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ORIGINAL ARTICLE

Response of Pistachio (*Pistacia vera* L.) Seedling Rootstocks to Salicylic Acid Foliar Application under Water Stress

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A R T I C L E I N F O

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A B S T R A C T

This research was conducted to determine the effect of salicylic acid on the drought tolerance of three pistachio (*Pistacia vera* L.) rootstocks. The treatments were included three irrigation levels (Control: 100 % ETc, medium stress: 65% ETc, and severe stress: 30% ETc), three pistachio rootstocks, and three salicylic acid concentrations (0, 1, and 3 mM). The salicylic acid solution was once applied as a foliar spray, starting water stress, and irrigation treatments continued for 75 days. Results showed that *P. vera* 'Badami Zarand' was less affected by water stress treatments than the other seedling rootstocks. Among the rootstocks studied, the highest photosynthesis rate (6.55 µmol CO_2 m^{-2} s^{-1}) was obtained in *P. vera* 'Badami Zarand' under severe water stress (30 % ETc) and 1 mM salicylic acid application. The lowest photosynthesis rate (2.75 µmol CO_2 m^{-2} s^{-1}) was observed in *P. vera* 'Sarakhs' under severe water stress (30 % ETc) and 3 mM salicylic acid treatment. In addition, the highest relative water content (66.16%) and the lowest ion leakage (33.29%) were obtained in 'Badami Zarand.' The 1 mM salicylic acid application effectively reduced the negative effects of water stress. According to the results, *P. vera* 'Badami Zarand' showed a better response to salicylic acid under stress conditions, and the highest photosynthesis, relative leaf water content, dry weight of organs (leaves, shoots, roots), and the lowest ion leakage under drought stress belonged to this rootstock.

Abbreviations

SA	Salicylic acid
ET_c	Crop evapotranspiration
E	Transpiration rate
RWC	Relative water content
RH	Relative humidity
Pn	Photosynthesis or CO_2 assimilation
g_s	Stomatal conductance
F_v/F_m	Photochemical efficiency of photosystem II

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Introduction

Drought stress is the most important non-biotic stress that occurs when water intake of the plant is less than its water loss (Mahajan and Tuteja, 2005; Behrooz *et al.*, 2019). Iran is among the dry and semi-arid countries with an average rainfall of about 250 mm per year (Lotfi *et al.*, 2019). In addition, having around 300,000 hectares of pistachio orchards, Iran is the major pistachio producer in the world (Arzani, 2002; Sharifkhah *et al*., 2020) and have a great share in pistachio export around the world (Alipour, 2018). Most of Iran's pistachio orchards are located in the arid and semi-arid regions affected by water stress conditions (Arzani, 2017; Ghasemi *et al*., 2017; Eslami *et al*., 2019). Despite pistachio is tolerant to drought stress (Norozi *et al*., 2019) the quality and productivity of pistachio trees are significantly reduced due to water stress (Goldhamer and Beede, 2004; Shamshiri et al., 2015; Behzadi Rad *et al.*, 2021). Plant dry weight, leaf area, and shoot elongation of three pistachio rootstocks decreased under mild (-0.5 MPa), moderate (-1 MPa) and severe (-1.5 MPa) water stress (Esmaeilpour *et al.*, 2016).

The tolerance of plants to environmental stresses is increased in different ways, including breeding or cultural management, such as plant growth regulators (Arzani, 2017; Husen *et al*., 2017; Husen *et al*., 2019). One of these ways is to use drought-tolerant rootstocks (Vahdati *et al.*, 2021). Tolerant rootstocks to drought enable the scion cultivar for better growth under a limited water supply (Serra *et al*., 2014).

