Grape response to salinity stress and role of iron nanoparticle and potassium silicate to mitigate salt induced damage under in vitro conditions

Ali-akbar Mozafari¹ · Ali Ghadakchi asl¹ · Nasser Ghaderi¹

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Abstract Grape softwood cuttings of Khoshnaw cultivar were cultured using tissue-culture methods to study the effect of iron nanoparticles and potassium silicate under salinity conditions during the 2015–2016 growing season. The treatments consisted of salinity stress (0, 50, and 100 mM NaCl), nanoparticles of iron (0, 0.08, and 0.8 ppm), and potassium silicate (0, 1, 2 mM). The results also showed that the application of iron nanoparticles and potassium silicate significantly increased the total protein content and reduced proline, enzymatic antioxidant activity and hydrogen peroxide. Salinity stress reduced membrane stability index while increased malondialdehyde content. Increase of membrane stability index and reduction of malondialdehyde content were obtained for 2 mM potassium silicate and 0.8 ppm iron nanoparticle. Iron and potassium silicate were shown to lower the sodium content and increase the potassium content under salinity-stress conditions. The highest ratio of sodium to potassium was observed in plants under salinity conditions (100 mM) treated with neither iron nanoparticles nor potassium silicate; conversely, the lowest ratio was achieved in plants treated with both 0.8 ppm iron nanoparticles with 1 mM and 2 mM potassium silicate under non-stress conditions. These results indicate that the application of micronutrients in stressful conditions is a suitable method to compensate for the negative effects of salinity stress. Tissue culture in this study was shown to be an economically efficient and

applicable technique for producing grape softwood cuttings to be used in experiments.

Keywords Nanoparticle · Iron · Potassium silicate · Salinity · Grape · Antioxidant

Introduction

Grown worldwide, grape is one of the highest-value agricultural products, significantly contributing to the economies of the countries in which it is cultivated. Some vineyards are located in semi-arid areas that commonly experience drought and salinity (Ahad and Inayatullah 2013; Benjak et al. 2005). Grape is relatively sensitive to salinity, and damage caused by saline conditions mostly results from the accumulation of chlorine and sodium ions (Fisarakis et al. 2001). Salinity stress is one of the most significant environmental stresses limiting agricultural production worldwide, and affects the anatomical, physiological, and enzymatic features of plants (Arslan et al. 2016; Nawaz et al. 2016). Salinity stunts grape branches and dramatically decreases the plants yield (Singh et al. 2000). In arid and semi-arid areas, salinity can be worsened by low precipitation, high evaporation and temperature, and poor management of water resources (Bernstein 1975). As water potential shrinks and ions become more unbalanced, salinity influences plant growth, reducing leaf expansion and stomatal closure. The resulting low water potential of the soil creates osmotic stress, which reduces photosynthesis (Seemann and Critchley 1985). Salinity decreases plants ability to absorb nutritional elements and increases the intra-cellular ionic concentrations, ultimately leading to failure of plant growth and development (Grattan and Grieve 1998).

✉ Ali-akbar Mozafari
a.mozafari@uok.ac.ir

¹ Department of Horticultural Sciences, Faculty of Agriculture, University of Kurdistan,
P. O. Box: 416, Sanandaj 66177-15175, Iran

Salinity also has a negative influence on plants nutritional content. Iron is a particularly crucial micronutrient in agricultural crops (George et al. 2008). Plants can quickly and completely take up nano-fertilizers as a compensation for shortages caused by plants growing conditions (Römheld and Marschner 1991). Use of nano-fertilizers can be considered as an effective step to attain sustainable agriculture, as they increase the efficiency of the nutritional elements and decrease soil toxicity relative to the use of chemical fertilizers and reduce the frequency of fertilizer application (Nadi et al. 2013). Micronutrients can also increase plants resistance to the negative effects of toxic ions. Specifically, higher concentrations of iron in nutritional solutions can compensate for salinity impacts (Uauy et al. 2006). Uauy et al. (2006) reported that iron, zinc, and magnesium spraying over wheat shoots led to their improved growth characteristics. In another study, Maciel et al. (2004) stated that the negative effects of saline could be relieved by the application of iron sulfate. Application of iron nano-fertilizer in plants can increase the plant resistance to salinity stress by simultaneously increasing the permeability of the root's selective plasma membrane and decreasing the absorption and accumulation of sodium, which improves the ratio of potassium to sodium in the shoot (Taiz et al. 2015).

