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Morphological and biochemical response of eight pomegranate (*Punica granatum* L.) cultivars under salinity stress

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ABSTRACT

Purpose: Identification of pomegranate cultivars with higher tolerance to salinity stress was the purpose of the current study. **Research Method**: One-year-old rooted cuttings of the pomegranate cultivars including 'Malase Saveh' ('M-Saveh'), 'Malase Isfahan' ('M-Isfahan'), 'Robabe Ghermeze Shiraz' ('Robab'), 'Gabrie Yazd' ('G-Yazd'), Gabrie Torshe Yazd' ('GT-Yazd'), 'Zaghe Sefide Yazd' ('ZS-Yazd'), 'Zaghe Torshe Yazd' ('ZT-Yazd') and 'Malase Torshe Pishva' ('M-Pishva') were cultured in plastic pots and treated with different concentrations of sodium chloride in irrigation water including 0 (control), 3, 6, 9 and 12 dS m^{-1}. **Findings:** At 12 dS m^{-1} salinity level, the lowest decrease in plant height was observed in 'Robab', and 'M-Pishva' and the lowest decrease in the plant diameter was recorded in 'ZT-Yazd' and 'G-Yazd'. At the same salinity level, 'GT-Yazd' showed the lowest percentage of increase in electrolyte leakage (EL) compared to the control (23%). The highest increase in proline content was observed at 9 dS m^{-1} salinity for 'ZS-Yazd' and at 12 dS m^{-1} salinity for 'GT-Yazd'. At high salinity levels, leaf chlorophyll content decreased in pomegranate cultivars compared to the control. The activity of peroxidase enzyme in 'M-Pishva', ZT-Yazd' and 'GT-Yazd' followed an increasing and then decreasing with increasing salinity levels. The activity of the superoxide dismutase enzyme was highest in 'G-Yazd', 'M-Isfahan' and 'Robab'. **Limitations**: Investigation of salinity tolerance in pots is one of the limitations of this study. **Originality/Value:** Yazd cultivars including 'G-Yazd', 'GT-Yazd', 'ZS-Yazd' and 'ZT-Yazd' were more tolerant to salinity stress.

INTRODUCTION

Iran has the richest pomegranate genetic resources in the world which are mainly distributed in the arid and semi-arid regions (Sarkhosh et al., 2006). Soil salinity in arid and semi-arid regions is one of the limiting factors of yield (Horie et al., 2012). Also in Iran, salinity is a limiting factor for sustainable agricultural production.

The cultivation of plants in saline soils or conditions causes morphological, physiological and biochemical changes. These changes include decreased leaf chlorophyll content and leaf ion toxicity (Acosta-Motos et al., 2017) and increased proline content (Khayyat et al., 2014). One of the important biochemical changes in higher plants under salinity stress is increased levels of reactive oxygen species (ROS) including superoxide, hydrogen peroxide and hydroxyl ion and their oxidative effect on cells (Mastrogiannidou et al., 2016). To scavenge the ROS, plants possess protective enzymes like peroxidase (EC 1.11.1.7) and superoxide dismutase (1.15.1.1) that protect plants from ROS damage (Wu et al., 2012). Therefore, plant survival under these conditions depends on the ability of the plant to understand the stimulus, the production, and transmission of messages and their various physiological and biochemical changes (Simaei et al., 2011).

There are major differences between plant species in salt tolerance (Koyro et al., 2013). Pomegranates have been reported semi-tolerant to salinity in field conditions (Francois & Maas, 1999). Okhovatian-Ardakani et al. (2010) showed that 'Voushik', 'Saravan', 'Malas Yazdi', and 'Tab-o-Larz' pomegranate cultivars were tolerant to salinity. As regards, salinity is one of the limiting factors of crop production, the study of the physiological and biochemical response of pomegranate cultivars to salinity stress helps us to identify the most tolerant ones and to use them in orchards development. The aim of this study was the screening of eight pomegranate cultivars exposed to different levels of salt stress based on some photosynthetic and biochemical markers.

MATERIALS AND METHODS

Plant material

This research was conducted at Isfahan Agricultural Research Station, Iran. In this study, cuttings of eight pomegranate cultivars including 'Malase Saveh' ('M-Saveh'), 'Malase Isfahan' ('M-Isfahan'), 'Robabe Ghermeze Shiraz' ('Robab'), 'Gabrie Yazd' ('G-Yazd'), Gabrie Torshe Yazd' ('GT-Yazd'), 'Zaghe Sefide Yazd' ('ZS-Yazd'), 'Zaghe Torshe Yazd' ('ZT-Yazd') and 'Malase Torshe Pishva' ('M-Pishva') were rooted in late February 2016. From maternal trees that were in the same nutrition and irrigation conditions. At the end of 2017, rooted cuttings that were similar in age, diameter and size, were removed from the culture media and planted in 20-liter plastic pots (35 cm height and 30 cm opening diameter) that filled with clay loam soil. The physical and chemical properties of the soil mixture (Table 1) were measured before the experiment.

