Effects of salicylic acid application on two almond cultivars under salinity stress

Hossein MOHAMMADI[1]
Ali IMANI[2,3] (✉)
Vahid ABDOSSI[2]
Mohammadreza ASGHARI[4]
Alireza TALAEI[2]

Summary

The effects of salinity stress on the physiological processes and biochemical compounds of plants were reported. Salicylic acid (SA), as one of the main phytohormones, is a signal molecule that alleviates the negative influences of salinity. This study was conducted to investigate the protective role of SA in improving the salinity tolerance of two almond cultivars. Two almond cultivars ('Tuono' and 'Sahand') grafted on the GN (Garnem) rootstock were exposed to different levels of salinity stress (0, 2, 6 and 8 $dS \cdot m^{-1}$) and treated with SA (0, 1 and 2 mM). The results showed that salinity stress significantly reduced the plant height, *Fv/Fm*, protein and total phenolic content (TPC), whereas Na and Cl content in roots, proline content and antioxidant enzymes activities, including superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), guaiacol peroxidase (POD) and glutathione peroxidase (GPX), significantly increased in response to salinity stress. Rootstock and scion diameter, and also leaves number of selected shoots significantly increased at 2 and 6 $dS \cdot m^{-1}$ of salinity, and then significantly decreased at 8 $dS \cdot m^{-1}$ of salinity. Furthermore, it was found SA treatment significantly alleviated the negative effects of salinity by enhancing morphological characteristics, *Fv/Fm*, accumulation of Na and Cl in roots, proline content, protein and TPC and also by enhancing the SOD, CAT, APX, POD and GPX activities. Taken together, the results showed that 'Sahand' cultivar treated with SA had a better response to salinity stress compared with 'Tuono' cultivar. Therefore, the use of 'Sahand' cultivar and application of SA could be recommended as a practical tool under salinity conditions.

Key words

ascorbate peroxidase, catalase, proline, 'Sahand', 'Tuono'

[1] PhD student of Horticulture, Science and Research Branch, Islamic Azad University, Tehran, Iran

[2] Department of Horticultural Science and Agronomy, Science and Research Branch, Islamic Azad University, Tehran, Iran

[3] Temperate Fruit Research Center, Horticultural Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Karaj, Iran

[4] Department of Horticulture, Agriculture Faculty, Urmia University, Urmia, Iran

✉ Corresponding author: imani_a45@yahoo.com; a.imani@areeo.ac.ir

Received: February 20, 2020 | Accepted: May 19, 2020

Introduction

Almonds (*Prunus dulcis* (Mill.) D. A. Webb), belongs to the Rosaceae family and is commercially cultivated worldwide. Almonds are rich contents of healthy nutrient and bioactive compounds and has been ranked as a nut of the highest nutritious value (Khadivi-Khub and Anjam, 2016; Torki-Harchegani, 2015). Unfortunately, in Iran, approximately 26 million hectares of arid and nine million hectares of and semiarid lands are affected by accumulation of sodium chloride (NaCl). Salinity was recognized as a main limiting factor of plant cultivation in arid and semiarid lands due to its negative impacts on soil traits (Bahrami et al., 2015; Hamzehpour and Bogaert, 2017).

The change in ions balance and their absorption are the main effects of extra NaCl content in the soil that causes the high production of reactive oxygen species (ROS). Reactive oxygen species can mainly interrupt plant organelles' metabolism via oxidative damages to membranes integrity, proteins production as well as DNA structure, photosynthetic apparatus and finally, plant growth and development (Liang et al., 2018). Thus, the study and improvement of salinity resistant crops over the selection and breeding of cultivars/ genotypes responsible to provide commercial yields during high NaCl conditions are necessary to reduce the negative effects of salinity (Zriget al., 2016).

Different factors affect the salinity resistance processes in plants. It was mentioned that plant cultivars have different behaviors regarding the alleviation of the negative effects of different stresses. In the meantime, the rootstock/ scion combination was shown to change salinity resistance by reducing the absorption and toxicity of harmful ions or the change of antioxidant activity and also the increase of osmotic adjustment compounds (Dejampour et al., 2012: Zrig et al., 2015b). The previous study reported that salinity significantly affected morphological characteristics, growth indices, pigments content and some photosynthesis-related parameters of different almond genotypes grafted on GF677 rootstock (Momenpour et al., 2018).

On the other hand, the exogenous application of natural compounds is a novel agro-technology used to improve the plant resistance to high concentration of NaCl (Wani et al., 2016). Salicylic acid (SA) is a phytohormone, which has been recognized as a major signal in endogenous resistance mechanisms and enhances the antioxidant activity of plants by expression of different genes that encode antioxidants as well as biosynthesis of secondary metabolites (Methenni et al., 2018; Wani et al., 2016). Previous studies mentioned that SA alleviates destructive effects of salinity by the enhancing of antioxidant activities as well as accumulation of secondary metabolites (Cag et al., 2009; Li et al., 2014; Liu et al., 2016).

There is paucity of information about the application of SA on the antioxidant enzymes activity and secondary metabolites accumulation of Iranian almond cultivars. Therefore, this study was conducted to evaluate the effects of SA application on some morpho-physiological traits and antioxidants enzymes activity of 'Sahand' and 'Tuono' almond cultivars grafted on GN rootstock under different salinity conditions.