Moreover, the external application of osmotic compounds, growth stimulants, and antioxidant compounds on plants offers a short-term solution to eliminate the negative effects of various stresses such as drought. Salicylic acid is one of the compounds involved in regulating various physiological processes in plants (Waseem *et al*., 2006; Husen *et al*., 2018; Miura and Tada, 2014; Khan *et al.*, 2015). In addition, salicylic acid plays a critical role in plant growth and development and protects the plants subjected to osmotic stress (Ignatenko *et al*., 2019; Heidarian and Roshandel, 2021; Mahdavian, 2022). Salicylic acid may increase the synthesis of plant hormones, enzymes, and photosynthesis rate (Fariduddin *et al.*, 2003; Ignatenko *et al*., 2019; Haghshenas et al., 2020). In water stress conditions, ethylene production increases, but salicylic acid reduces ethylene synthesis by converting 1-amino-cyclopropane-carboxylic acid to ethylene (Leslie and Romani, 1986; Yousefzadeh Najafabadi and Ehsanzadeh, 2017). Depending on the concentration, plant genotype, growth stage, and environmental conditions, salicylic acid may have different stimulating or preventing effects on physiological processes (Husen *et al*., 2019). Salicylic acid (0.1 mM) effectively protected tomato plants against drought stress and improved their growth performance (Senaratna *et al*., 2000). Shafiei *et al*. (2019) reported that the use of salicylic acid at a concentration of 2 mM alleviated the adverse effects of drought stress (66 and 33 % ETcrop) in young olive trees (*Olea europaea* 'Konservalia') through the improvement of chlorophyll content, enhancing leaf total soluble carbohydrate and proline content which can lead to osmotic adjustment. Leventtuna *et al*. (2014) showed that salicylic acid reduced the amount of electrolyte leakage in Maize under water stress. Increasing salicylic acid concentration up to 0.5 mM decreased the amount of ion leakage in cucumber (Bayat *et al*., 2011) and grape (Abbaspour and Babaee, 2017) leaves under water stress. Reducing membrane damage due to the salicylic acid application may be associated with an increase in the antioxidant activity to prevent membrane lipid peroxidation (Chu *et al.*, 2010). Khan *et al*. (2003) reported that exogenous salicylic acid application on corn and soybean plants increased the photosynthesis rate. Salicylic acid may also increase the synthesis of plant hormones, enzymes, and photosynthesis (Fariduddin *et al*., 2003). Nemeth *et al*. (2002) also stated that the positive effects of salicylic acid might be due to an increase in the amount of putrescine, spermidine, and spermine in the plant, which helps maintain membrane stability under water stress.

This research was conducted to determine the effect of salicylic acid on the drought tolerance of three pistachio rootstocks and identify drought-tolerant rootstocks. The treatments were included three irrigation levels (without stress, medium stress, and severe stress), three pistachio rootstocks, and three salicylic acid concentrations (0, 1, and 3 mM).

Material and Methods

The experiment was carried out in the 2012-2013 growing season at the department of horticultural science, Tarbiat Modares University (TMU), Tehran, Iran. The greenhouse environments were set up to the 27 to 35 and 19 to 25ºC during day and night, respectively, with 37±5 %RH. The split nuts of pistachio (*P. vera*) rootstocks were sown in the 11-liter pots in March 2012. The soil texture was sandy loam with 15, 65, and 20% clay, sand, and silt. Because in pistachios, seed rootstocks are preferable, so to increase the uniformity between seedlings obtained from seed cultivation, many seeds per genotype were planted, and finally, uniform seedlings were selected.

The moisture of the pots was kept at field capacity for four months until the treatments were started. Treatments included three irrigation levels (Control: 100 % ETc, medium stress: 65% ETc, and severe stress: 30% ETc), three pistachio seedling cultivars (rootstocks) included 'Badami Zarand' (`Badami'), 'Ghazvini,' and 'Sarakhs, and three salicylic acid concentrations (0, 1 and 3 mM). Treatments were arranged in a factorial experiment in a completely randomized block design with three replications (Three plants used in each replication). The salicylic acid solution was sprayed once on plants for four months, and irrigation treatments continued for 75 days. Note that salicylic acid is first dissolved in a small amount of ethanol and then increased to the desired volume by distilled water. Evapotranspiration (ETc) of the experimental plants was recorded based on weighing the pots and weight loss during two irrigation time intervals. For applying irrigation treatments, pots were irrigated, allowed to drain the excess water reached to the constant weight (field capacity or 100% water holding capacity). Then, pots were reweighed in two-day intervals; ETc was calculated for each pot to irrigate experimental plants based on the selected treatments (%ETc) (Arzani and Arji, 2000). 100%, 65%, and 30% of lost water were returned to plants in control, moderate stress, and severe stress treatments, respectively (Ghasemi *et al*., 2017).