Salinity treatments

Salinity treatments were begun two months after transferring young plants to the pot and continued for 130 days. To avoid of sudden shock and to create gradual equilibrium in the soil, the pots were irrigated four times with 3 dS m^{-1} saline water for seven days. The electrical conductivity ratio of inlet and outlet water was measured at all four times and recorded as leaching percentage. The consumed water in each pot during the growing season was measured by calibrated containers. Irrigation depth (dn) was calculated to determine the irrigation period and net irrigation requirement (In). To prevent salt accumulation, more water was added to the pots (15% of the field capacity) so that the drainage water was removed from the bottom of the pots. Salinity treatments were applied for eight cultivars of pomegranate young plants in five salinity levels with different concentrations, including 0 (control, the water without salt addition), 3, 6, 9 and 12 dS m^{-1} of sodium chloride in irrigation water. The qualitative characteristics of irrigation water for salt stress treatment and urban water analysis for control treatment are presented in Tables 2 and 3. The used sodium chloride in this study was from lake salt that was analyzed before use (Table 4). Any fertilizer (nutrient solution) was not applied to the irrigation water. The soil chemical properties of each salinity level were measured at the end of the experiment (Table 5).

Table 1. Some physical and chemical properties of soil mixture

Property	Unit	Value	Property	Unit	Value
pH	-	7.75	K	mg kg^{-1}	709
SP	%	17	P	mg kg^{-1}	2.26
FC	%	30	Zn	mg kg^{-1}	0.64
PWP	%	7.25	Mg	mg kg^{-1}	10.23
OC	%	10.14	Fe	mg kg^{-1}	5.01
N	%	0.096	Cu	mg kg^{-1}	1.23
TNV	%	32.5	B	mg kg^{-1}	2.66
Silt	%	35	Na	meq l^{-1}	4.53
Clay	%	35	Cl	meq l^{-1}	7.19
Sand	%	30	Salinity	dS m^{-1}	3.24
Tissue	-	Clay Loam	-	-	-

Table 2. The chemical analysis of used water for salt stress treatment

EC (dS m^{-1})	pH	K (mg l^{-1})	Mg (mg l^{-1})	Ca (mg l^{-1})	Cl (mg l^{-1})	Na (mg l^{-1})
3	7.45	5.09	66	1.98	1320	770
6	7.84	12.49	162	4.86	3240	1890
9	7.90	19.66	255	7.65	5100	2975
12	8.20	25.45	330	9.9	6600	3850

Table 3. Properties of the urban water for control treatment

Total cations (meq l^{-1})	Na (meq l^{-1})	Mg (meq l^{-1})	Ca (meq l^{-1})	Total anions (meq l^{-1})	Sulfate (meq l^{-1})	Chloride (meq l^{-1})	Bicarbonate (meq l^{-1})	pH	EC dS m^{-1}
6.8	2.8	1.2	2.8	6.8	0.6	3	3.2	6.9	0.38

Table 4. Some chemical properties of used lake salt for salinity treatments

Co	Cd	Cr	Ni	Pb	Fe	Cl	Na	Mg	Ca	K	P
		(mg kg^{-1})							(%)		
3	2	0	8	15	0	60	35	3	0.09	0.23	0

Table 5. Chemical properties of soil mixture in each salinity level at the end of the experiment

E.C (Water)	E.C. (Soil)	pH.	O.C	T.N.V	Total N	Na	Cl
(dS m^{-1})	(dS m^{-1})	-	%	%	%	(meq l^{-1})	(meq l^{-1})
0	6.47	7.85	1.19	32.5	0.099	28.1	46.81
3	11.18	7.73	1.13	32	0.095	64.5	79.63
6	16.57	8.02	1.04	31.5	0.088	128.4	121.61
9	24.6	7.92	1.020	30.5	0.102	195.4	194.80
12	26	8.08	0.97	30.5	0.101	216.7	197.02
$K_{ava.}$	$P_{ava.}$	$Zn_{ava.}$	$Mn_{ava.}$	$Fe_{ava.}$	$Cu_{ava.}$	$B_{ava.}$	
(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})	
582	21.9	0.71	11.04	4.96	1.45	2.41	
382	24.9	0.66	9.88	5.12	1.18	2.69	
191	21.9	0.67	11.25	4.25	1.39	2.77	
152	25.2	0.70	11.84	5.16	1.47	2.45	
108	20.1	0.69	10.37	4.87	1.18	2.31	

Measurement of traits

All traits were evaluated and recorded at the end of the salinity treatment period (130 days after the start of the stress period). Plant height from the soil surface and plant diameter at a distance of 2 cm above soil surface were measured by the meter and a digital caliper, respectively.

The measure of electrolyte leakage (EL): Hamed et al. (2007) method was used to measure electrolyte leakage (EL). Ten discs of the fresh leaf (0.5 cm diameter) were cut from the fully expanded leaves and the samples were washed with deionized water to remove surface-adhered electrolytes. The leaf discs were placed in closed tubes containing 5 ml of deionized water and incubated at 10 °C for 24 h. Subsequently, the initial electrical conductivity of the solution (EC1) was determined using a Conductometer GRYF 158 (GRYF HB, Ltd., Czech Republic). The samples were then incubated in a water bath at 95 °C for 20 min to release all electrolytes, cooled down to 25 °C and their final electrical conductivity (EC2) was measured. EL was calculated using the following equation.

