

Research Article





Effects of manganese on physiological characters of grapevine cultivars under salinity stress

Abstract

Salinity is one of the most important environmental factors which have a significant effect on the growth and fertility. Manganese is an essential element that plays a key role as a nutrient in many plant metabolic processes. The present study was carried out to investigate the effects of manganese (1ppm) on Yaghoti and Ghara uzum - two cultivars of grapevine - which were planted and grown under salt stress (0, 50 and 100mmol L-1 NaCl) using greenhouse and hydroponics methods. Results on saline concentration showed led to a significant decrease in length, fresh and dry weight, photosynthesis pigments content, K^+ and nitrate content, and K^+/Na^+ ratio of the plants. It also caused a significant increase in Na+ and Cl- ion concentration and soluble sugar content in shoots and roots of both cultivars. Ghara uzum was more sensitive than Yaghoti to salinity stress. Applying manganese to the medium, resulted in low level of salinity toxicity and sodium ion accumulation in the shoots for Ghara uzum cultivar. For Yaghoti, however, the application of manganese did not reduce the sodium ion accumulation in the shoots, but increased the salinity toxicity. Our findings suggest that Ghara uzum and Yaghoti cultivars had different responses to salinity, manganese and their interaction.

Keywords: salinity, manganese, grapevine, ions accumulation, nitrate content, photosynthesis pigments

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Introduction

Plants grown in arid and semi-arid regions are usually subjected to adverse environmental factors such as high salinity or drought. Salinity has unfavorable effects on the growth and development of plants in 20percent of arable lands of the world. The most common effect of saline soils on plants is growth inhibition, often related to high density of Na⁺ and deficiency of K⁺. The accumulation of Cl⁻ in the shoots is also harmful to the plant growth. Salinity has a variety of effects on plants' physiological processes reduces the leaf net CO₂ assimilation rate and efficiency of photosynthesis.

In other study accomplished by R, M Steven in 2011, Cl uptake was greatest when saline irrigation was applied early in organ formation, whereas Na⁺ uptake reflected seasonal salt load in irrigation water.

José M and Intrigliolo 2017 in a study reviewed the main findings obtained regarding the effects of deficit irrigation strategies, as well as irrigation with saline water, on the berry composition of both red and white cultivars, as well as on the final wine. A meta-analysis was performed using published data for red and white varieties; a general liner model accounting for the effects of cultivar, rootstock, and midday stem water potential was able to explain up to 90% of the variability in the dataset, depending on the selected variable. In both red and white cultivars, berry weight, must titratable acidity and pH were fairly well simulated, whereas the goodness-of-fit for wine attributes was better for white cultivars.

Manganese is an essential micronutrients for plant growth.⁵ It acts as a cofactor to many enzymes like Mn-superoxide dismutase, Mn-catalase, pyruvate arboxylase, and phosphol pyrovate.⁶ The deficiency

and toxicity of manganese are two nutritional diseases which influence the growth of various plants. Imiting the photosynthetic rate and efficiency. Manganese is essential for chlorophyll synthesis and photosynthesis, nitrogen and carbohydrate metabolism, oxidation, and reduction and activation of some enzymes. However, a high level of Mn seems to damage the photosynthetic apparatus.

The availability of Mn for plants depends on its amount in the soil, easily reducible Mn oxides, pH redox potential, soil humidity, and microbial activity. If Mn is available in excess, its high concentration in the plant tissue causes the appearance of toxicity symptoms and affects plant growth.⁵ The toxic symptoms of Mn include limited growth and photosynthesis rate, and appearance of chlorosis and necrotic brown spots on the leaves.⁹

Mn toxicity reduces photosynthesis, chlorophylls a and b contents, and other compounds such as carotenoids in plants.¹⁰ The damage threshold of Mn varies among to species and within genotypes of given species.¹¹