Several studies have shown that potassium silicate has a positive impact on plant growth and productivity. It has also been proven that silicon improves plant growth, increases photosynthesis, decreases evapo-transpiration, stiffens the leaves, increases the chlorophyll concentration in a given leaf area, and improves the quality of the products (Rodrigues et al. 2009). When deposited inside the cell wall, it combines with calcium and pectin to create a cellulose-silicon layer that increases the stability of the cell wall against damage by pathogens. Moreover, unlike the calcium, silicon can be considered as a complementary element in the growth of a wide range of plants (Vinal and Lander 1955). Under saline conditions, potassium silicate heightens the enzymatic activities of antioxidants, thus reducing the permeability of plasma membrane and increasing root absorption of nutrient elements (Liang et al. 2003). Silicon, by both limiting transportation of sodium to leaves and storing the sodium inside the root, can improve plants stability against salinity stress. Furthermore, silicon can reduce the toxic effect of sodium, thus improving plants growth and development by reducing the degree to which it is taken up (Gong et al. 2005).

In the examination of resistance to salinity stress, using tissue cultures is preferable to using plants because experimental conditions are easier to control and a larger number of genotypes can be examined in the same space (Yeo et al. 1990). A number of studies have shown that in vitro cultivation is an effective method for salinity-stability experiments (Daub 1986; Malusá et al. 2012; Rolli

et al. 2015); these include studies carried out on grape (Elbotaty 2012), tomato (Mohammed et al. 2006), citrus (Ben-Hayyim et al. 1985), pistachio (Barghchi and Alderson 1989), plum (Diaz-Vivancos et al. 2013), kiwi fruit (Liu et al. 2015), apple (Tabart et al. 2015), pear (Shibli et al. 2000), and peach (Dimassi-Theriou 1998).

Because of both the potential severe effects of salinity stress, the economic importance of grape cultivation, and the increasing use of tissue-culture techniques in agricultural production, the current study aimed to survey the effect of salinity stress on the biochemical and nutritional properties of grape var. Khoshnaw under in vitro conditions, and to consider the effect of iron and potassium-silicate nanoparticles in compensating for the effects of salinity stress on this plant.

Materials and methods

Plant materials

Grape softwood cuttings of Khoshnaw cultivar were used in this experiment, which occurred during the 2015–2016 growing season. Herbaceous branches that had reached a certain level of growth were separated from the mother plant, then placed in clean, wet fabric, and immediately transported to the tissue-culture laboratory of the University of Kurdistan. Leaves were cut off the cuttings using a scalpel, and micro samples containing an active bud (one-node cuttings) were prepared for planting. To remove dirt and pathogens from the samples, they were washed for about 30 min in tap water and a washing liquid. The samples were then placed in a laminar hood as a sterile environment and placed in ethanol 70% for about 30 s. In the next stage, the samples were placed in sodium hypochloride 1.2% for 15 min. When these stages of sterilization were finished, the samples were washed three times (for periods of 5, 10, and 15 min respectively) with distilled water. The prepared samples were then placed separately in their medium and cultivated. The treatments consisted of salinity stress at three levels (0, 50, and 100 mM NaCl), iron nanoparticles at three levels (0, 0.08, and 0.8 ppm), and potassium silicate at three levels (0, 1, and 2 mM). The experiment was carried out for 60 days. The samples used for measuring biochemical traits were frozen with liquid nitrogen and kept in freezers at -80°C until measuring.

Trait measurement

Proline and total protein contents

To determine the content of proline in each sample, 0.5 g fresh tissue was weighed and finely ground with liquid

nitrogen in a mortar. 10 mL of sulfo-salicylic acid 3% were added to the well-ground extract, and the extracts were centrifuged for 5 min at 6000 rpm. After centrifugation, 2 mL of supernatant were separated and placed in falcons, and 2 mL dimenhydrinate and 2 mL of glacial acetic acid were added to the supernatant. Next, 2 mL of toluene were added to the falcon containing the solution. The falcons were then gently shaken for 30 s. At the final stage, the supernatant from the red solution, which contained proline, was separated and its light absorption was spectrophotometrically recorded at a wavelength of 520 nm to determine the proline content (Bates et al. 1973). Total protein content was measured using the method described by Bradford (1976).