$$EL = \left(\frac{EC1}{EC2}\right) \times 100\ (\%) \quad (1)$$

The measure of chlorophyll content: To test chlorophyll content, 0.1 gr fresh leaf were placed into 10 ml of dimethylsulfoxide (DMSO) and pigments were allowed to extract in the dark at 65 °C for 30 min. The absorbance of the extracts was measured using Spectrophotometer (T80 UV/Visible) at 647 and 663 nm. The content of chlorophyll a, b and total was calculated by Equation 2, 3 and 4 (Hiscox & Israelstam, 1979).

$$Chl_a = 12.25A_{663} - 2.79A_{647} \quad (2)$$
$$Chl_b = 21.5A_{647} - 5.1A_{663} \quad (3)$$
$$Chl_T = Chl_a + Chl_b \quad (4)$$

The measure of proline content: The proline content was determined by the method given by Bates et al. (1973). For this, 10 ml of 3% (w/v) sulphosalicylic acid was added to 0.4 gr leaf materials. The homogenate was filtered through filter paper. Two ml acetic acid and 2 ml acidic ninhydrin reagent were added to a 2 ml aliquot. The mixture was thoroughly stirred and incubated in a boiling water bath for 1h and then the reaction was stopped by using an ice bath and warmed to room temperature. The mixture was extracted with toluene and the absorbance of the fraction with toluene extracted from the liquid phase was read at 518 nm. The concentration of proline in the sample was computed from a standard curve of proline.

The measure of activity enzyme: The SOD activity was assayed by monitoring its ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT). One unit of SOD was defined as the amount of enzyme necessary to cause 50% inhibition of the rate of NBT reduction at 560 nm. In order to 1 ml reaction mixture contained 75 µM NBT, 2 µM riboflavin, 13 mM methionine, 0.1 mM EDTA, 50 mM phosphate buffer (pH 7.0) and 50 µl of enzyme extraction. The test cells containing the mixture were placed on a shaker under light at 78 µmol photons s-1 m-1 for 15 min and absorbance at 560 nm was recorded. A nonirradiated reaction mixture that did not develop color served as the control and its absorbance was subtracted from A560 of the reaction solution (Dhindsa et al., 1981).

For Guaiacol POD, the oxidation of guaiacol was measured by the increase in absorbance at 470 nm for 1 min. The reaction solution contained 450 µl of 225 mM guaiacol, 100 µl of 50 mM phosphate buffer (pH 7.0) and 100 µl enzyme extract. The reaction was started with 450 µl of 45 mM H2O2 (Hemeda & Kelin, 1990).

Data analysis

The experiment was conducted as a factorial experiment in a randomized complete block design (RCBD) with three replications. The first factor was pomegranate cultivar with eight levels and the second factor was salinity treatment with five levels. After data collection, analysis of variance was carried out using SAS software (version 9.3) and the means were compared using the LSD method.

RESULTS

Plant height and diameter stem

All cultivars showed the lowest plant height in 12 dS m^{-1} with the exception of 'Rabab'. The height of 'Rabab' had no significant differences among 3, 6, 9 and 12 dS m^{-1} treatments. The highest height in 'M-Isfahan' and 'Robab' was observed in control treatment, but in other cultivars, the highest height was observed in 3, 6 or 9 dS m^{-1} concentrations. In 12 dS m^{-1} salinity, 'ZS-Yazd' (65.16%) and 'G-Yazd' (65.09%) showed the highest decrease in plant height compared to control. At this salinity level, the lowest decrease in plant height compared to the control was observed in 'Robab' (41.4%) and 'M-Pishva' (43.24%) (Table 6). The lowest percentage of plant height reduction compared to the control was observed in 9 dS m^{-1} salinity in 'Robab' and 'G-Yazd' (20.23% and 16.51%, respectively). 'ZS-Yazd' and 'GT-Yazd' had a higher height than control plants in this salinity level. 'M-Pishva' in salinity of 6 dS m^{-1} and 'ZT-Yazd' in salinity of 3 dS m^{-1} had the lowest decrease percentage of height compared to the control treatment (Table 6).

The comparison of means showed that the lowest stem diameter in all cultivars except 'ZT-Yazd' was observed in 12 dS m^{-1} salinity. 'ZT-Yazd' showed the lowest diameter in 9 dS m^{-1}, but did not show a significant difference with the salinity of 12 dS m^{-1} (Table 6). In salinity of 12 dS m^{-1}, plant diameter in 'Robab', 'GT-Yazd', 'M-Isfahan', 'M-Saveh', 'ZS-Yazd', 'M-Pishva', 'ZT-Yazd' and 'G-Yazd' decreased by 84.59, 84.35, 82.39, 71.09, 59.23, 53.85, 47.47, and 36.57 percent compared to control, respectively. In salinity of 9 dS m^{-1}, the lowest diameter reduction compared to control was observed in 'Robab'. In this salinity level, 'ZS-Yazd' and 'G-Yazd' had a higher diameter than control plants. In 6 dS m^{-1} treatment, 'M-Pishva' and 'G-Yazd' showed the lowest diameter reduction compared to the control. 'ZS-Yazd', 'M-Isfahan' and 'Robab' had a higher diameter than control plants at this salinity level. In 3 dS m^{-1} treatment, 'ZT-Yazd' and 'M-Isfahan' showed the lowest decrease in diameter compared to control. The diameter of 'ZS-Yazd' and 'M-Saveh' in this salinity level was higher than the control treatment.