Materials and methods

Plant materials and treatments

A factorial experiment was performed according to the completely randomized design (CRD) with four levels of NaCl (0, 2, 6 and 8 $dS{\cdot}m^{-1}$) and three concentrations of SA (0, 1 and 2 mM) in Temperate Fruit Research Center, Horticultural Research Institute, Karaj, Iran, in 2016. One-year-old plants of GN were obtained from Ita-Sadra Tissue Culture Company (FarsPprovince, Iran), planted in pots filled with 25 kg soil in early March. Some physicochemical characteristics of soil mixture were: texture = Loamy, electrical conductivity (EC) = 1.28 $dS{\cdot}m^{-1}$, pH= 7.4 and organic carbon = 1.43 %. Pots were placed in the research greenhouse of Temperate Fruit Research Center, Horticultural Research Institute, Karaj, Iran (temperature: 25 ± 2°C; photoperiod: 16-h light: 8-h dark; light intensity: 500-700 micromoles m^{-2} s^{-1}). After six months, when plants have grown enough and completely established, two commercial almond cultivars ('Sahand' and 'Tuono') were grafted on rootstocks by shield budding in middle of May. After the establishment and sufficient growth of the scions (eight weeks after budding), plants were treated twice (with one week intervals) with SA (Sigma, Germany) at concentrations of 0 (control), 1 and 2 mM. The salinity stress was obtained by adding NaCl to the nutrient solution to obtain 2, 6 and 8 $dS{\cdot}m^{-1}$ concentrations. Control treatment consisted of no NaCl added. To avoid osmotic shock, the concentration of the nutrient solution was increased by 1 $dS{\cdot}m^{-1}$ per day until the final salinity level for each treatment was reached. From the moment when the final concentration of NaCl was obtained for the most severe stress level, the concentrations of nutrient solutions were kept constant (Zrig et al., 2016). Sixty days after applying the salt treatment, when signs of salinity (leaves chlorosis and necrosis) appeared on the plants, some morphological characteristics, root Na^+ and Cl^- contents, the maximum quantum efficiency of PSII photochemistry (*Fv/Fm*), antioxidant enzymes activities and secondary metabolites accumulation were measured in the leaf samples of each cultivar.

Plant measurements

Morphological characteristics

Some morphological characteristics including plant height, rootstock diameter, scion diameter and leaves number of selected shoot were evaluated.

Na and Cl content in root

To measure Na and Cl content in roots, roots tissues were milled into a fine powder to pass a 60-mesh sieve, then, 20 mg of the powder was extracted with 20 mL of 0.1M HNO_3 (Waling et al., 1989). After filtration, Na was determined by a flame photometer (JENWAY, PFP-7, Staffordshire, UK). Content of Cl was determined by titration of 10 ml filtrated solution with silver nitrate ($AgNO_3$).

Chlorophyll fluorescence

The conditions for determining chlorophyll fluorescence were as follows: the amount of photosynthetic active radiation (PAR) was 1895-1425 micromoles m^{-2} s^{-1}, the ambient temperature was 32.8 ± 1.2°C, atmospheric pressure was 101.45 KPa, daily average relative humidity was 27% ± 1.9%, air CO_2 content was 387 ± 1.9 ppm and the leaf area contained within the chamber was 6.25 cm^2. Chlorophyll fluorescence in each plant was measured by sampling the 10th leaf from the top of the shoots between 9:00 and 11:00 am, approximately two hours after the plants were subject to the sunlight. Chlorophyll fluorescence meter (Model Hansatech) was attached to the leaves so that a portion of the leaf was placed under the clip and in the darkness for 30 minutes, then we used a fluorescence measuring apparatus. The light was applied to the leaf and minimal fluorescence (F_0) and maximum fluorescence (F_m) values were read. The variable fluorescence (F_v), value of the difference between F_m and F_0 and the F_v/F_m ratio were also calculated (Maxwell and Johnson, 2000).

Secondary metabolites

Proline content was evaluated based on the Bates et al. (1973) method at 528 nm using a UV/Visible spectrophotometer (BT600 Plus, Canada). The protein amount was measured spectrophotometrically based on the Bradford (1976) at 595 nm. Total phenolic content (TPC) was estimated using the Folin–Ciocalteu method according to Singleton et al. (1999) at 765 nm.

Antioxidant enzymes activities

Enzyme extraction in leaf samples was performed based on the Ozden et al. (2009) method by the potassium-phosphate buffer. The supernatant was used to determination of superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), guaiacol peroxidase (POD) and glutathione peroxidase (GPX).

The SOD activity was measured by determining the capacity of the enzyme to prevent the photochemical reduction of nitro blue tetrazolium (NBT) at 560 nm using an UV/Visible spectrophotometer (BT600 Plus, Canada) as introduced by Giannopolitis and Ries (1977). The CAT activity was assayed by the decrement of hydrogen peroxide at 240 nm as defined by Chance and Maehly (1955). Activity of APX was analyzed by recording the reduction in ascorbate amount at 290 nm as reported by Nakano and Asada (1981). Activity of POD was assayed by determining the oxidation of guaiacol in the existence of hydrogen peroxide with a rise in absorbance at 470 nm as defined by Sorkheh et al. (2012). Finally, GPX was evaluated as reported by Rotruck et al. (1973) method according to the color increment at 412 nm.

Statistical analysis

The data were analyzed using SAS software (version 9.1 2002–2003, SAS Institute, Cary, NC, USA); the description was performed for traits with double and triple interactions that were significant. The comparison of the meanings was done by the least significant difference (LSD) test ($P < 0.05$) with MSTAT-C software and the graphs were drawn also with Minitab software (version 17.3).