Measurements

The parameters such as gas exchanges, photochemical efficiency of photosystem II (Fv/Fm), percent relative water content (% RWC), ion leakage, and plant biomass were evaluated**.**

Gas exchange parameters

Seventy-five days after stress (approximately six months old seedlings), gas exchange parameters were monitored before noon between 10 to 12 am on the two fully developed leaves from the middle canopy according to the method described by Arzani (1994) and Roozban *et al.* (2010). Photosynthesis (Pn), transpiration rate (E), and stomatal conductance (g_s) were measured using LI-6200 Portable Photosynthesis System (LiCor-6200 Portable, Inc., Lincoln, NE, USA).

Photochemical efficiency of photosystem II (Fv/Fm)

The Fv/Fm ratio was recorded in the mid-point of fully developed leaves according to the method described by Khaleghi *et al*. (2012) by portable plant stress meter (PSM, Bio Monitor S.C.I. AB, Sweden). The measurements were taken after 30 minutes of darkness exposed on the leaves by special clips under the red light (695 nm). Note that Fv/Fm ratio is the most common parameter used for water stress monitoring, so is a dark-adapted record reveals the maximum quantum efficiency of photosystem II under stress conditions.

Relative water content (RWC)

RWC was measured according to the method described by Arzani (1994). Ten leaf discs with 8 mm diameter were taken from each replication, then immediately fresh weighed. The leaf samples were transferred in the distilled water in the Petri dishes, placed under dark for 4 hours to reach and measure turgor weight. The dry weight of the samples was recorded after oven-dried at 70°C for 24 hours. The percent relative water content of the leaves was calculated using the following equation (1):

RWC (%) = ((fresh weight-dry weight) / (turgor weight- dry weight)) ×100

Ion leakage

In order to record the ion leakage, the Blum and Ebercon (1981) method were used with some modifications. Leaf samples were taken from the middle canopy of each plant. The leaf samples were washed with distilled water and then transferred to the test tubes containing 15 ml distilled water. Tubes with leaf samples were placed on a shaker at 120 rpm for 24 hours at 25°C, and electrical conductivity was measured by EC meter (EC_1). Then the samples were autoclaved for 20 minutes at 121°C to exit all contents of the cell; when samples temperature reached the ambient temperature (25°C), the electrical conductivity of the solution was again recorded (EC_2), and the ion leakage percentage was calculated from equation (2):

Ion leakage (%) = ((EC_1)/ (EC2)) ×100

Growth parameters

Plants were destructively harvested at the end of the experiment, divided into root, shoot, and leaf, placed in the oven at 70°C for 48 hours for dry weight measurements (Arzani, 1994).

Statistical analysis

Data were analyzed using SPSS software version 16, and results were statistically evaluated by analysis of variance (ANOVA) and expressed as treatments mean. Means comparisons were made using Duncan's multiple range test (DMRT), and the differences were considered statistically significant at $P \leq 0.05$.

Results

Gas exchanges

The results of variance analysis of data are shown in Table 1. Water stress significantly reduced photosynthesis, stomatal conductance, and transpiration rates (Table 2). In the present research, the photosynthesis rate of pistachio genotypes was increased using one mM salicylic acid under both normal and stress conditions. In addition, the highest photosynthesis rate (11.85 μmol CO_2 m^{-2} s^{-1}) was obtained in `Badami' rootstock under no stress condition with one mM salicylic acid application. The lowest photosynthesis rate (2.75 μmol $CO_2$ $m^{-2}$ $s^{-1}$) was recorded in the `Sarakhs' rootstock under severe water stress and 3 mM salicylic acid concentration. The highest stomatal conductance (600.9 mmol H_2O $m^{-2}s^{-1}$) was monitored in `Ghazvini' rootstock under full irrigation treatment (Table 2).