The final destination of Mn^{2+} during the transportation in plant cells is the chloroplast and the deficiency of Mn^{2+} can cause photosynthesis reduction which in turn leads to growth inhibition by salinity. The ionic activity inhibition can occur during absorption of salinity major cations, namely Na^+ and K^+ . 12

Our research aimed to study the effects of Mn on the growth, metabolism and ion accumulations in two cultivars of the grapevine under saline stress conditions. Grape is one of the major crop of West Azarbaijan's especially around the Urmia. Due to the shortage drop in rainfall, increasing drough, the growing danger of Urmia drying process, and the increase of the salt percentage of the nearby farmlands,



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specifying the proper cultivar matching with saltiness of the lands is of paramount importance and for the economic sustainability of viticulture in the region.

Material and methods

Plant growth and experimental design

Hardwood cuttings of two grapevines (Vitis vinifera L.) cultivars (Ghara uzum and Yaghoti) were obtained from Kahriz vineyard (Agricultural Research Center, Urmia, Iran). The cuttings were disinfected and then their basal parts were soaked in IBA (indol-3butyric acid) 0.1% (w/v) for 5-10s. All cuttings were placed in a mist chamber (relative humidity 80%) at 25-35°C. After two weeks, the rooted cuttings were planted in pots filled with perlite and irrigated with 1/8 strength Hoagland's solution containing 0.125mM KNO₃, 0.125mM Ca(NO₃)₂, 0.05mM MgSO₄.7H₂O, 0.0125mM KH₂PO₄, EDTA. Cuttings with opened leaf bud were transferred to 2-1 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 4-5 fully expanded leaves were treated with NaCl (0, 50, 100mM NaCl), Mn (1ppm) and their interactions with NaCl (Mn+50mM NaCl and Mn + 100mM NaCl) in full strength Hoagland solution for 2 weeks. NaCl was incrementally added to the nutrient solution at certain concentrations until the final desired concentrations were reached. Plants were harvested after 2 weeks and plant parts were weighed separately and dried at 70°C for 48 hours.

This experiment was conducted as based on completely randomized design (CRD) with two cultivars of grapevine and 6 treatments and 4 replications and each replication including three plants.

Photosynthetic pigments

The concentration of chlorophyll was determined using the spectrophotometeric method of Lichtenthaler and Wellburn. ¹³ For this purpose, 5mL of acetone (80%) was added to 0.1g leaf fresh weight and, then crushed and ground using mortar and pestle. After centrifugation of the samples at 3000g (Dynamica model; Velocity, made in UK) for 10min, the supernatants were removed and the absorbance of the samples was read at wavelengths of 470, 647 and 663nm in a specterophotometers (Dynamica model; Halo DB-20, made in UK). The amount of these pigments was calculated according to the formulas of Lichtenthaler and Wellburn. ¹³

Determination of Na+, K+ and Cl-

The dried samples of shoot or root were powdered with mortar and pestle and then were digested following Allen et al. ¹⁴ A flame photometer (Fater 405) was used for Na⁺ and K⁺ determination. A chloride analyzer (Corning 926) was used for determining Cl⁻ contents in the extracts. 100mg of dry shoot or root materials was extracted in 10mL of distilled water, heated at 70°C till the volume was almost halved and before determination the volume was again reached to 10mL with distilled deionized water.

Statistical analysis

Data were analyzed with SPSS (ver. 19) package. In all graphs and tables, the results were expressed in average values of four replications \pm SE. The treatment effects were compared using the ANOVA and Duncan test at the p<0.05 level of significance.

Results

Shoot and root lengths and fresh and dry weights decreased significantly (p<0.05) in both cultivars under salt and Mn stress (Table 1).