Malondialdehyde (MDA) content

To measure MDA content, 0.3 g of ground fresh leaf was mixed with 5 mL of trichloroacetic acid (TCA 0.1%) and its extract was centrifuged at 10,000 rpm for 5 min at 4 °C. The supernatant was then mixed with a malondialdehyde solution containing trichloroacetic acid 20% and thiobarbituric acid (TBA: 5%). The resulting solution was kept in a bain marie bath for 30 min at 95 °C. The solution was quickly cooled in an ice bath and centrifuged again at 10,000 rpm at 4 °C. Finally, the light absorption of the solution was measured at a wavelength of 523 nm to estimate MDA content. The extinction coefficient for MDA measurement was 153 (Ahmad et al. 2008).

Hydrogen peroxide (H₂O₂) content

In accordance with the method described by Pick and Mizel (1981) to measure H₂O₂, 0.3 g fresh tissue was ground with liquid nitrogen and 5 mL of trichloroacetic acid (TCA: 0.1%). The extraction was centrifuged at 10,000 rpm for 5 min at 4 °C. Next, 250 µL of the supernatant was mixed with 250 µL of the phosphate buffer (pH = 7) 100 mM and 500 µL potassium iodide (KI) 1 M. Light absorption at a wavelength of 390 nm was then read to estimate H₂O₂ content.

Peroxidase activity (POD)

In accordance with the method of Flohé and Günzler (1984) for measuring POD, phosphate potassium buffer was mixed with 780 µL potassium phosphate buffer 50 mM (pH = 7), 90 µL guaiacol 1%, and 90 µL hydrogen peroxide 0.3% in a cuvette placed on ice surface as the main solution for measuring POD activity. After adding 40 µL of the total proteins extracted from total protein content stage, the light-absorption exchange was read for

120 s. POD activity based on micromole decomposed H₂O₂ per min per mg protein was reported.

Ascorbate peroxidase activity (APX)

To measure the activity of APX enzyme in samples, the method of Nakano and Asada (1981) was used. Solutions containing 100 µL sodium phosphate buffer 50 µmM (pH = 7), 50 µL H₂O₂ 10 mM, 50 µL ascorbate 10 mM, and 790 µL double-distilled water were mixed inside a cuvette placed on ice. Then 10 µL of extracted total protein was placed in the prepared sodium phosphate buffer on the ice surface. The curve change for the light absorption of the final solution was read over 120 s at 290 nm. The activity of APX was reported based on the content of H₂O₂ decomposed by APX per min per mg protein.

Superoxide dismutase activity (SOD)

For measuring SOD activity based on Beauchamp and Fridovich (1971), a solution containing 50 mM phosphate potassium (pH = 7), 75 µL nitroblue tetrazolium (NBT), 13 mol methionine, 100 µL riboflavin 12 µm, 0.1 mm EDTA, and 10 µL protein extraction was mixed and placed under fluorescent light for 15 min. After 15 min of reaction under the fluorescent light, the light absorption of these samples was read at 560 nm using a spectrophotometric device. The concentration of the enzyme that limits decreases in NBT photochemical activity was taken as one unit of enzymatic activity. The enzyme-activity unit was then expressed as absorption changes per mg protein per minute per fresh weight.

It should be mentioned that all enzymatic measurements used the spectrophotometer of UV-2100 model suv (New Jersey, USA) and the centrifuge of HETTCH model MICRO (Germany).

Mineral elements measurements

To mineral elements including sodium and potassium, the ash of samples was mixed with HCl. Sodium and potassium content were measured using the flame photometric method described by Gupta et al. (2007). 2 g sample of the plant, that was previously dried using an oven at 75 °C for 24 h, was incubated in 550 °C for 6 h.

Statistical analysis

This study was performed as a three-way ANOVA factorial experiment with four replications on the basis of a completely randomized design (CRD). Each replication consisted of three sub-samples of each vitro sample. Mean comparison for treatments was carried out based on least

significant difference (LSD) at a 5% level of probability. SAS statistical software v. 9.3 was used to analyze the data and Excel 2007 was used to draw the charts.