Table 6. Interaction of salinity and pomegranate cultivar on measured vegetative traits

Cultivar	Salinity (dS m^{-1})	Plant height (cm)	Plant diameter (mm)
'ZS-Yazd'[1]	0	51.67^{e-m}	5.25^{e-k}
	3	56.33^{d-l}	6.48^{c-g}
	6	56.67^{d-l}	6.90^{a-f}
	9	61.33^{d-h}	5.55^{e-j}
	12	18.00^{o}	2.14^{n-q}
'M-Pishva'[2]	0	74.00^{cde}	6.74^{b-g}
	3	105.00^{ab}	3.35^{k-o}
	6	71.00^{c-f}	6.15^{d-h}
	9	50.33^{e-n}	3.13^{k-o}
	12	42.00^{g-n}	3.11^{l-p}
'M-Isfahan'[3]	0	69.33^{c-f}	5.68^{e-i}
	3	54.00^{d-l}	5.13^{f-l}
	6	59.67^{d-j}	5.99^{e-h}
	9	36.67^{h-o}	2.94^{m-p}
	12	32.33^{k-o}	1.00^{pq}
'ZT-Yazd'[4]	0	62.33^{d-h}	6.74^{b-g}
	3	60.67^{d-i}	6.56^{c-g}
	6	71.33^{c-f}	3.32^{k-o}
	9	33.33^{j-o}	2.21^{n-q}
	12	23.67^{no}	3.54^{j-n}
'G-Yazd'[5]	0	70.67^{c-f}	7.30^{a-e}
	3	91.00^{abc}	6.64^{c-g}
	6	71.00^{c-f}	6.65^{c-g}
	9	59.00^{d-k}	8.42^{abc}
	12	24.67^{mno}	4.63^{g-m}
'M-Saveh'[6]	0	80.67^{bcd}	8.13^{a-d}
	3	66.67^{c-g}	8.95^{a}
	6	111.33^{a}	3.56^{i-n}
	9	61.67^{d-h}	4.05^{h-n}
	12	29.67^{l-o}	2.35^{n-q}
'Robab'[7]	0	57.67^{d-k}	4.87^{f-m}
	3	44.33^{f-o}	5.81^{e-h}
	6	24.67^{mno}	5.18^{e-l}
	9	46.00^{f-n}	3.55^{j-n}
	12	34.00^{i-o}	0.75^{q}
'GT-Yazd'[8]	0	63.00^{d-h}	8.82^{ab}
	3	53.00^{e-l}	6.43^{c-g}
	6	92.33^{abc}	2.99^{m-p}
	9	70.33^{c-f}	5.53^{e-j}
	12	32.00^{k-o}	1.38^{opq}

Similar letters in each column indicate no significant difference at the 5% level of LSD test.
1- 'Zaghe Sefide Yazd', 2- 'Malase Torshe Pishva', 3- 'Malase Isfahan', 4- 'Zaghe Torshe Yazd', 5- 'Gabrie. Yazd', 6- 'Malase Saveh', 7- 'Robabe Ghermeze Shiraz', 8- 'Gabrie Torshe Yazd'.

Electrolyte leakage (EL) and proline

According to Table 7, in salinity of 12 dS m^{-1}, the lowest percentage of EL increase was observed in 'GT-Yazd' with an average of 23%. 'GT-Yazd' and 'G-Yazd' showed the lowest increase in EL (by 12 and 13%, respectively) compared to the control treatment at 9 dS m^{-1} salinity. In 6 dS m^{-1} salinity, 'ZS-Yazd', 'GT-Yazd' and 'Robab' showed the lowest increase in

EL compared to the control with the averages of 10, 5.5 and 4%, respectively. 'GT-Yazd' had the lowest increase of EL than control (7.5%) in salinity of 3 dS m^{-1}. The lowest EL was observed in 'ZS-Yazd' and 'G-Yazd' in 6 and 3 dS m^{-1} treatment, respectively. Other cultivars in the control treatment had the lowest EL.

The highest increase of proline in 9 dS m^{-1} salinity level for 'ZS-Yazd' (with the average of 5.47 µmol g^{-1} FW) and in 12 dS m^{-1} salinity for 'GT-Yazd' (with the average of 5.13 µmol g^{-1} FW) were observed. By applying salinity at the level of 9 dS m^{-1}, 'ZS-Yazd' and 'M-Saveh' showed the highest increase in proline compared to the control treatment with the averages of 54 and 46%, respectively (Table 7). 'M-Isfahan' and 'GT-Yazd' cultivars showed the highest proline in 12 dS m^{-1} treatment with the averages of 4.85 and 5.13 µmol g^{-1} FW, respectively. Other cultivars produced the highest proline content at lower salinity levels and their proline content decreased at higher salinity levels. So that, 'ZS-Yazd' and 'M-Saveh' in 9 dS m^{-1} salinity and 'M-Pishva', 'ZT-Yazd' and 'G-Yazd' in 6 dS m^{-1} salinity showed the highest proline content. The highest proline content in 'Robab' belonged to 3 and 6 dS m^{-1} treatments (Table 7).