Table I Effects of Mn (Ippm) on the growth parameters (root and shoot lengths and fresh and dry weights) in two grapevine cultivars (Yaghoti and Ghara uzum) under saline stress (0,50 and 100mM NaCl)

Cultivar	NaCl and Mn level	Shoot Root		Shoot fresh	Root fresh	Shoot dry	Root dry
		length (cm)	length (cm)	weight (g)	weight (g)	weight(g)	weight (g)
Yaghoti	0 (Control)	65 b	35b	26.84c	7.41d	0.96bc	0.162c
	50mM NaCl	46 de	23.6e	21.21de	5.53e	0.58de	0.075fg
	100mM NaCl	38.8f	21.3f	7.41g	2.52h	0.49e	0.049h
	Mn (Ippm)	59c	28.1cd	26.30c	5.90e	1.06b	0.131d
	Mn+50mM NaCl	48d	29.4c	16.01e	4.91f	0. 79 cd	0.110e
	Mn+100mM NaCl	42e	26.2d	12.32f	3.63g	0.59de	0.077fg
Ghara uzum	0 (Control)	70a	42.3a	37.25a	11.10a	2.1a	0.332a
	50mM NaCl	57.6c	33.5b	25.83c	9.73b	0.88c	0.140cd
	100mM NaCl	42.7e	23e	13.11ef	2.81h	0.69d	0.079f
	Mn (Ippm)	63.1 bc	30.1c	33.05b	10.30b	0.89c	0.261b
	Mn+50mM NaCl	56.2cd	33.02bc	23.21d	8.52c	0.67d	0.082f
	Mn+100mM NaCl	38.4f	32.2bc	12.50f	4.31f	0.44e	0.061g

Each value is the mean (\pm SE) of four replicates. Different letters in each column show significant difference at p<0.05 by Duncan's Test

Compared to the control groups, the concentration of NaCl (50 and 100mM) in Yaghoti cultivar caused a decrease of 29-40% in shoot length, 32-39% in root length, 19-72% in shoot fresh weight, 25-66% in root fresh weight, 40-49% in shoot dry weight, and 54-70% in root dry weight. Reading Ghara uzum cultivar, there was a decrease of 18-40% in shoot length, 21-45% in root length, 32-65% in shoot fresh weight, 13-74% in root fresh weight, 58-67% in shoot dry weight, and 58-76% in root dry weight. Fresh and dry shoot biomasses of Yaghoti cultivar under salinity stress were higher than those of Ghara uzum (Table 1).

The interaction of Mn and salinity in the Ghara uzum cultivar led to a decrease of (2-10% in shoot length, 4-8% in fresh shoot weight, 24-36% in dry shoot weight, and 25-43% in dry root weight. In the Yaghoti cultivar, this led to an improvement and increase in some growth parameters (4-10% in shoot length, 77% in fresh shoot weight, 20-36% in dry shoot weight and 46-57% in root dry weight) compared to salinity alone (Table 1). The responses of the two studied grapevine cultivars were significantly different under salinity treatment alone and with interaction of Mn and salinity.

Due to salinity and Mn stress (Table 2), Chlorophylls a and b (Chl a and b), the ratio of chl a/b, and the total carotenoids content of the leaves decreased significantly compared to the controls in both cultivars. Compared to the plants in the control group, the salinity stress decreased chlorophyll a content by nearly 38-55% in Ghara uzum and 34-42% in Yaghoti, and reduced chlorophyll b content by nearly 29-60% in Ghara uzum and 41-50% in Yaghoti. It also reduced carotenoids content about 68-92% in Ghara uzum and 64-58% in Yaghoti.

Table 2 Effects of Mn (Ippm) on total chlorophyll content (Total Chl), Chlorophyll a (Chl a), Chlorophyll b (Chl b), Chlar Chl b ratio and carotenoid (Cx+c) (T car) in two grapevine cultivars (Yaghoti and Ghara uzum) under saline stress (0, 50 and 100mM NaCl)