Results and discussion

Proline content

Watanabe et al. (2000) demonstrated that in vitro culture is an acceptable way for studying salinity tolerance in many plants, since in most cases the results are in orientation of those obtained under indoor and outdoor conditions. In present experiment, salinity stress was shown to increase the proline content in grape var. Khoshnaw (Table 1). The highest content of free proline was obtained at the 100 mM salinity level, while the lowest was recorded in the control treatment. All three levels of salinity differed significantly from each other. Under salinity stress, higher levels of glutamate as a pre-product for chlorophyll and proline were consumed to generate proline (Ahmad 2014). In this respect, the activity of glutamate ligase escalated under stress conditions, and glutamine was converted into proline. Proline can adjust the osmotic pressure of cells under various stresses. Additionally, free proline can remove and detoxify the reactive oxygen species (ROS) generated as a result of stresses, thus protecting cell membranes against these radicals (Saed-Moucheshi et al. 2014).

The application of iron nanoparticles increased the content of proline under both stressful and non-stressful conditions (Table 2). Potassium-silicate application increased the content of proline and carbohydrate compared to the control treatment, albeit less than the other

treatments. Applying potassium silicate and iron nanoparticles together as a treatment increased proline content, but the effect was lower than that for iron nanoparticles alone (Tables 1, 2). The results of this study indicated that silicon application reduces proline content under both stress and control conditions. The reason for this is the decrease of salt absorption and increased cell growth caused by silicon.

Hydrogen peroxide (H_2O_2)

The highest content of H_2O_2 was observed for 100 mM salinity without application of iron nanoparticles, and the lowest for 2 mM potassium silicate and 0.8 ppm iron-nanoparticle treatment under the non-stress condition (Table 3). In this study severe salinity generated higher hydrogen peroxide in grape var. Khoshnaw. Hossain et al. (2015) have explained that under conditions of very severe salinity stress, high levels of hydrogen peroxide can dramatically damage plant tissue, and accordingly cause plant death. Under severe salinity stress, without detoxification from hydrogen peroxide, this results in the Haber–Weiss reaction and the generation of hydroxyl radicals and instability of biological macro-molecules such as lipids and proteins (Taiz et al. 2015). Application of iron nanoparticles and potassium silicate separately as well as together reduced hydrogen-peroxide levels under both saline and control conditions (Table 2). One of the most important mechanisms leading to H_2O_2 production is the activity of the enzyme superoxide dismutase (SOD), which converts dismutase superoxide radicals into H_2O_2 in chloroplasts (Hossain et al. 2015). Catalase, on the other side, detoxifies H_2O_2 into water and oxygen. Silicon increases the activity of both SOD and CAT enzymes, thus reducing hydrogen-

Table 1 Influence of salinity stress, Iron and potassium silicate on some nutritional elements and physiological characteristics of grapevine under in vitro conditions

	H_2O_2 ($\mu\text{mol g}^{-1}$ FW)	MDA ($\mu\text{mol g}^{-1}$ FW)	MSI (%)	Proline (mg g^{-1} FW)	Na (%)	K (%)	Na/K
Salinity (mM)							
0	2.38583c	0.4925c	84.0267a	0.31278c	0.232222c	1.70528a	14.41c
50	4.42806b	1.36611b	68.9639b	0.90444b	0.754167b	0.99278b	65.71b
100	6.22417a	2.66028a	52.3547c	1.13528a	1.303611a	0.47417c	377.2a
Iron (ppm)							
0	5.35917a	1.80083a	65.4425c	0.59972c	0.860278a	0.93361c	165.96a
0.08	4.32944b	1.48528b	68.4056b	0.79778b	0.775b	1.02806b	156.42ab
0.8	3.34944c	1.23278c	71.4972a	0.95500a	0.654722c	1.21056a	134.94b
Potassium silicate (mM)							
0	4.74528a	1.72583a	66.3086c	0.87222a	0.830556a	0.93917c	181.95a
1	4.32417b	1.48222b	68.6575b	0.80139b	0.754722b	1.08083b	145.09b
2	3.96861c	1.31083c	70.3792a	0.67889c	0.704722c	1.15222a	130.28b

Different letters indicate significances by LSD tests at $P < 0.05$

Table 2 Influence of salinity stress, Iron and potassium silicate on some physiological characteristics of grapevine under in vitro conditions