The highest transpiration rate (11.64 mmol H_2O m^{-2} s^{-1}) belonged to the `Badami' rootstock under the normal condition using three mM salicylic acid. The lowest transpiration rate (0.91 mmol $H_2O$ $m^{-2}$ $s^{-1}$) was recorded in `Sarakhs' rootstock under severe water stress and three mM salicylic acid treatment. Pistachio `Badami' rootstock showed more photosynthetic efficiency than the `Sarakhs' and `Ghazvini' rootstocks under water stress conditions (Table 2). Based on the results, the photosynthesis rate was improved by one mM salicylic acid compared to the three mM application.

Table 1. Analysis of variance (ANOVA) of different measured parameters in three pistachio seedling rootstocks grown under different levels of irrigation and salicylic acid (SA) concentrations in greenhouse conditions.

Variation source	df	Photosynthesis	Stomatal conductance	Transpiration	Fv/Fm	RWC	Ion Leakage	Leaf dry weight	Shoot dry weight	Root dry weight
Irrigation (A)	2	286.604 **	2088224.38 **	983.95 **	0.005 **	910.882 **	2022.287 **	1591.617 **	1948.304 **	595.338 **
Rootstock (B)	2	6.834 **	20337.59 **	0.364 ns	0.001 ns	79.82 **	976.328 **	61.477 **	64.04 **	13.90 **
SA (C)	2	51.915 **	8280.549 *	4.473 **	0.007 **	9.733 ns	17.002 ns	24.367 **	132.274 **	38.234 **
A×B	4	5.84 **	12952.776 **	1.848 **	0.005 *	44.602 ns	39.949 ns	19.904 *	29.818 **	48.221 **
A×C	4	8.471 **	12317.039 **	2.966 **	0.006 **	7.886 ns	24.655 ns	5.233 *	23.222 **	10.583 **
B×C	4	3.856 **	29207.835 **	3.715 **	0.001 ns	8.682 ns	76.826 *	3.814 ns	5.739 ns	1.706 ns
A×B×C	8	3.343 **	28274.954 **	3.991 **	0.002 **	4.76 ns	36.76 ns	1.715 ns	5.093 ns	2.393 ns

**, * and ns, significant at level 1%, 5%, and non-significant, respectively.

Table 2. Effect of different water stress levels and salicylic acid concentrations on photosynthesis rate (Pn), stomatal conductance (gs), transpiration (E), and photochemical efficiency of photosystem II (Fv/Fm) in three pistachio seedling rootstocks under greenhouse conditions.

Irrigation Treatments	Rootstock seedlings	Salicylic acid mM	Photosynthesis (Pn) μmol CO_2 m^{-2} s^{-1}	Stomatal conductance (gs) mmol H_2O m^{-2} s^{-1}	Transpiration (E) mmol H_2O m^{-2} s^{-1}	Fv/Fm
No Stress	Badami	0	8.2 d	483.48 cd	10.4 bc	0.82 a
		1	11.85 a	442.32de	8.37 e	0.80 abc
		3	8.23 d	592.37 a	11.64 a	0.76bcdef
	Ghazvini	0	6.78 ef	493.69 cd	10.07cd	0.76bcdef
		1	11.41 ab	600.9a	9.49 d	0.79 abc
		3	8.96 d	527.89 bc	8.42 e	0.76 bcdef
	Sarakhs	0	9.85 c	584.23 ab	10.99ab	0.80abc
		1	10.98 b	583.14ab	9.47 d	0.80 abc
		3	8.45 d	390.3 ef	8.66 e	0.72 ef
Mild stress	Badami	0	6.75 ef	337.48 fg	4.33 f	0.76 abcdef
		1	7.16 e	202.53hi	3.47 g	0.82 ab
		3	6.03 fgh	209.89 hi	3.56 fg	0.77 abcdef
	Ghazvini	0	5.36 hij	291.66 g	3.47 g	0.77 abcdef
		1	6.74 ef	313.83g	3.62 fg	0.80 abc
		3	6.00 fgh	348.5 fg	3.95 fg	0.77 abcdef