Cultivar	NaCl and Mn level	Chla	Chl <i>b</i>	Total chl	Chla/chlb	T Car
	0 (Control)	29.5a	16.3a	45.8a	1.81d	5.21b
	50mM NaCl	19.5d	9.6c	29. l de	2.03c	1.89f
Vh4:	100mM NaCl	17.2de	8.1d	25.3f	2.2bc	2.2de
Yaghoti	Mn (Ippm)	25.6b	9.01cd	34.61c	2.84a	3.5c
	Mn+50mM NaCl	22.3c	9.3c	31.6d	2.39b	1.46g
	Mn+100mM NaCl	18.1d	9cd	27.1e	2.01c	2.4d
	0 (Control)	25.1b	13.68b	38.78b	1.83d	6.55a
	50mM NaCl	15.66e	9.67c	25.33f	1.62e	2.11e
Ch	100mM NaCl	11.2g	5.5e	16.7h	2.03c	0.54i
Ghara uzum	Mn (Ippm)	12.6fg	7.9de	20.5g	1.59f	2.34d
	Mn+50mM NaCl	17.22de	8.5d	25.72f	2.02c	2.2de
	Mn+100 mM NaCl	13.18f	5.8e	18.98gh	2.27bc	0.81h

Each value is the mean (±SE) of four replicates. Different letters in each column show significant difference at p<0.05 by Duncan's Test

The application of Mn together with saline stress increased chlorophyll a content in both cultivars (5-14% in Yaghoti and 10-18% in Ghara uzum) compared to salinity alone (Table 2).

Applying Mn and saline stresses also increased the total carotenoids content in both cultivars; (Table 2).

The combination of salinity and Mn stresses increased soluble sugars content in both shoot and root (Figure 1). Compared to the control group, the saline stress plants increased shoot soluble sugars in Ghara uzum and Yaghoti by, 70-87% and 25-38%, respectively (Figure 1A). Mn and salinity increased shoot soluble sugars in Yaghoti cultivar by 14% more than salinity alone. The salinity and Mn caused a decrease of 53-80% in root soluble sugars in Ghara uzum and an increase of nearly 28% in the 100mM salinity treatment in Yaghoti when compared to salinity alone (Figure 1B).

The effects of salinity on Na+, Cl- and K+ content (mgg-1DW) and K⁺/Na⁺ ratio are shown in Figures 2–5 in the shoots and roots of 45-day-old plants after 30days of NaCl and Mn treatment.

The concentration of 50 and 100mmol L-1 NaCl in Yaghoti plants increased by 200-450% in shoot Na+ (Figure 2A), 17-21% in root Na⁺ (Figure 2B), 240-400% in shoot Cl⁻ (Figure 4A), and 22-128% in root Cl⁻ (Figure 4B). In Ghara uzum this increased was 560-1200% in shoot Na+(Figure 2A), 260-450% in root Na+(Figure 2B), 500-1100% in shoot Cl⁻ (Figure 4B), and 500-8200% in root Cl⁻ (Figure 4B). In contrast, salinity in Yaghoti cultivar decreased by 39-44% in shoot K⁺ (Figure 3A), 85-91% in root K⁺ (Figure 3B), 80-90% in shoot K⁺/ Na⁺ ratio (Figure 5A) and 85-91% in root K⁺/Na⁺ ratio (Figure 5B) compared to the control group, and by about 27-32% in shoot K+, 34-87% in root K+, 89-95% in shoot K+/Na+ ratio, and 82-98% in root K⁺/Na⁺ ratio in Ghara uzum cultivar compared to the control group (Figure 5).

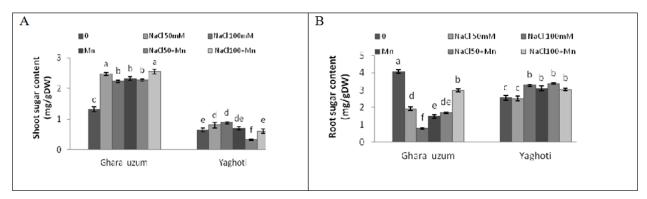


Figure I Effects of Mn (Ippm) on soluble sugar content in shoots (A) and roots (B) in two cultivars of grapevine (Yaghoti and Ghara uzum) plants cultivated under saline stress (0, 50 and 100mM NaCl). Each value is the mean (±SE) of four replicates. Different letters on each column show significant difference at p<0.05 by Duncan's Test.