Salinity (mM)	Iron (ppm)	Potassium silicate (mM)	APX ($\mu\text{M H}_2\text{O}_2$ min ⁻¹ mg ⁻¹ protein)	POD ($\mu\text{M H}_2\text{O}_2$ min ⁻¹ mg ⁻¹ protein)	SOD (U g ⁻¹ protien)	Protein (mg g ⁻¹ Fw)	Proline (mg g ⁻¹ FW)
0	0	0	0.039o	3.89f	0.21q	78.82e	0.71klm
		1	0.063n	3.4g	0.23pq	81.78d	0.67klm
		2	0.081lm	2.94hi	0.18q	84.15bcd	0.56lmn
	0.08	0	0.069mn	2.64i	0.29op	82.24cd	0.61lm
		1	0.089l	2.19j	0.35no	84.22bcd	0.5lmn
		2	0.092l	1.78kl	0.23pq	85.18abc	0.45mn
	0.8	0	0.088l	2.05jk	0.41mn	84.72bcd	0.53lmn
		1	0.108k	1.53l	0.52l	87.09ab	0.3mn
		2	0.118jk	1.05m	0.43m	88.05a	0.12n
50	0	0	0.084l	6.03b	0.87hi	64.22j	1.71gh
		1	0.109k	5.43cd	0.8j	67.25i	1.53ghi
		2	0.128ij	5.21d	0.68k	68.04hi	1.48ghi
	0.08	0	0.122ijk	4.55e	1.01ef	67.74hi	1.55ghi
		1	0.136hi	4.41e	0.92gh	68.6ghi	1.3hij
		2	0.144gh	4.34e	0.78j	70.83fgh	1.24hij
	0.8	0	0.126ij	3.53fg	1.18d	69.58ghi	1.43hi
		1	0.148gh	3.24gh	1.04e	71.38fg	1.1jk
		2	0.164ef	3.14gh	0.87hi	73.06f	0.96jkl
100	0	0	0.134hi	7.27a	0.96fg	45.61p	3.65a
		1	0.173de	7.13a	0.84ij	48.43o	3.16b
		2	0.179de	6.94a	0.64k	50.69no	2.74bcd
	0.08	0	0.157fg	6.98a	1.41b	49.98no	2.81bc
		1	0.183d	6.15b	1.15d	52.65mn	2.59cde
		2	0.199c	5.94b	1.06e	54.22lm	2.33def
	0.8	0	0.186cd	5.77bc	1.53a	53.9lm	2.54cde
		1	0.225b	5.46cd	1.37b	56.52kl	2.19ef
		2	0.251a	4.39e	1.26c	59.2k	1.93fg

Different letters in each column indicate significances by LSD tests at $P < 0.05$

peroxide content and phospholipase-acid activity. Furthermore, iron in stressful conditions increases the activity of antioxidant enzymes such as SOD and CAT, which reduces free radicals in plants under stressful conditions (Saed-Moucheshi et al. 2014).

Malondialdehyde (MDA)

The lowest membrane stability and highest MDA content were obtained for 100 mM salinity stress without any special treatment; the highest membrane stability and the lowest MDA content were obtained for 2 mM potassium silicate and 0.8 ppm iron nanoparticle under non-stressful conditions (Table 3). Salinity stress reduced membrane stability while increasing malondialdehyde content in grape var. Khoshnaw (Table 1). Lipid peroxidation of the cell membrane is one of the most important signs of damage and ionic leakage in salinity-stress conditions.

Under salinity stress, as a result of lipid peroxidation, cell membranes are damaged and the selective permeability of the membrane is disrupted, which leads to higher permeability and electrolyte leakage in the cells (Saed-Moucheshi et al. 2015).