Results

Morphological characteristics

The results showed that plant height significantly decreased from 104.83 ± 3.3 cm in non-stressful conditions to 85.67 ± 4.9 cm at 8 dS·m^{-1} of salinity (Table 1). Among all treatments, 'Sahand' cultivar treated with 1mM of SA had the highest height. As compared with normal conditions (without any salinity, 0 dS·m^{-1}), the rootstock and the scion diameter, as well as the leaves number of selected shoot significantly increased at 2 and 6 dS·m^{-1} of salinity, while at 8 dS·m^{-1-1} of salinity the rootstock and the scion diameter and also the leaves number of selected shoot significantly decreased (Table 1). Under normal conditions (0 dS·m^{-1}), untreated 'Sahand' cultivar had the highest rootstock and scion diameter, while at 8 dS·m^{-1} of salinity the highest rootstock and scion diameter was observed in 'Sahand' cultivar treated with 2 mM of SA (17.17 ± 0.85 and 14.27 ± 1.05 mm, respectively). At both normal conditions (0 dS·m^{-1}) and at the most severe levels of salinity (8 dS·m^{-1}) the maximum leaves number of the selected shoot was found in 'Sahand' cultivar treated with 1 mM of SA (Table 1).

Root Na and Cl contents

As shown in Table 1, along with increasing salinity level, root Na and Cl content significantly enhanced. Root Na content significantly increased from 0.078% ± 0.005 to 0.083% ± 0.004, 0.116% ± 0.007 and 0.148% ± 0.006 at 2, 6 and 8 dS·m^{-1} of salinity, respectively. Furthermore, Cl content significantly increased from 0.235% ± 0.014 to 0.323% ± 0.018, 0.733% ± 0.043 and 0.908% ± 0.051 at 2, 6 and 8 dS·m^{-1} of salinity, respectively. Among all treatments, 'Sahand' cultivar treated with SA accumulated the highest Na and Cl contents in roots, except for the Na content at 8 dS·m^{-1} of salinity, in which no significant differences were observed among all the treatments (Table 1).

The maximum quantum efficiency of PSII photochemistry (*Fv/Fm*)

As compared with normal conditions (0 dS·m^{-1}), *Fv/Fm* linearly declined from 0.776 ± 0.042 to 0.736 ± 0.040, 0.646 ± 0.038 and 0.566 ± 0.035 at 2, 6 and 8 dS·m^{-1} of salinity, respectively (Table 1). Our results showed that among all salinity concentrations, 'Sahand' cultivar treated with SA had the highest *Fv/Fm* levels of salinity.

Proline content

It was revealed that the simple and the interaction effects of cultivar, salinity and SA treatment significantly affected proline content. As shown in Table 2, proline content significantly increased in response to salinity and the highest proline content (336.5 µg·g·FW^{-1}) was found at 8 dS·m^{-1} of salinity. 'Sahand' cultivar untreated with SA showed the highest proline content at 2 and 6 dS·m^{-1} of salinity, while at 8 dS·m^{-1} of salinity, 'Sahand' cultivar treated with 1 mM of SA had the highest amount (Table 2).

Table 1. Changes in some morphological characteristics, Na and Cl content of root and the maximal efficiency of PSII photochemistry (*Fv/Fm*) of 'Tuono' and 'Sahand' almond cultivars in response to different concentration of salinity and salicylic acid (SA) application