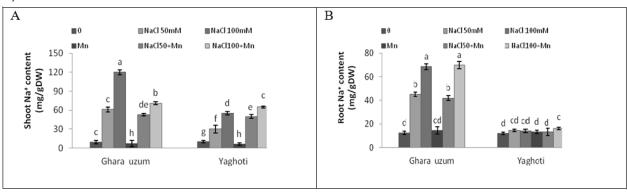


Figure 2 The effects of Mn (Ippm) on concentration of Na⁺ in shoots (A) and roots (B) in two grapevine cultivars (Yaghoti and Ghara uzum) cultivated under saline stress (0, 50 and I00mM NaCl). Each value is the mean (±SE) of four replicates. Different letters on each column show significant difference at p<0.05 by Duncan's Test.

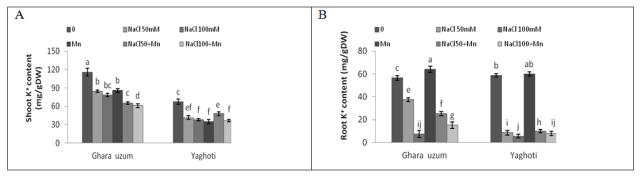


Figure 3 Effects of Mn (Ippm) on concentration of K^+ in shoots (A) and roots (B) in two grapevine cultivars (Yaghoti and Ghara uzum) plants cultivated under saline stress (0, 50 and 100 mM NaCl). Each value is the mean (\pm SE) of four replicates. Different letters on each column show significant difference at p<0.05 by Duncan's Test.

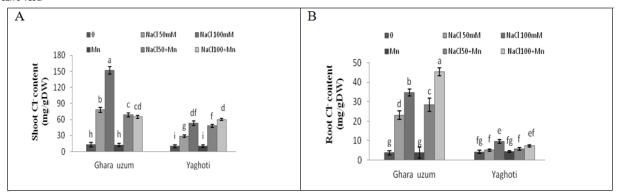


Figure 4 Effects of Mn (Ippm) on concentration of Cl⁻ in shoots (A) and roots (B) in two grapevine cultivars (Yaghoti and Ghara uzum) cultivated under saline stress (0, 50 and 100mM NaCl). Each value is the mean (±SE) of four replicates. Different letters on each column show significant difference at p<0.05 by Duncan's Test.

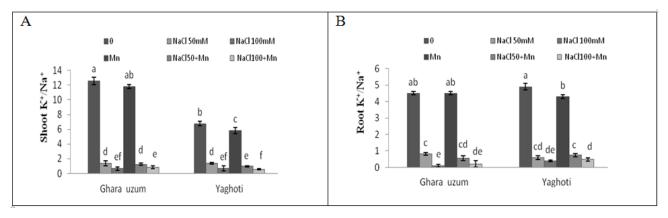


Figure 5 Effects of Mn (Ippm) on K^+/Na^+ ratio in shoots (A) and roots (B) in two grapevine cultivars (Yaghoti and Ghara uzum) cultivated under saline stress (0,50 and 100mM NaCl). Each value is the mean (\pm SE) of four replicates. Different letters on each column show significant difference at p<0.05 by Duncan's Test.