Chlorophylls a, b and total chlorophyll

Chlorophyll a, b and total chlorophyll in 'G-Yazd' and 'Robab' had no significant changes at different stress levels. Chlorophyll a in 'ZS-Yazd' and Chlorophyll b in 'ZT-Yazd' also had no significant difference at different stress levels. The highest content of chlorophyll a and total chlorophyll in 'ZT-Yazd' was observed in 3 dS m^{-1} salinity and their content decreased at higher salinity levels. Other pomegranate cultivars showed the highest chlorophyll content in 6 dS m^{-1} and then decreased in the higher salinity levels. Chlorophyll content increased in 9 and 12 dS m^{-1} in 'G-Yazd', 'ZT-Yazd' and 'M-Saveh' compared to the control treatment. Chlorophyll content in these two salinity levels was significantly decreased compared to the control in 'M-Isfahan', 'GT-Yazd', M-Pishva' and 'ZS-Yazd' cultivars (Table 7).

Enzyme activities

According to the results of mean comparisons, the differences in the activity of POD at different salinity levels were not significant in 'ZS-Yazd', 'M-Isfahan' and 'Robab' cultivars. The activity of POD in 'M-Pishva', 'ZT-Yazd', 'G-Yazd' and 'GT-Yazd' followed an increasing-decreasing trend. With increasing salinity levels, the activity of this enzyme decreased in 'M-Saveh'. In general, 'GT-Yazd' showed the highest activity of this enzyme with a mean of 0.39 unit g^{-1} FW min in 3 dS m^{-1} treatment. The lowest activity of this enzyme was 0.11 unit g^{-1} FW min that observed in the control treatment of 'ZT-Yazd' and also in the treatment of 12 dS m^{-1} in 'G-Yazd' (Table 7).

Superoxide dismutase activity initially increased slightly. The activity of this enzyme in 6 dS m^{-1} salinity decreased and in 9 dS m^{-1} increased and then its activity remained constant (Fig. 1). Superoxide dismutase activity at the end of the salinity period was the highest in 'G-Yazd', 'M-Isfahan' and 'Robab' with mean values of 3.006, 2.927 and 2.923 unit g^{-1} FW min, respectively. 'ZT-Yazd', 'M-Saveh', 'ZS-Yazd' and 'M-Pishva' showed the lowest activity of this enzyme with the mean values of 2.669, 2.673, 2.683 and 2.696 unit g^{-1} FW min, respectively.

Table 7. Interaction of salinity and pomegranate cultivar on measured leaf biochemical traits