In the current study, iron nanoparticles, potassium silicate, and their application together lowered malondialdehyde content and raised the membrane stability of grape cells in the treated plants. It has been explained that silicon brings about lower permeability of leaves plasma walls in line with decreasing lipid peroxidation under salinity stress (Saed-Moucheshi et al. 2015). Silicon application in salinity-stress conditions reduces electrolyte leakage by both decreasing absorption of salt, and by accumulating in cell walls, thus increasing stability. It is also demonstrated that by inducing amino acids such as proline toward detoxification of free radicals, silicon inhibits lipid peroxidation of cell membranes, which reduces malondialdehyde

Table 3 Interaction effects of salinity, Iron and potassium silicate on some nutritional elements and physiological characteristics of grapevine under *in vitro* conditions

Salinity (mM)	Iron (ppm)	Potassium silicate (mM)	H ₂ O ₂ (μmol g ⁻¹ FW)	MDA μmol g ⁻¹ FW)	MSI (%)	Na (%)	K (%)	Na/K
0	0	0	3.88f	0.704l–n	78.8f	0.33l	1.54e	12.1e
		1	3.4gh	0.665mn	81.7e	0.28lm	1.63cd	13.9e
		2	2.94ij	0.562mn	84.1c–e	0.24mn	1.63cd	12.6e
	0.08	0	2.64j	0.609mn	82.2de	0.24mn	1.58de	19.7e
		1	2.19k	0.494no	84.2c–e	0.23m–o	1.68c	15.6e
		2	1.78lm	0.449no	85.1bc	0.2no	1.78b	14.6e
	0.8	0	2.05kl	0.531no	84.7b–d	0.21no	1.65cd	14.1e
		1	1.53m	0.297no	87.0ab	0.2no	1.9a	13.6e
		2	1.04n	0.116o	88.05a	0.18o	1.98a	13.3e
50	0	0	6.03b	1.713gh	64.2k	0.97g	0.83k	94.6e
		1	5.42cd	1.529g–i	67.2j	0.81h	0.86jk	57.6e
		2	5.20d	1.475h–j	68.0ij	0.78hi	0.93ij	52.2e
	0.08	0	4.54e	1.545g–i	67.3j	0.82h	0.8k	73.1e
		1	4.40e	1.30h–k	68.6h–j	0.74ij	0.96i	57.5e
		2	4.33e	1.238i–k	70.8g–i	0.69jk	1.05h	57.7e
	0.8	0	3.52fg	1.432h–j	69.5h–j	0.71j	0.99hi	76.5e
		1	3.23g–i	1.096j–l	71.3gh	0.65k	1.2g	67.6e
		2	3.13hi	0.959k–m	73.0g	0.64k	1.32f	54.2e
100	0	0	7.27a	3.645a	45.6q	1.51a	0.24p	529.7a
		1	7.12a	3.161b	48.4p	1.41c	0.38o	382.2bc
		2	6.93a	2.741cd	50.6op	1.43bc	0.38o	338.3cd
	0.08	0	6.98a	2.811bc	49.9op	1.48ab	0.38o	435.9b
		1	6.14b	2.592c–e	52.6no	1.36d	0.49mn	380.8bc
		2	5.94b	2.327d–f	54.2mn	1.24e	0.54m	352.6c
	0.8	0	5.78bc	2.538c–e	53.8mn	1.22e	0.45no	381.7bc
		1	5.45cd	2.191ef	56.5lm	1.14f	0.63l	316.5cd
		2	4.39e	1.927fg	59.2l	0.97g	0.78k	276.6d

Different letters in each column indicate significances by LSD tests at $P < 0.05$

content (Liang et al. 2003). The action of iron in electron redox reactions makes it an important element with a central role in essential biochemical reactions, including oxygen transportation, sharp reaction with free radicals, DNA synthesis, electron transportation in chloroplasts and mitochondria, and general energy production. Iron and zinc induce activity of enzymatic antioxidants in plants, which contributes to the mitigation of the effects of free radicals on membrane systems (Sperotto et al. 2008).

Total protein content

The highest amount of total soluble protein was obtained with the application of 2 mM potassium silicate and 0.8 ppm iron nanoparticles under non-stressful conditions (Table 2). The lowest content of total soluble protein was observed in 100 mM salinity stress without the application

of any compensating elements (Fig. 1). Moreover, the application of iron was shown to be an effective way to increase enzymatic antioxidant activities (Figs. 1, 4). Reduction of total protein content under salinity stress may be caused by the effect of generated oxidative stress on protein structure and the destruction of protein synthesis paths. The reaction of free radicals with proteins and the resulting changes in the protein structures, increased activity of degrading enzymes, and reductions in the generation of some types of free amino acids could be named as the most important factors in decline in protein content under stress conditions (Saed-Moocheshi et al. 2014). Application of potassium silicate and iron nanoparticles individually and together under both salinity and control conditions increased the amount of total protein (Fig. 3). Since iron and zinc have roles in nitrogen metabolism and protein structure, their application can increase the amount