Application of Mn together with saline stress led to an increase in the accumulation of Na $^+$ and Cl $^-$ ions in Yaghoti cultivar about 18-66% in shoot Na $^+$, 15.7% in root Na $^+$ (Mn+100mM NaCl), 12-65% in shoot Cl $^-$, 11% in root Cl $^-$ (Mn+50mM NaCl), 18-41% in root K $^+$, and 23-29% in root K $^+$ /Na $^+$ ratio. Similarly in Ghara uzum cultivar this led to an increase of 2% in root Na $^+$, 106% in root K $^+$ (Mn+100mM NaCl), 23-30% in root Cl $^-$, and 96% in root K $^+$ /Na $^+$ ratio (Mn + 100 mM NaCl). In contrast, applying Mn together with salinity in Ghara uzum cultivar led to a decrease in shoot Na $^+$ (14-40%), root Cl $^-$ (12-

57%), and root K⁺/Na⁺ ratio (30% in Mn+50mM NaCl). Similarly in Yaghoti cultivar this led to a decrease in root Na⁺ (9% in Mn+50mM NaCl), root Cl⁻ (24% in Mn+100mM NaCl), and shoot K⁺/Na⁺ ratio (19-31%).

Based on our findings, however, salinity caused a significant decrease in NO₃ content in both cultivars (Figure 6). Applying Mn with salinity, compared with salinity alone, decreased the nitrate ion content in shoot of both grapevine cultivars (Figure 6).

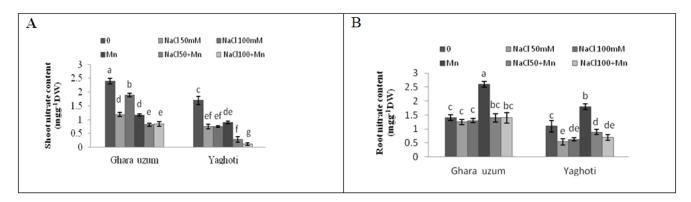


Figure 6 Effect of Mn (Ippm) on nitrate content in shoots (A) and roots (B) in two grapevine cultivars (Yaghoti and Ghara uzum) cultivated under saline stress (0,50 and 100mM NaCl). Each value is the mean (±SE) of four replicates. Different letters on each column show significant difference at p<0.05 by Duncan's Test.

Discussion

The current study of indicated that saline stress and increased concentration of manganese resulted in a decrease in a vegetative growth. In support of these observations, Yasar et al.¹⁵ reported the amount-based decrease in the growth of the green bean plants under the influence of salinity. Although manganese is necessary for plant growth, its excessive amount, as reported in rice for example, can be an inhibitor.¹⁶

Mn excess in plants decreases the root and shoot lengths.¹⁷ The accumulation of salt and ions leads to osmotic and water stress which decreases water absorption in plant tissue leading to growth decrease in cells. The lower growth of shoot in high concentration of Mn can be related to its presence in some specific metabolic processes.⁶ Manganese may decrease leaf growth in some plants due to the inhibition in elongation and cell divisions.¹⁸

Simultaneous effects of NaCl and Mn on cell division and cell elongation intensified the reduction in shoot growth in Ghara uzum. In Yaghoti, however, the application of manganese together with salinity increased growth in the shoots more than salinity alone. It also adjusted the reduction caused by salinity. This contradiction can occur to some extent due to the variety of plant species and their genotypes and their various responses.¹⁹

NaCl and Mn treatments, separately or simultaneous, led to the accumulation of soluble sugars in the shoots of both Ghara uzum and Yaghoti cultivars, and also in the roots of Yaghoti. This is a prevailing event under stress. Garg et al.²⁰ reported that total soluble sugars increase as a result of salinity increase, playing an important role in osmosis adjustment.²¹ Since salinity, besides ion toxicity, creates osmosis stress as a result of reduction in osmotic potential of soil matrix, the plants decrease their osmotic potential to adjust the osmosis. The osmotic potential reduction is the result of the increase

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in cell soluble materials, which is an adjustment and adaptation mechanism in plants against external stresses¹⁷ and helps the plant to maintain its tolerance in saline stress conditions. Increasing in soluble sugar concentrations can be regarded as a response to relative water content and the leaves' water potential, because increase of sucrose and other soluble sugars under saline conditions plays an important role in tolerance to salinity. This finding was consistent with Geholt et al.²² results (2005). The concentration of organic soluts, can contribute to the maintenance of ionic homeostasis and C/N ratio; the removal of free radicals; and the stability of organs and macromolecules like proteins, protein complexes, and membranes.²³