Cultivar	Salinity ($dS\ m^{-1}$)	Chlorophyll a ($mg\ g^{-1}FW$)	Chlorophyll b ($mg\ g^{-1}FW$)	Total chlorophyll ($mg\ g^{-1}FW$)	EL (%)	Proline ($\mu mol\ g^{-1}FW$)	POD ($g^{-1}FW\ min$)
'ZS-Yazd'[1]	0	1.76^{d-j}	0.67^{d-i}	2.43^{d-j}	24.26^{hij}	2.48^{lm}	0.27^{a-f}
	3	1.60^{e-k}	0.65^{d-i}	2.26^{d-k}	28.40^{g-j}	2.38^{m}	0.23^{b-i}
	6	1.94^{c-i}	0.87^{c-f}	2.81^{c-g}	21.66^{ij}	2.37^{m}	0.23^{b-i}
	9	1.04^{jk}	0.35^{i}	1.4^{jk}	47.23^{b-f}	5.47^{a}	0.21^{c-i}
	12	1.53^{e-k}	0.65^{d-i}	2.19^{d-k}	54^{abc}	4.04^{b-h}	0.14^{ghi}
'M-Pishva'[2]	0	2.24^{b-f}	0.86^{c-f}	3.1^{b-f}	24.16^{hij}	2.58^{j-m}	0.26^{b-g}
	3	1.98^{c-h}	0.61^{e-i}	2.59^{d-i}	34.86^{f-i}	4.14^{a-g}	0.32^{abc}
	6	3.21^{a}	1.28^{a}	4.5^{a}	33.26^{g-j}	4.68^{a-e}	0.27^{a-f}
	9	1.67^{d-j}	0.69^{d-i}	2.37^{d-k}	31.70^{g-j}	3.29^{f-m}	0.14^{ghi}
	12	1.41^{k-f}	0.55^{e-i}	1.96^{f-k}	41.40^{c-g}	4.24^{a-g}	0.13^{hi}
'M-Isfahan'[3]	0	1.77^{d-j}	0.68^{d-i}	2.46^{d-j}	20.66^{j}	3.15^{f-m}	0.18^{e-i}
	3	1.57^{e-k}	0.58^{e-i}	2.16^{e-k}	32.93^{g-j}	4.13^{a-g}	0.23^{b-i}
	6	3.04^{ab}	1.20^{abc}	4.25^{ab}	34.63^{f-i}	2.57^{klm}	0.19^{d-i}
	9	1.59^{e-k}	0.66^{d-i}	2.25^{d-k}	34.90^{f-i}	4.03^{b-h}	0.23^{b-i}
	12	0.82^{k}	0.42^{hi}	1.24^{k}	48.60^{a-e}	4.85^{a-d}	0.21^{c-i}
'ZT-Yazd'[4]	0	1.32^{h-k}	0.53^{f-i}	1.84^{g-k}	28^{g-j}	3.68^{c-m}	0.11^{i}
	3	2.47^{a-d}	0.89^{b-e}	3.36^{a-d}	41.46^{c-g}	3.37^{e-m}	0.27^{a-f}
	6	2.08^{c-h}	0.79^{d-g}	2.88^{c-g}	39.80^{d-g}	4.70^{a-e}	0.23^{b-i}
	9	1.65^{d-k}	0.66^{d-i}	2.32^{d-k}	39.06^{d-g}	3.70^{c-m}	0.16^{f-i}
	12	1.52^{k-e}	0.58^{e-i}	2.11^{f-k}	60.93^{a}	3.93^{b-i}	0.18^{e-i}
'G-Yazd'[5]	0	1.31^{h-k}	0.52^{f-i}	1.83^{g-k}	34.20^{f-i}	2.92^{g-m}	0.24^{b-h}
	3	1.13^{ijk}	0.43^{hi}	1.57^{h-k}	22.26^{hij}	3.36^{e-m}	0.27^{a-f}
	6	1.28^{h-k}	0.52^{f-i}	1.81^{g-k}	30.50^{g-j}	4.09^{b-g}	0.34^{ab}
	9	1.68^{d-j}	0.72^{d-h}	2.41^{d-k}	39.20^{d-g}	3.92^{b-k}	0.16^{f-i}
	12	1.64^{k-d}	0.65^{d-i}	2.29^{d-k}	57.63^{ab}	3.93^{b-j}	0.11^{i}
'M-Saveh'[6]	0	1.38^{g-k}	0.48^{ghi}	1.86^{g-k}	24.16^{hij}	2.66^{i-m}	0.31^{a-d}
	3	1.73^{d-j}	0.68^{d-i}	2.42^{d-k}	35.60^{e-h}	3.86^{b-k}	0.32^{abc}
	6	2.62^{abc}	1.23^{ab}	3.84^{abc}	32.16^{g-j}	4.91^{abc}	0.24^{b-h}
	9	2.18^{c-g}	0.81^{d-g}	2.99^{c-g}	34.63^{f-i}	4.95^{abc}	0.26^{b-g}
	12	1.40^{k-g}	0.57^{e-i}	1.97^{f-k}	60.40^{ab}	4.30^{a-f}	0.13^{hi}
'Robab'[7]	0	1.44^{f-k}	0.52^{f-i}	1.96^{f-k}	24.30^{hij}	3.51^{d-m}	0.14^{ghi}
	3	1.95^{c-i}	0.73^{d-h}	2.69^{c-h}	40.46^{d-g}	3.73^{c-l}	0.19^{d-i}
	6	1.90^{c-i}	0.76^{d-h}	2.66^{c-h}	25.40^{hij}	3.73^{c-l}	0.27^{a-f}
	9	1.59^{e-k}	0.68^{d-i}	2.27^{d-k}	50.46^{a-d}	2.71^{h-m}	0.18^{e-i}
	12	1.32^{k-h}	0.6^{e-i}	1.91^{g-k}	48.90^{a-e}	3.40^{e-m}	0.24^{b-h}
'GT-Yazd'[8]	0	1.78^{d-j}	0.67^{d-i}	2.45^{d-j}	30.60^{g-j}	3.21^{f-m}	0.23^{b-h}
	3	2.03^{c-h}	0.74^{d-h}	2.77^{c-g}	33.10^{g-j}	4.35^{a-f}	0.39^{a}
	6	2.34^{b-e}	0.99^{a-d}	3.34^{a-e}	32.36^{g-j}	2.73^{h-m}	0.24^{b-h}
	9	1.56^{e-k}	0.65^{d-i}	2.21^{d-k}	34.70^{f-i}	3.14^{f-m}	0.29^{a-e}
	12	1.05^{jk}	0.41^{hi}	1.47^{ijk}	39.86^{d-g}	5.13^{ab}	0.16^{f-i}

Similar letters in each column indicate no significant difference at the 5% level of LSD test.

1- 'Zaghe Sefide Yazd', 2- 'Malase Torshe Pishva', 3- 'Malase Isfahan', 4- 'Zaghe Torshe Yazd', 5- 'Gabrie. Yazd', 6- 'Malase Saveh', 7- 'Robabe Ghermeze Shiraz', 8- 'Gabrie Torshe Yazd'.

DISCUSSION

Plant height and stem diameter

Salt stress leads to osmotic stress and results in a decrease in the water content of the cells and their elongation. So that even after osmotic equilibration and re-osmotic pressure in cells, their expansion and elongation take place slowly (Munns & Tester, 2008). In the present study, only the height of 'M-Isfahan' and 'Robab' in control treatment was more than salinity treatments. The height of other cultivars increased at some salinity concentrations compared to the control treatment, but eventually, the plant height decreased with higher salinity concentrations (Table 6), which is consistent with the results of Naeini et al. (2006). The response of pomegranate cultivars to different salinity levels and experiment conditions is different. A decrease in the height of 'Manfalouti' in 11.8 dS m^{-1} NaCl was 5% compared to the control (Mastrogiannidou et al., 2016).

'M-Pishva', 'ZT-Yazd', and 'GT-Yazd' in control treatment, 'M-Saveh' and 'Robab' in salinity of 3 dS m^{-1}, 'M-Isfahan' and 'ZS-Yazd' in 6 dS m^{-1} salinity and 'G-Yazd' in 9 dS m^{-1} salinity had the highest average of plant diameter. 'ZS-Yazd' in 3, 6 and 9 dS m^{-1} salinity levels had greater diameter than control treatment (Table 6). The decrease in plant diameter in the studied cultivars did not follow a similar trend. Mastrogiannidou et al. (2016) reported that 'Wonderfull' in salinity of 4, 8.5 and 11.8 dS m^{-1} showed a decrease in plant height, diameter and vegetative growth. However, in the present study, the decrease in height and diameter of the plant in the studied cultivars was not in full compliance with the salinity level.