Salinity ($dS \cdot m^{-1}$)	Cultivar	SA (mM)	Plant height (cm)	Rootstock diameter (mm)	Scion diameter (mm)	Leaves number of selected shoot	Root Na content (%)	Root Cl content (%)	*Fv/Fm*
0	-	-	104.83±3.3a	15.55±0.76b	13.05±0.74c	55.17±4.1c	0.078±0.005d	0.235±0.014d	0.776±0.042a
2	-	-	91.50±5.3b	15.00±0.81c	12.75±0.70d	58.33±3.2b	0.083±0.004c	0.323±0.018c	0.736±0.040b
6	-	-	90.50±4.2b	15.68±0.90a	13.52±0.62a	61.67±3.5a	0.116±0.007b	0.733±0.043b	0.646±0.038c
8	-	-	85.67±4.9c	14.98±0.83c	13.20±0.73b	44.67±2.3d	0.148±0.006a	0.908±0.051a	0.566±0.035d
0	'Tuono'	0	75.67±4.0d	15.37±0.65b	14.07±0.65b	42.67±1.9d	0.075±0.004c	0.246±0.014b	0.777±0.045abc
	'Tuono'	1	112.67±5.8b	14.27±0.64d	13.17±0.66c	54.67±2.6c	0.074±0.003c	0.199±0.007c	0.771±0.033bc
	'Tuono'	2	101.67±6.2c	15.17±0.70b	12.47±0.53d	36.67±1.8e	0.065±0.002d	0.206±0.009c	0.767±0.041c
	'Sahand'	0	100.67±6.9c	19.97±0.43a	14.87±0.50a	42.67±1.6d	0.083±0.004b	0.199±0.008c	0.770±0.035bc
	'Sahand'	1	137.67±9.1a	13.77±0.37e	12.67±0.51d	79.67±3.8a	0.092±0.003a	0.245±0.012b	0.788±0.036a
	'Sahand'	2	100.67±5.2c	14.77±0.69c	11.07±0.47e	74.67±3.4b	0.078±0.002c	0.316±0.015a	0.781±0.047ab
2	'Tuono'	0	74.67±2.6d	15.07±0.76b	13.07±0.58c	37.67±2.0c	0.075±0.004c	0.191±0.007f	0.732±0.037bc
	'Tuono'	1	74.67±4.0d	15.27±0.61b	13.27±0.61c	66.67±3.3b	0.077±0.004c	0.316±0.014e	0.737±0.034b
	'Tuono'	2	91.67±4.1c	14.77±0.53c	13.97±0.70a	35.67±1.5c	0.081±0.003b	0.344±0.017c	0.731±0.036c
	'Sahand'	0	74.67±3.9d	15.67±0.52a	10.17±0.56e	66.67±4.1b	0.101±0.005a	0.330±0.015d	0.727±0.031c
	'Sahand'	1	123.67±7.1a	15.57±0.64a	13.67±0.68b	75.67±2.4a	0.082±0.003b	0.361±0.015b	0.741±0.039a
	'Sahand'	2	109.67±4.3b	13.67±0.51d	12.37±0.62d	67.67±3.7b	0.084±0.004b	0.397±0.019a	0.744±0.035a
6	'Tuono'	0	74.67±3.2e	16.07±1.09b	13.27±0.81d	46.67±2.8e	0.108±0.006e	0.674±0.45d	0.643±0.046cd
	'Tuono'	1	88.67±5.2d	19.27±0.95a	14.37±0.97a	44.67±3.5e	0.115±0.007d	0.663±0.051d	0.646±0.043c
	'Tuono'	2	96.67±4.0c	13.67±0.84d	12.67±0.72e	66.67±4.2c	0.099±0.006f	0.702±0. 038c	0.625±0.037c
	'Sahand'	0	61.67±2.6f	14.37±0.75c	13.07±0.70d	57.67±3.9d	0.125±0.006b	0.727±0.032b	0.631±0.036d
	'Sahand'	1	115.67±4.6a	14.57±0.78c	14.06±0.88b	74.67±4.2b	0.132±0.005a	0.908±0.045a	0.656±0.039ab
	'Sahand'	2	105.67±5.7b	16.67±0.72b	13.67±0.89c	79.67±5.6a	0.116±0.007c	0.723±0.059b	0.679±0.042a
8	'Tuono'	0	84.67±3.7d	16.67±1.12b	13.67±0.66b	37.67±1.4c	0.150±0.007a	0.691±0.033e	0.549±0.025c
	'Tuono'	1	89.67±4.5c	14.27±1.05d	13.67±0.71b	37.67±2.7c	0.147±0.006a	0.695±0.031e	0.547±0.038c
	'Tuono'	2	75.67±3.6e	13.77±0.74e	11.77±0.59e	37.67±1.8c	0.146±0.007a	0.737±0.054d	0.549±0.021c
	'Sahand'	0	66.67±3.0f	15.27±0.90c	13.17±0.60c	45.67±2.9b	0.147±0.004a	1.106±0.038b	0.556±0.026c
	'Sahand'	1	101.67±3.8a	13.27±0.91f	12.67±0.57d	62.67±3.0a	0.150±0.004a	1.163±0.057a	0.588±0.033b
	'Sahand'	2	95.67±4.4b	17.17±0.85a	14.27±0.73a	46.67±3.5b	0.149±0.006a	1.056±0.046c	0.609±0.029a

* For each column and salinity concentration, means followed by the same letters are not significantly different at $P \leq 0.05$ according to the LSD test. Slicing was performed based on the salinity concentration

Table 2. Changes in secondary metabolites and antioxidant enzymes activities of 'Tuono' and 'Sahand' almond cultivars in response to different concentration of salinity and salicylic acid (SA) application