In this study, the chlorophyll a and b, and the total carotenoids contents decreased under saline conditions and with the application of manganese. Chlorophyll is membrane-bound, and rarely remains healthy under saline stress.²⁴ Decrease in chlorophyll content due to salinity has been reported by other researchers, and its decrease can be related to chlorophyll degrading enzyme (Chlorophyllase).²⁵ High levels of sodium concentration in plant tissues have been reported as a major factor in the reduction of photosynthetic pigments and photosynthesis velocity.²⁶

The decrease in chlorophyll content under heavy metal stress is only caused by the inhibition of biosynthesis since. The central Mg element existing in the chlorophyll structure is replaceable by other elements such as manganese, copper, zinc and nickel. This displacement is the major destruction caused by heavy metals in chlorophyll structure.²⁷

In this study with increasing salinity, Cl- and Na+ concentrations increased in the shoots and roots of the two cultivars of two grapevine cultivars , while $K^{\scriptscriptstyle +}$ and $NO_3^{\scriptscriptstyle -}$ concentrations decreased. This finding was consistent with Meloni et al.¹⁷ who reported that sodium and chlorine ions increased as potassium decreased. The accumulation of sodium and chlorine in plants leads to osmosis increase, which can help the plants deal with the osmosis potential decrease in the root environment. Being an essential element for plant growth, potassium absorption is inhibited by an increase in salinity; consequently the plant has no choice but to face its deficiency. In saline soils in which sodium concentration is high, the plants absorb high amounts of sodium instead of potassium and calcium, and this leads to calcium and potassium deficiency. Due to the structural similarity of sodium and potassium, and because of sodium's competition to replace potassium, potassium related metabolic processes are controlled in cytoplasm, and this indicates that cell sodium amounts must be kept at minimum level.28

The reason for nitrate reduction in plants exposed to manganese toxicity might be that their nitrate absorption has reduced because of toxicity. The low sodium concentration in plants under simultaneous Mn²+ and NaCl stress in comparison with those exposed to NaCl stress alone can be attributed to this toxicity-reducing effect of the metal which has been observed for other heavy metals like cadmium and zinc as well.²9,30 Previously conducted studies reported that heavy metals such as manganese, zinc and cadmium in sufficient amounts are essential for plant resistance against NaCl toxicity.³¹ These elements are essential for cell membrane strength in roots, and thus control the membrane permeability.³²-³²

Conclusion

According to the results obtained from the present study, especially those concerning the growth characteristics, photosynthetic pigments content, the way the deleterious ions of Na⁺ and Cl⁻ accumulate, and

the K^+/Na^+ ratio which is a very determining factor in sensitivity and resistance of plants against salinity, it can generally be stated that Ghara uzum experienced much more reduction in growth characteristics and pigments content compared to Yaghoti; and Na^+ and Cl^- ions accumulation in the shoots was higher in the former cultivar.

Manganese caused growth limitation in both cultivars, and it was found that Yaghoti was more resistant to Mn than Ghara uzum.

In the simultaneous application of salinity and manganese, and through examining Na⁺ and Cl⁻ accumulation, it was observed that the application of manganese had more impact on decreasing salinity toxicity in Ghara uzum compared to that in Yaghoti. Through the application of Manganese, Na⁺ accumulation was reduced by 40% in Ghara uzum, while in Yaghoti not only there was no decrease in Na⁺ accumulation in the shoot, but also the salinity stress was intensified in this cultivar. The results also indicated that the responses of the two cultivars to saline, manganese, and their interactivity were different. This difference in responses was to some extent due to the species of the plants, their genotypes and their different responses.

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Conflicts of interest

The authors declare there are no conflicts of interest.

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