	Sarakhs	0	5.55^{hij}	293.57^{g}	3.71^{fg}	0.79^{abcd}
		1	5.94^{fghi}	213.86^{h}	3.51^{g}	0.79^{abcd}
		3	5.77^{ghi}	294.74^{g}	4.26^{fg}	0.73^{ef}
	Badami	0	5.05^{ij}	172.92^{hij}	1.39^{hij}	0.76^{abcdef}
		1	6.55^{efg}	135.66^{jk}	1.10^{ij}	0.75^{cdef}
		3	5.41^{hij}	174.5^{hij}	1.37^{hij}	0.72^{ef}
Severe Stress	Ghazvini	0	4.79^{j}	166.83^{hij}	2.03^{h}	0.76^{abcdef}
		1	5.64^{hij}	152.02^{hijk}	1.77^{hi}	0.78^{abcde}
		3	5.21^{hij}	98.48^{kl}	1.36^{hij}	0.75^{cdef}
	Sarakhs	0	3.82^{k}	91.57^{kl}	1.28^{hij}	0.71^{f}
		1	5.48^{hij}	147.07^{ijk}	1.86^{hi}	0.80^{abc}
		3	2.75^{l}	56.37^{l}	0.91^{j}	0.73^{def}

According to Duncan's multiple range tests,*Means with similar letters in each column are not significantly different at 5% probability level.

Photochemical efficiency of photosystem II

The results showed that the photochemical efficiency of photosystem II was significantly affected by water stress and salicylic acid. There was no significant difference among studied seedling rootstocks (Table 1). As shown in Table 2, the lowest value (0.716) was recorded in `Sarakhs' rootstock under severe water stress without salicylic acid application. Foliar application of 1 mM salicylic acid increased the photochemical efficiency of photosystem II by 0.785, while three mM salicylic acid decreased it by 0.755 comparisons with control treatment (0.779) (Table 2). In addition, the photochemical efficiency of photosystem II of `Badami' and `Sarakhs' rootstocks was less and more affected by water stress, respectively.

Leaf relative water content (RWC)

The simple effect of irrigation and genotype on leaf relative water content was significant (P≤0.01), but the effects of salicylic acid and also the interaction of irrigation, genotype, and salicylic acid were not significant (Table 1). The RWC in control, moderate and severe water stress treatments was 70.67, 62.38, and 59.48%, respectively (Fig. 1).

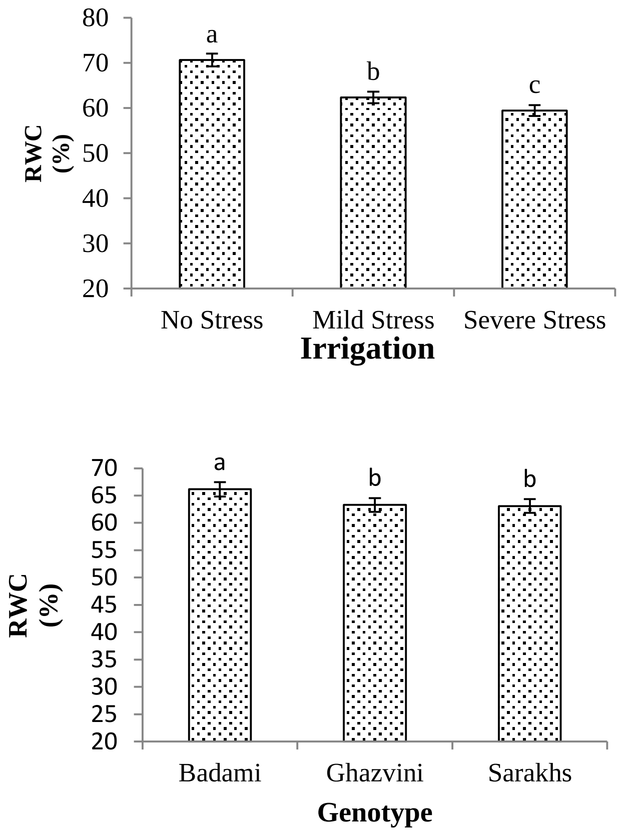

Fig. 1. The effect of irrigation and rootstock (**genotype)** on the percentage of relative leaf water content (RWC). Vertical bars indicate the standard error of the means (± SE).