Salinity ($dS \cdot m^{-1}$)	Cultivar	SA (mM)	Proline ($\mu g \cdot g \cdot FW^{-1}$)	Protein ($mg \cdot g \cdot FW^{-1}$)	TPC ($mg \cdot g \cdot FW^{-1}$)	SOD ($U \cdot mg \cdot protein^{-1}$)	CAT (DA $m \cdot mg \cdot protein^{-1}$)	APX (DA $m \cdot mg \cdot protein^{-1}$)	GPX (DA $m \cdot mg \cdot protein^{-1}$)
0	-	-	168.6±9.1d*	0.371±0.020a	0.949±0.053a	7740±406c	44.3±2.7b	3.82±0.21b	3.57±0.29b
2	-	-	222.2±11.7b	0.328±0.018b	0.804±0.042c	7016±370d	70.8±3.6a	3.09±0.16bc	3.68±0.18b
6	-	-	192.5±10.6c	0.249±0.013c	0.952±0.051a	12103±602a	72.5±3.9a	6.40±0.32a	4.73±0.27a
8	-	-	336.5±21.1a	0.317±0.016b	0.929±0.047b	8147±428b	68.4±3.1a	5.80±0.30a	3.71±0.16b
0	'Tuono'	0	158.3±7.5c	0.413±0.024b	0.817±0.046d	9321±536a	21.9±1.3c	1.65±0.09c	2.15±0.16d
	'Tuono'	1	234.1±14.3a	0.740±0.041a	0.987±0.059c	4159±2316c	23.0±1.4c	0.28±0.01d	2.21±0.10d
	'Tuono'	2	123.8±7.8d	0.180±0.011e	0.657±0.037f	4789±274c	107.5±6.8a	10.83±0.65a	0.64±0.04e
	'Sahand'	0	182.7±9.7b	0.233±0.012d	0.697±0.041e	8576±502b	34.9±2.0b	0.51±0.03d	6.87±0.48a
	'Sahand'	1	157.2±10.6c	0.327±0.022c	1.333±0.075a	9987±279a	41.3±2.6b	4.54±0.27b	5.42±0.36b
	'Sahand'	2	155.5±9.1c	0.333±0.018c	1.207±0.072b	9611±569a	36.9±2.1b	5.12±0.31b	4.11±0.25c
2	'Tuono'	0	155.5±7.4e	0.467±0.023a	0.723±0.035f	6413±331c	46.7±2.5d	2.35±0.12b	0.80±0.5d
	'Tuono'	1	152.2±8.5e	0.280±0.014b	0.813±0.040c	12543±658a	67.2±3.7c	1.86±0.08c	1.91±0.10c
	'Tuono'	2	240.4±14.3c	0.460±0.027a	0.733±0.037e	8557±443b	34.2±1.2e	6.70±0.36a	1.08±0.05d
	'Sahand'	0	317.6±17.0a	0.227±0.012c	0.850±0.044b	584±35e	101.4±5.1a	0.32±0.01e	5.47±0.30b
	'Sahand'	1	193.8±11.9d	0.273±0.011b	0.803±0.042d	8284±423b	77.1±4.7b	0.87±0.05d	5.70±0.37b
	'Sahand'	2	273.7±13.7b	0.260±0.014b	0.900±0.049a	5713±279d	98.2±5.3a	6.46±0.034a	7.13±0.25a
6	'Tuono'	0	145.5±6.1c	0.260±0.012b	1.257±0.053b	16573±784a	35.4±1.7d	7.65±0.37b	4.66±0.21b
	'Tuono'	1	152.7±8.5b	0.180±0.009c	0.697±0.033e	15674±740a	114.0±5.5a	3.47±0.17c	2.77±0.14c
	'Tuono'	2	113.9±5.4d	0.230±0.010b	0.663±0.310f	7599±364c	72.8±3.6b	10.60±0.51a	0.72±0.02d
	'Sahand'	0	486.9±26.6a	0.393±0.023a	0.887±0.043d	11093±521b	54.7±2.8c	3.43±0.18c	3.98±0.19bc
	'Sahand'	1	145.5±6.9c	0.173±0.007c	0.903±0.047c	9014±437c	109.1±5.0a	10.13±0.42a	12.24±0.66a
	'Sahand'	2	110.6±5.6d	0.260±0.012b	1.307±0.060a	12665±595b	49.2±3.2cd	3.11±0.15c	4.01±0.23bc
8	'Tuono'	0	383.71±9.1b	0.513±0.027a	0.943±0.045c	6694±332c	6.9±0.5e	2.40±0.09de	0.05±0.01e
	'Tuono'	1	272.1±13.2d	0.220±0.012c	0.583±0.024e	2543±128d	117.4±5.2a	7.97±0.43b	1.38±0.06d
	'Tuono'	2	328.7±16.7c	0.227±0.011c	0.776±0.037d	9543±470b	102.0±5.6a	10.29±0.49a	5.16±0.27c
	'Sahand'	0	386.9±20.0b	0.240±0.012c	0.883±0.041c	6375±372c	89.7±4.8b	3.72±0.16d	8.09±0.44a
	'Sahand'	1	446.9±22.1a	0.347±0.017b	1.097±0.058b	12208±602a	34.2±1.9d	5.01±0.26c	1.79±0.09d
	'Sahand'	2	200.5±9.9e	0.353±0.019b	1.293±0.066a	11520±571a	60.0±3.2c	5.37±0.28c	5.77±0.31b

* For each characteristic and salinity concentration, means followed by the same letters are not significantly different at $P \leq 0.05$ according to the LSD. Slicing was performed based on the salinity concentration

Protein content

The results showed that the protein content of almond cultivars significantly declined from 0.371 ± 0.020 to 0.249 ± 0.013 mg·g·FW^{-1} along with increasing salinity from 0 to 6 dS·m^{-1}, whereas at 8 dS·m^{-1} of salinity protein content significantly increased to 0.317 ± 0.016 mg·g·FW^{-1} (Table 2). Almond cultivars showed different responses to SA treatment under different concentrations of salinity. Generally, at 8 dS·m^{-1} of salinity, the highest protein content was found in 'Tuono' cultivar untreated with SA.

Total phenolic content

The simple and the interaction effects of cultivar, salinity and SA treatment significantly influenced the TPC. As shown in Table 2, the highest TPC was found at 6 and 0 dS·m^{-1} of salinity, while the lowest TPC was observed at 2 dS·m^{-1} of salinity. In non-salinity conditions, 'Sahand' cultivar treated with 1 mM of SA had the highest TPC (1.333 mg·g·FW^{-1}), while under salinity conditions, 'Sahand' cultivar treated with 2 mM of SA showed the highest TPC (Table 2).

SOD activity

The results showed that SOD activity of almond cultivars was significantly affected by the simple and the interaction effects of cultivar, salinity and SA treatment. Superoxide dismutase (SOD) activity significantly declined from 7740 to 7016 U·mg·protein^{-1} at 2 dS·m^{-1} of salinity, whereas it increased to 12203 U·mg·protein^{-1} at 6 dS·m^{-1}. Finally, SOD activity significantly decreased again to 8147 U·mg·protein^{-1} at 8 dS·m^{-1} of salinity (Table 2). At the severe level of salinity, 'Sahand' cultivar treated with 1 and 2 mM of SA showed the highest SOD activity.