Electrolyte leakage (EL) and proline

One of the mechanisms of adaptation to salinity under stress conditions is to maintain membrane stability. Treating the plants with sodium chloride increases EL of cell membranes (Sun et al., 2018). In all studied salinity treatments, 'GT-Yazd' showed the least amount of EL reduction compared to control. In the 6 dS m^{-1} salinity treatment, 'Robab' also had a lower EL than the control treatment, such as 'GT-Yazd' (Table 7). In studied cultivars, EL had an increasing trend, but this increase did not follow a regular pattern, which can be attributed to the genetic differences between cultivars and their different response to different levels of stress. Differences in EL rates of different pomegranate cultivars under salinity stress were also reported by Jamali and Bonyanpour (2017). Naeini et al. (2006) reported that with increasing drought stress, EL of leaf cells in pomegranate cultivars was significantly increased. According to this, 'Robab Neiriz' and 'Malas Yazdi' pomegranate cultivars that had lower EL in their leaves than 'Ardestani Mahvalat' and 'Naderi Badroud' cultivars, had a higher tolerance to drought stress. Salinity stress in 'Wonderfull' pomegranate cultivar also increased leaf proline content (Sarafi et al., 2014).

Salinity increased proline levels, but this increase was different between cultivars and salinity levels. Some cultivars, such as 'M-Isfahan' and 'GT-Yazd', showed the highest proline content in 12 dS m^{-1} treatment. Other cultivars produced the highest proline at lower salinity levels and it's value decreased with increasing salinity (Table 7). Proline acts in the cytoplasm as an osmotic regulator in maintaining the structure of molecules in an environment where ionic equilibrium is disturbed (Nayyar, 2003). It is also considered as a store of energy and nitrogen in the plant and plays a role in trapping reactive oxygen species (Verbruggen & Hermans, 2008). According to Khayyat et al. (2014), applying salt stress in pomegranate cvs. 'Malas Saveh' and 'Shishe Gap' increased proline. It seems that proline as an osmoprotectant and reactive oxygen species scavenger in some cultivars in lower salinity levels and in some cultivars in higher salinity levels is efficient and this is related to cultivar tolerance to salinity stress.

Chlorophylls a, b and total chlorophyll

Salinity stress impairs ion exchange and reduces nitrate uptake due to increased chloride ion in the root medium or decreased magnesium uptake, which results in a decrease in chlorophyll synthesis. The decrease in chlorophyll content under salinity stress has been reported due to higher activity of chlorophyllase enzyme (Garcia-Sanchez et al., 2002). Salinity stress increased the activity of reactive oxygen species, so the decrease in chlorophyll content of each cultivar indicates the extent of oxidative damage (Ashraf & Ali, 2008). Most of the pomegranate cultivars showed their highest chlorophyll content in 6 dS m^{-1} salinity and their chlorophyll decreased at higher salinity levels. The reduction of photosynthetic pigments affected by salinity stress has been reported in 'Malas Saveh' and 'Shishe Gap' pomegranate cultivars (Khayyat et al., 2014). Chlorophyll content in 9 and 12 dS m^{-1} salinity in 'G-Yazd', 'ZT-Yazd' and 'M-Saveh' increased and in 'M-Isfahan', 'GT-Yazd', 'M-Pishva' and 'ZS-Yazd' decreased compared to control treatment (Table 7). Similarly, in a research, increasing salt levels resulted in a decrease in leaf chlorophyll concentration in 'Wonderfull' (Mastrogiannidou et al., 2016). An increase in chlorophyll content in some cultivars can be due to decreased leaf area under salinity stress. Decreasing leaf area due to salinity stress (Bhantana & Lazarovitch, 2010) increases chlorophyll content per unit area of the leaf. It should be noted that increased proline also leads to an increase in leaf chlorophyll content in stressed plants (Hayat et al., 2012).

Enzyme activities

One of the important biochemical changes in higher plants under salinity stress is the increase of free radicals such as superoxide, hydrogen peroxide, and hydroxyl ions and their oxidative effect on cells (Corpas et al., 2015). Increased activity of antioxidant enzymes such as POD and SOD is one of the mechanisms of scavenging ROS (Dai et al., 2009). In the present study, the change in POD activity in all cultivars did not follow the same pattern. Cultivars at different levels of sodium chloride showed trends of decreasing, increasing-decreasing or no significant change (Table 7). An increasing-decreasing trend of POD activity in 'Tunisi' pomegranate cultivar has also been reported by Sun et al. (2018). It seems that increasing the concentration of sodium chloride lead to a decrease in enzyme precursors which results in a decrease in enzyme activity. For this reason, lower salinity levels in 'M-Pishva', 'ZT-Yazd', 'G-Yazd', and 'GT-Yazd' resulted in increased activity of this enzyme due to the production of reactive oxygen species, but over time, the antioxidant activity decreased due to further degradation and reduce their production. In 'M-Saveh', the decrease in the activity of this enzyme can be due to a decrease in their synthesis or an increase in their degradation due to the accumulation of reactive oxygen species (Fidalgo et al., 2004). The activity of antioxidant enzymes is directly correlated with plant resistance to stress (Guo et al., 2006). In this study, the highest POD activity was observed in 'GT-Yazd' and 'G-Yazd' in 3 and 6 dS m^{-1}, respectively. These two cultivars also showed the lowest EL under salinity stress (Table 7).

