-134.

Ferguson L., Poss J.A., Grattan S.R., Grieve C.M., Wang D., Wilson C. and Donovan T., 2002. Pistachio rootstocks influence scion growth and ion relations under salinity and boron stress. *J. Amer. Soc. Hort. Sci.*, **127**, 194-199.

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.*, **12**, 2117-2134.

Franklin J.A. and Zwiazek J.J., 2004. Ion uptake in *Pinus banksiana* treated with sodium chloride and sodium sulphate. *Physiol. Plant.*, **120**, 482-490.

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

Hayat Sh., Mori M., Pichtel J. and Ahmad A., 2010. *Nitric Oxide in Plant Physiology*. Wiley Blackwell.

Hoagland D.R. and Arnon D.I., 1950. *The Water-Culture Method for Growing Plants without Soil*. California Agricultural Experiment Station, Circular no. 347.

Hu Y. and Schmidhalter U., 2005. Drought and salinity: a comparison of their effects on mineral nutrition of plants. *J. Plant Nutr. Soil Sci.*, **168**, 541-549.

Kopyra M. and Gwozdz E.A., 2003. Nitric oxide stimulates seed germination and counteracts the inhibitory effect of heavy metals and salinity on root growth of *Lupinus luteus*. *Plant Physiol. Biochem.*, **41**, 1011-1017.

Lamotte O., Courtois C., Dobrowolska G., Besson A., Pugin A. and Wendehenne D., 2006. Mechanisms of nitric-oxide-induced increase of free cytosolic Ca^{2+} concentration in *Nicotiana plumbaginifolia* cells. *Free Radic. Biol. Med.*, **40**, 1369-1376.

Lara M.V., Disante K.B., Podesta F.E., Andreo C.S. and Drincovich M.F., 2003. Induction of a Crassulacean acid like metabolism in the C_4 succulent plant, *Portulaca oleracea* L.: physiological and morphological changes are accompanied by specific modifications in phosphoenolpyruvate carboxylase. *Photosynth. Res.*, **77**, 241-254.

Läuchli A. and Grattan S.R., 2007. Plant growth and development under salinity stress. *In: Advances in Molecular Breeding toward Drought and Salt Tolerant Crops*. Jenks M.A., Hasegawa P.M. and Jain S.M. (eds.), Springer. pp. 1-32.

Martinez-Beltran J. and Manzur C.L., 2005. Overview of salinity problems in the world and FAO strategies to address the problem. *In: Proceeding of the International Salinity Forum*, Riverside, California, pp. 311-313.

Melgar J.-C., Syvertsen J.P., Martinez V. and Garcia-Sanchez F., 2008. Leaf gas exchange, water relations, nutrient content and growth in citrus and olive seedlings under salinity. *Biol. Plant.*, **52**, 385-390.

Moya J.L., Gomez-Cadenas A., Primo-Millo E. and Talon M., 2003. Chloride absorption in salt-sensitive Carrizo citrange and salt-tolerant Cleopatra mandarin citrus rootstocks is linked to water use. *J. Exp. Bot.*, **54**, 825-833.

Munns R., James R.A. and Läuchli A., 2006. Approaches to increasing the salt tolerance of wheat and other cereals. *J. Exp. Bot.*, **57**, 1025-1043.

Neumann P., 1997. Salinity resistance and plant growth revisited. *Plant Cell Environ.*, **20**, 1193-1198.

Parida A.K. and Das A.B., 2005. Salt tolerance and salinity effects on plants: a

review. *Ecotoxic. Environ. Safety*., **60**, 324-349.

Picchioni G.A., Miyamoto S. and Storey J.B., 1991. Rapid testing of salinity effects on Pistachio seedling rootstock. *J. Amer. Soc. Hort. Sci.*, **116**, 555-559.

Qiao W. and Fan L.M., 2008. Nitric oxide signaling in plant responses to abiotic stresses. *J. Integr. Plant Biol.*, **50**, 1238-1246.

Rogers M.E., Grieve C.M. and Shannon M.C., 2003. Plant growth and ion relations in Lucerne (*Medicago sativa* L.) in response to the combined effects of NaCl and P. *Plant Soil*, **253**, 187-194.