CAT activity

The changes in CAT activity depended on the simple and the interaction effects of cultivar, salinity and SA treatment. Salinity significantly enhanced the CAT activity of almond cultivars as compared with normal conditions (0 dS·m^{-1}) (Table 2). At the normal conditions, 'Tuono' cultivar treated with 2 mM of SA had the highest CAT activity (107.45 DA m·mg protein^{-1}), while at the severe salinity, the same cultivar treated with 1 and 2 mM of SA showed the highest activity, respectively.

APX activity

It was found that the simple and the interaction effects of cultivar, salinity and SA treatment significantly influenced APX activity. As compared with non-salinity conditions, APX activity significantly ($P \leq 0.05$) decreased from 3.82 to 3.09 DA m·mg·protein^{-1} at 2 dS·m^{-1} of salinity, but then significantly increased to 6.40 and 5.80 DA m·mg·protein^{-1}, respectively at 6 and 8 dS·m^{-1} of salinity (Table 2). SA treatments significantly enhanced APX activity in all salinity and non-salinity conditions, with the highest APX activity obtained in the 'Tuono' cultivar treated with 2 mM of SA.

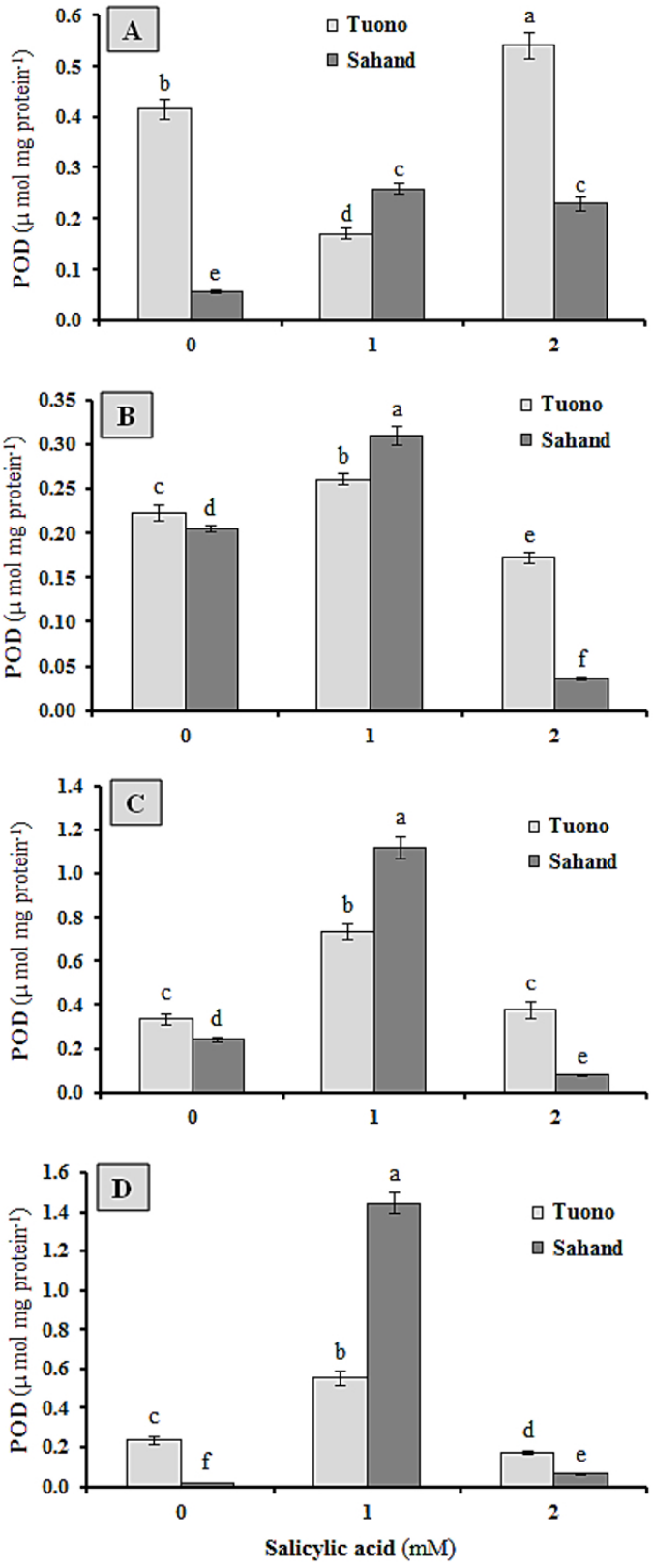

Figure 1. Changes in guaiacol peroxidase (POD) activity of 'Tuono' and 'Sahand' almond cultivars in response to different salinity concentration [0 dS·m^{-1} (A), 2 dS·m^{-1} (B), 6 dS·m^{-1} (C), 8 dS·m^{-1} (D)] at three treatments with salicylic acid. Vertical bars indicate standard deviation (n = 3). Different letters indicate significant difference ($P \leq 0.05$) according to the LSD test

POD activity

Our results mentioned that POD activity was significantly affected by the simple and the interaction effects of cultivar, salinity and SA treatment. Generally, POD activity significantly ($P \leq 0.05$) reduced from 0.277 to 0.201 μmol·mg protein^{-1} at 2 dS·m^{-1} of salinity but then significantly increased to 0.480 μmol·mg protein^{-1} at 6 dS·m^{-1} of salinity, and finally, it decreased again to 0.414 μmol·mg protein^{-1} at 8 dS·m^{-1} of salinity. In non-salinity conditions, 'Tuono' cultivar treated with 2 mM of SA had the highest POD activity (0.539 μmol·mg protein^{-1}), while in all salinity concentrations the highest POD activity was observed in 'Sahand' cultivar treated with 1 mM of SA (Fig. 1).