of total protein (Saed-Moucheshi et al. 2014). As some scientists have pointed out, silicon in salinity-stress conditions increases the content of soluble proteins in plants. The possibility here is that adding potassium silicate to the media of the plants increases the content of chlorophylls, and for this reason, increases photosynthesis capacity, ultimately leading to higher protein content. Also, the potassium silicate increases the effectiveness of antioxidant systems and thus protects proteins from degradation.

Antioxidant enzymes activity

The highest activities of peroxidase (POD), ascorbate peroxidase (APX), and superoxide dismutase (SOD) were observed in salinity stress (100 mM) with the application of potassium silicate (2 mM) and iron nanoparticles together, while their lowest activities were measured in control treatment (Figs. 1, 2, 3, 4). The increase in enzymes activities indicates a defense response to tissues damage induced by NaCl in the culture media. Similar results in another study in apple under in vitro condition have been reported (Molassiotis et al. 2006). Sivanesan and Park (2014) reported that the application of potassium silicate in shoot-proliferation media in ajuga significantly increased the activity of SOD, POD, CAT, and APX. Ferreira et al. (2002) and Laspina et al. (2005) separately found that the activities of peroxidase iso-enzymes decreased in soybean and sunflower in media with no iron in response to higher hydrogen peroxide. These results indicate that POD, CAT, and SOD activities depend on iron content. In the current study, salinity stress increased the activities of the three measured antioxidants in grape var. Khoshnaw. Also, applications of potassium silicate and iron nanoparticles individually and together increased the activities of antioxidant enzymes (Fig. 3).

Fig. 1 Influence of salinity stress on ascorbic peroxidase (APX), peroxidase (POD) activity and protein content in grapevine under in vitro conditions. Different letters indicate significance by LSD tests at $P < 0.05$

Fig. 2 Influence of iron application on ascorbic peroxidase (APX), peroxidase (POD) activity and protein content in grapevine under in vitro conditions. Different letters indicate significance by LSD tests at $P < 0.05$

Fig. 3 Influence of potassium silicate application on ascorbic peroxidase (APX), peroxidase (POD) activity and protein content in grapevine under in vitro conditions. Different letters indicate significance by LSD tests at $P < 0.05$

In an experiment by Miao et al. (2010), potassium silicate increased antioxidant enzymes activities and decreased hydrogen-peroxide content, thus compensating for some negative effects of potassium shortage in soybean. Amirjani (2010) reported that application of potassium-silicate accomplice in either salinity-stress or non-saline conditions resulted in the highest SOD activity. Becana et al. (1998) explained that a shortage of iron, as a cofactor in the structure of antioxidant enzymes, results in lower antioxidant activity and higher susceptibility of plants in response to environmental stresses. Applications of iron sulfate, zinc sulfate, copper sulfate, and manganese sulfate in the study of Papadakis et al. (2007) carried out on citrus resulted in higher activity for SOD, CAT, and GPX. In the presence of hydrogen peroxide, plants cannot continue life and growth under conditions of severe iron shortage; conversely, plants with sufficient iron survive (Hossain et al. 2015). Choi and Lee (2009), in a study related to the effect of potassium silicate on antioxidant enzyme activities in two grape species (*V. vinifera* L.) under salinity

Fig. 4 Influence of salinity, iron and potassium silicate application on superoxide dismutase (SOD) activity in grapevine under in vitro conditions. Different letters indicate significance by LSD tests at $P < 0.05$

stress, found that potassium silicate decreased SOD and CAT activities but induced APX activity.

Sodium and potassium

Salinity stress (100 mM) with the application of neither potassium silicate nor iron nanoparticles showed the highest levels of sodium and lowest levels of potassium; in contrast, the control condition (no stress) with the application of potassium silicate and iron nanoparticles together had lowest content of sodium and highest content of potassium (Table 3). Salinity stress significantly increased the amount of sodium in the aerial parts of the grape var. Khoshnaw; alternatively, it decreased the content of potassium. As shown in Table 3, application of either iron nanoparticles or potassium silicate, in addition to their application together, on grape under both non-stress and stress conditions reduced sodium content but increased potassium content.