Romero-Aranda R., Moya J.L., Tadeo F.R., Legaz F., Primo-Millo E. and Talon M., 1998. Physiological and anatomical disturbances induced by chloride

salts in sensitive and tolerant citrus: beneficial and detrimental effects of cations. *Plant Cell Environ.*, **21**, 1243-1253.

Ruiz D., Martinez V. and Cerda A., 1997. Citrus responses to salinity: growth and nutrient uptake. *Tree Physiol.*, **17**, 141-150.

Shabala S. and Cuin T.A., 2008. Potassium transport and plant salt tolerance. *Physiol. Plant.*, **133**, 651-669.

Shi Q. and Zhu Z., 2008. Effects of exogenous salicylic acid on manganese toxicity, element contents and antioxidative system in cucumber. *Environ. Exp. Bot.*, **63**, 317-326.

Siadat H., Bybordi M. and Malakouti M.J., 1997. Salt affected soils of Iran: a country report. *In: International Symposium on Sustainable Management of Salt Affected Soils in the Arid Ecosystem*. Cairo, Egypt.

Singh S.K., Sharma H.C., Goswami A.M., Datta S.P. and Singh S.P., 2000. *In vitro* growth and leaf composition of grapevine cultivars as affected by sodium chloride. *Biol. Plant.*, **43**, 283-286.

Sivritepe N., Sivritepe H.O., Celik H. and Katkat A.V., 2010. Salinity responses of grafted grapevines: effects of scion and rootstock genotypes. *Not. Bot. Hort. Agrobot. Cluj.*, **38**, 193-201.

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. Amer. Soc. Hort. Sci.*, **121**, 569-575.

Storey R., Schachtman D.P. and Thomas M.R., 2003. Root structure and cellular chloride, sodium and potassium distribution in salinized grapevines. *Plant Cell Environ.*, **26**, 789-800.

Sykes S.R., 1992. The inheritance of salt exclusion in woody perennial fruit species. *Plant Soil*, **146**, 123-129.

Tan J., Zhao H., Hong J., Han Y., Li H. and Zhao W., 2008. Effects of exogenous nitric oxide on photosynthesis, antioxidant capacity and proline accumulation in wheat seedlings subjected to osmotic stress. *World J. Agric. Sci.*, **4**, 307-313.

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

Troncoso A., Matte C., Cantos M. and Lavee S., 1999. Evaluation of salt tolerance of *in vitro*-grown grapevine rootstock varieties. *Vitis*, **38**, 55-60.

Uchida A., Jagendrof A.T., Hibino T., Takabe T. and Takabe T., 2002. Effects of hydrogen peroxide and nitric oxide on both salt and heat stress tolerance in rice. *Plant Sci.*, **163**, 515-523.

Walker R.R., Blackmore D.H., Clingeleffer P.R. and Iacono F., 1997. Effect of salinity and Ramsey rootstock on ion concentrations and carbon dioxide assimilation in leaves of drip-irrigated, field-grown grapevines (*Vitis vinifera* L. cv. Sultana). *Aust. J. Grape Wine Res.*, **3**, 66-74.

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.

Wu X., Zhu W., Zhang H., Ding H. and Zhang H.J., 2011. Exogenous nitric oxide protects against salt-induced oxidative stress in the leaves from two genotypes of tomato (*Lycopersicom esculentum* Mill.). *Acta Physiol. Plant.*, **33**, 1199-1209.

Zhang F., Wang Y., Yang Y., Wu H., Wang D. and Liu J., 2007. Involvement of hydrogen peroxide and nitric oxide in salt resistance in the calluses from *Populus euphratica*. *Plant Cell Environ.*, **30**, 775-785.

Zhang Y.Y., Liu J. and Liu Y.L., 2004. Nitric oxide alleviates the growth inhibition of maize seedlings under salt stress. *J. Plant Physiol. Mol. Biol.*, **30**, 455-459.

Zhao L., Zhang F., Guo J., Yang Y., Li B. and Zhang L., 2004. Nitric oxide functions as a signal in salt resistance in the calluses from two ecotypes of reed. *Plant Physiol.*, **134**, 849-857.

Zhao M.G., Tian Q.Y. and Zhang W.H., 2007. Nitric oxide synthase-dependent nitric oxide production is associated with salt tolerance in *Arabidopsis*. *Plant Physiol.*, **144**, 206-217.