GPX activity

It was revealed that changes in GPX activity depended on the simple and the interaction effects of cultivar, salinity and SA treatment. As shown in Table 2, GPX activity significantly enhanced at 6 dS·m^{-1} of salinity, while no significant difference was obtained between non-salinity conditions, 2 and 8 dS·m^{-1} of salinity. The results showed that 'Sahand' cultivar untreated with SA had the highest GPX activity in non-salinity conditions and 8 dS·m^{-1} of salinity. Furthermore, at 2 and 6 dS·m^{-1} of salinity, 'Sahand' cultivar treated with 2 and 1 mM of SA showed the highest GPX activity, respectively (Table 2).

Discussion

Effect of salinity on almond cultivars

A higher concentration of Na^+ and Cl^- in the rhizosphere may result in a passive accumulation of these ions in root and shoot. The lower Na/K ratio will lead to metabolic disorders such as a reduction in protein synthesis and enzymatic activities (Kumar et al., 2018; Liang et al., 2018). Therefore, the uptake and accumulation of Na^+ and Cl^- in roots and the inhibition of their transfer to the shoot and the leaves are important parameter for discrimination and identification among salt-tolerant and salt-sensitive genotypes (Munns and Tester, 2008; Liang et al., 2018).

In term of plant physiology, the *Fv/Fm*, along with other parameters, can be used to estimate the severity of the damages under stressful conditions (Munns and Tester, 2008; Yang et al., 2020). Therefore, the *Fv/Fm* ratio is an index of salinity (Liang et al., 2018; Yang et al., 2020). It seems that during salinity rapid stomatal closure results in a reduction of CO_2 uptake rate that is associated with a decrease in photosynthesis. In tolerant cultivars, the stress-relief mechanism, such as delay in stomatal closure and/or partial closure, prevents a CO_2 deficit (Munns and Tester, 2008; Liang et al., 2018; Yang et al., 2020).

Salinity is one of the most limiting factors to plant growth and development as well as to agricultural yield (Liang et al., 2018). Plants use different physiological and biochemical defensive approaches against salinity (Isayenkov and Maathuis, 2019). These approaches involve the accumulation of compatible solutes and osmolytes compounds in the cytosol and activation of antioxidant defense systems (Liang et al., 2018; Isayenkov and Maathuis, 2019). Plants need to control their inner water content below that of the soil to keep the turgor and the water uptake for growth and development. This needs an increase in osmotic content in the cells and organelles throughout the uptake of inorganic ions or the production of different compatible compounds such as proline (Liang et al., 2018). Proline is a good index of salinity tolerance for almond trees (Zrig et al., 2016). Cytosolic compatible compounds act also a major role in cell osmoregulation, they regulate and reduce the water flow to the apoplast and to the vacuole (Zrig et al., 2016). Furthermore, proline is one of the main multi-purpose amino acids, and as a signaling molecule had a major role in the controlling of plant growth by causing cascade signaling systems (Singh et al., 2014; Rajasheker et al., 2019). Proline improves plant tolerance against different stresses such as salinity by increasing their endogenous content and their intermediate enzymes in plants. Proline controls the expression of some genes associated with antioxidant enzymes during salinity (Singh et al., 2014). Ghasemi et al. (2016) reported that the increase of the proline content is due to high protein destruction during abiotic stresses. Similar to our results, Zrig et al. (2015b) and Zrig et al. (2016) concluded that salinity increased the proline content of different almond cultivars.

Our results revealed that protein content significantly decreased under salinity (Table 1). Salinity induces different signaling systems causing changes in gene expression and protein content (Kosová et al., 2013). Proteins cause main changes in energy metabolism resulting under salinity adaptations (Kosová et al., 2013). One aspect of salinity is the elimination of potassium (K) ions by plant root cells, which results in a physiological imbalance because potassium is necessary for protein synthesis. The K deficit results in decreased plant growth and development. If the salinity is continued, it could affect protein synthesis and finally cause it to decrease. On the other hand, the protein content may have increased due to plants have many tolerance mechanisms under different NaCl concentrations (Ayala-Astorga and Alcaraz-Meléndez, 2010). The decrease in protein content in response to salinity is in agreement with Jiang et al. (2006) and Ayala-Astorga and Alcaraz-Meléndez (2010).

It was found that TPC of almond cultivars is significantly reduced in response to 2 and 8 dS·m^{-1} of salinity (Table 1). Phenolic compounds are the important groups of plant secondary metabolites, which have different biochemical and biological activities (Valifard et al., 2017). The decrease in TPC under salinity might be due to the oxidation of TPC by POD (Valifard et al., 2017). Moreover, during salinity absorption of phosphor and potassium (as main substances of secondary metabolites biosynthesis) is reduced, which can cause a decrease in TCP (Rezazadeh et al., 2012). Reduction of TPC under salinity conditions was previously reported by Yuan et al. (2010), Rezazadeh et al. (2012) and Minh et al. (2016).