High concentrations of sodium ions disrupted osmotic balance and membrane structure, reduced growth, and prevented cell division. In addition, high concentrations of sodium ions in nutritional solutions destroys hydraulic conductivity and/or tissue permeability related to water, thus decreasing plant survival (Abadía et al. 1999). Sotiropoulos et al. (2006) reported that explants are under stress in two ways under in vitro salinity: by the increase of culture media osmotic potential as a result of high Na content, and by Na toxic effects. According to the explanation of Yeo et al. (1999), accumulation of silicon in plant leaves triggers the generation of two layers in these leaves, which reduces transpiration maintains water in the plant. David (1960) argued that because a great amount of sodium absorption is passively performed by transpiration

in plants, reduction of sodium absorption by applying potassium silicate may result from the effects of potassium silicate on plant's transpiration. The study of Romero-Aranda et al. (2006) on tomato and spinach found that silicon application reduced the destructive effects of sodium toxicity in these plants, and that the application of iron-chelate EDDHA in sunflower reduced the content of sodium and chlorine and increased the content of potassium under salinity stress.

The small diameter of iron in the form of nanoparticles makes the iron more readily absorbed, aiding the plant's nutritional balance. By providing a situation in which energy is produced in the plant through suitable photosynthesis compartments, the absorption of elements is increased (Ursache-Oprisan et al. 2011).

The highest ratio of sodium to potassium was observed in plants under salinity conditions (100 mM) treated with neither iron nanoparticles nor potassium silicate; conversely, the lowest ratio was achieved in plants treated with both 0.8 ppm iron nanoparticles with 1 mM and 2 mM potassium silicate under non-stress conditions. The ratio of sodium to potassium was significantly high in salinity-stress conditions. In contrast, the application of iron nanoparticles and potassium-silicate under both stress and non-stress conditions reduced the ratio of sodium to potassium.

Maintaining actively selective properties for potassium in relation to sodium is one of the most important factors in plant resistance to salinity stress. Considering the content of potassium in relation to content of sodium under salinity stress is a consequential and far-reaching criterion in plant resistance to salinity, as a higher ratio of potassium to sodium can compensate for the negative effects of sodium toxicity under saline conditions (Liang 1999). With

increasing of NaCl concentration in the culture medium, the Na^+/K^+ ratio increased in stem, leaf and root (Amini and Ehsanpour 2005). The K^+ uptake is influenced by Na^+ due to both ions chemical similarities. Therefore, the plants that have good selectivity of K^+ over Na^+ can be considered as salt tolerant (Amini and Ehsanpour 2005). Tahir et al. (2006) have argued that by accumulating inside the apoplastic space, silicon prohibits sodium transportation toward areal parts of the plant, reduces salt absorption, and improves actively selective absorption in regard to ratio of potassium per sodium. In a study on wheat plants, foliar spraying with iron and manganese led to higher concentrations of potassium, iron, and manganese, and a higher ratio of potassium to sodium in the areal parts of the plants (Hauser and Horie 2010). In another study, using iron increased the selectivity coefficient of potassium in leaves and increased the in ratio of potassium to sodium, resulting in higher salinity tolerance and higher growth. Sabaghnia and Janmohammadi (2015) noted that the ratio of potassium to sodium is affected by interaction between genotypes by iron chelated in the form of nano-fertilizer.

Conclusion

The overall results indicated that salinity heightened the production of ROSs such as hydrogen peroxide and increased the ratio of proline and total protein in line with increased activity of enzymatic antioxidants. In contrast, application of iron nanoparticles and potassium silicate could significantly increase the content of free proline and total protein as well as enzymatic antioxidant activity, lowering the content of hydrogen peroxide. Iron and potassium silicate were found to lower sodium content and increase potassium content under salinity-stress conditions. These results suggest that micronutrient application in stressful conditions is a suitable and effective method for somewhat compensating for the negative effects of salinity stress. Tissue culture in this study was found to be an economically efficient and applicable method for producing grape scions and conducting experiments on grape. The application of iron nanoparticles together with potassium silicate on grape trees in natural environments of drought or salinity to improve the quality and quantity of grape fruits is suggested for future works.

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