The activity of SOD in salinity levels of 3, 6, 9 and 12 dS m^{-1} showed a slight increase, decrease, increase and then stability, respectively (Fig. 1). The SOD enzyme acts as the first defense barrier against the attack of reactive oxygen species. The decrease in SOD activity in salinity of 6 dS m^{-1} could be due to that the amount of produced hydrogen peroxide at this stage increased so much that it reduced the activity of SOD enzyme to less than that of control plants. It seems that in 9 dS m^{-1} salinity, other scavengers of ROS eliminate the produced hydrogen peroxide and lead to increased SOD activity.

The activity of SOD was highest in 'GT-Yazd', 'M-Isfahan' and 'Robab' (Fig. 2). Various studies have shown that resistance to oxidative stress is related to salt resistance in plants (Guo et al., 2006; Mousavi et al., 2008). In other words, salt-tolerant cultivars have shown a better defense mechanism against oxidative stress than susceptible cultivars through increased antioxidant activity (Yildiz & Terzi, 2013).

CONCLUSION

By increasing salinity of sodium chloride concentration in irrigation water, the percentage of decrease in vegetative characteristics, increase of proline, POD, SOD and decrease of EL and chlorophyll content were significantly dependent on pomegranate cultivar and salinity concentration. Generally, Yazd cultivars included 'G-Yazd' (with the lower plant diameter and EL as well as higher chlorophyll content, POD and SOD), 'GT-Yazd' (with lower EL and higher POD), 'ZS-Yazd' (more proline) and 'ZT-Yazd' (with the lower plant diameter and more chlorophyll content and POD) showed higher tolerance to salt stress. Based on the results of this study, it can be concluded that pomegranate is relatively salinity tolerant and the threshold salinity value for pomegranate cultivars can be between 3 and 6 dS m^{-1} concentrations of sodium choloride in water irrigation.

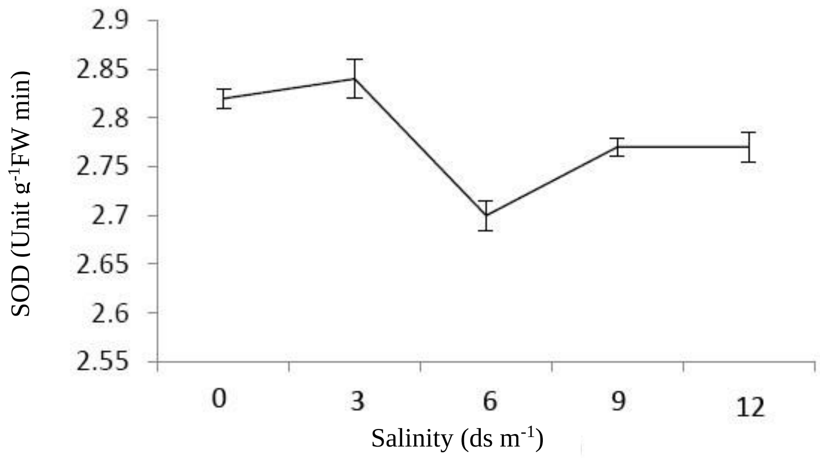

Fig. 1. Comparison of SOD activity at different salinity levels.

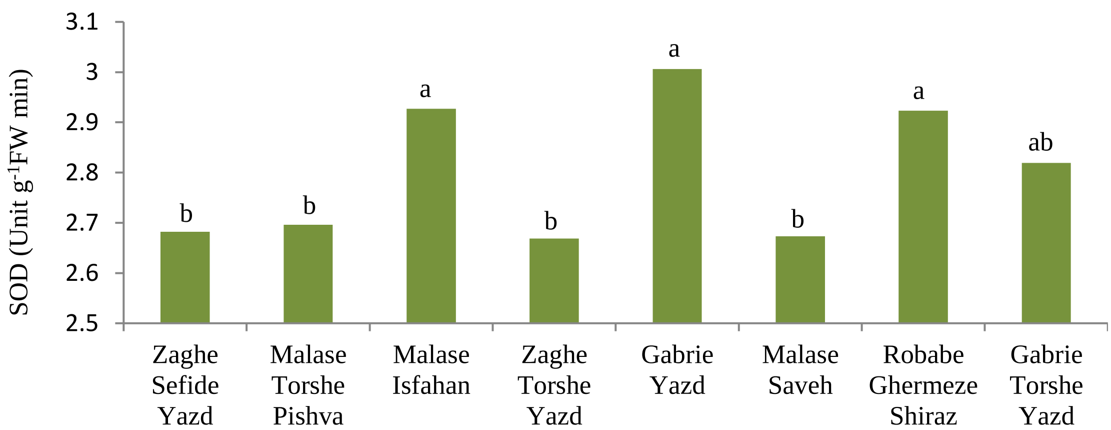

Fig. 2. Mean comparison of SOD activity in different pomegranate cultivars.

Conflict of interest
The authors have no conflict of interest to report.

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