Plants exposed to the high concentration of NaCl were prone to oxidative stress due to the production of the different types of ROS, such as superoxide (O_2^-), hydroxyl radical ($^{\bullet}OH$) and hydrogen peroxide (H_2O_2) (Caverzan et al., 2016). Especially, it is recognized that H_2O_2 is a powerful suppressor of the Calvin cycle, therefore, it must be scavenged as soon as possible (Caverzan et al., 2016). Therefore, plants enhance their enzymatic and non-enzymatic antioxidant systems to reduce the harmful effects of this oxidative stress (Gupta et al., 2018). Superoxide dismutases (SODs) are the first step of the cellular defense system to scavenge ROSs; they convert O_2^- and water (H_2O) to H_2O_2 and molecular

oxygen O_2. Then the produced H_2O_2 is quickly converted to H_2O and ½ O_2 by the CAT activity. Ascorbate peroxidase (APX), which acts mainly in the chloroplast, has an important role in the convert H_2O_2 to water, by ascorbate as a special electron donor. POD is a heme-containing protein that preferably oxidizes aromatic electron donors such as guaiacol and pyrogallol at the present of H_2O_2. Additionally, GPX located in the cytosol, vacuole, cell wall, and apoplast is believed to be associated to reduce lipid hydroperoxides to their corresponding alcohols and to reduce free H_2O_2 to water (Gupta et al., 2018). In this study, we found that the activities of antioxidant enzymes significantly increased under salinity conditions (Table 1 and Fig. 1). These results are in agreement with Sorkheh et al. (2012) and Zrig et al. (2015 a), who reported that salinity significantly enhanced the activities of antioxidant enzymes in different almond cultivars.

Effect of SA

Phytohormones have a major role in controlling stress tolerance by changing different physiological and biochemical mechanisms. The role of SA as being important in changing physiological processes that result in acclimatization and adaptation of different plant species to the undesirable environmental conditions has been reported (Rajeshwari and Bhuvaneshwari, 2017).

The photosynthetic yield was negatively affected by salinity may be due to the stomatal closure and CO_2 deficiency, but SA can delay the stomatal closure and enhance the CO_2 entrainment rate that improves the photosynthetic yield (Khoshbakht and Asghari, 2015). The higher proline content in response to SA treatment might be associated with the role of SA in the defense again different abiotic stresses. SA can enhance the abscisic acid concentration, which results in an increase of proline content (Yeganehpoor et al., 2017). Misra and Misra (2012) reported that increase in the activity of proline production enzymes [like pyrroline-5-carboxylate reductase (P5CR) and γ-glutamyl kinase (GK)] and reduction of proline oxidase activity were associated for increased proline content in plants treated with SA. Furthermore, similarly to our results, Cag et al. (2009), Li et al. (2014) and Liu et al. (2016) mentioned that SA treatment could enhance the amount of compatible solutes such as proline and the protein content under salinity. The increase of protein content in SA treated plants could be related to the effects of SA on the changes in the expression of some protein synthesis genes (Tarchevsky et al., 2011).

In our study, SA treatment significantly enhanced TPC of almond cultivars, which are in agreement with Misra et al. (2014), El-Esawi et al. (2017) and Grzeszczuk et al. (2018). It was reported that SA induces changes in the gene expression associated with the biosynthesis of phenylpropanoids, such as phenylalanine ammonia-lyase (PAL), the first enzyme of the during phenylpropanoid biosynthetic pathway (Kovacik et al., 2009).

As shown in Table 1 and Fig. 1, SA significantly increased the antioxidant enzyme activities of almond cultivars. These results are in agreement with Li et al. (2014), Misra et al. (2014), Liu et al. (2016) and Ma et al. (2017). Increases in the antioxidant enzymes activities in response to SA treatment might be due to the effects of SA on the expression of antioxidant enzymes genes El-Esawi et al. (2017) concluded that the expression levels of some antioxidant enzymes genes such as CAT, SOD and APX were enhanced in SA-treated rosemary under salinity conditions.

The increase of the maximum quantum efficiency of PSII photochemistry (*Fv/Fm*), accumulation of Na and Cl in the root, proline content and antioxidant enzymes activities could help plants to reduce the adverse effects of salinity and to have better growth and development (Munns and Tester, 2008; Liang et al., 2018). Furthermore, we found some differences between the two cultivars in response to salinity and also SA treatment. These results are in agreement with Sorkheh et al. (2012), Zrig et al. (2015a), Zrig et al. (2015b) and Zrig et al. (2016), who reported that different cultivars and/or species had a different response to salinity.

Conclusion

Our study showed the negative impacts of salinity on two cultivars of Iranian almonds, such as decreases in plant height, *Fv/Fm*, protein and TPC.

On the other hand, salinity significantly enhanced root Na and Cl content, proline content and antioxidant enzymes activities (SOD, CAT, APX, POD and GPX). Moreover, it was revealed that SA treatment significantly ameliorate the adverse effects of salinity, by increasing morphological characteristics, *Fv/Fm*, accumulation of Na and Cl in the root, the content of proline, protein and TPC, as well as the activities of SOD, CAT, APX, POD and GPX. Overall, we found that 'Sahand' cultivar treated with SA showed a better response to salinity as compared to 'Tuono' cultivar.

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aCS85_35