Ion leakage

Analysis of variance (Table 1) showed that only the simple effect of irrigation treatments and genotypes was significant in the case of the ion leakage (P≤0.01). The one mM salicylic acid application reduced the ionic leakage, but this decrease was not statistically significant (Table 1). The highest and lowest ion leakage was observed in severe water stress (30 % ETc) and non-stress treatment (100 % ETc), respectively (Fig. 2). In this study, among the rootstocks, the highest and lowest ionic leakage was observed in `Sarakhs' and `Badami,' respectively (Fig. 2).

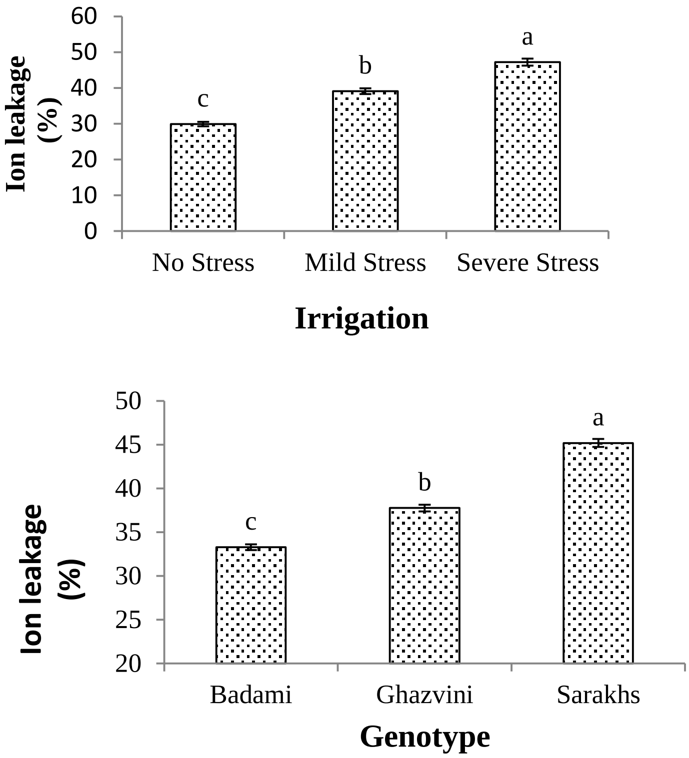

Fig. 2. Effect of irrigation treatments and rootstock (genotype) on Ion leakage of three pistachio seedling rootstocks grown under greenhouse conditions. Vertical bars indicate the standard error of the means (± SE).

Growth parameters

The simple effect of irrigation, genotype, salicylic acid, the interaction effect of irrigation and genotype, and irrigation and salicylic acid on shoot root and leaf dry weight was significant (Table 1). Water stress significantly decreased shoot, root, and leaf dry weight, showing significant differences among irrigation treatments, although leaves were more affected by water stress than the roots. The interaction effect of irrigation and genotype on the shoot, leaf, and root dry weight of three pistachio genotypes showed that under normal conditions, the highest biomass belonged to Ghazvini genotype but in severe water stress, leaf, shoot and root dry weight of Badami genotype was more than Ghazvini and Sarakhs genotypes (Fig. 3). The growth parameters increased with one mM salicylic acid application compared to control, although growth reduced with the higher salicylic acid concentration. The interaction of irrigation and salicylic acid showed that under drought stress, application of 1 mM salicylic acid increased the dry weight of leaves, shoots, and roots compared to the non-foliar application but application of 3 mM concentration was not effective (Fig. 4).

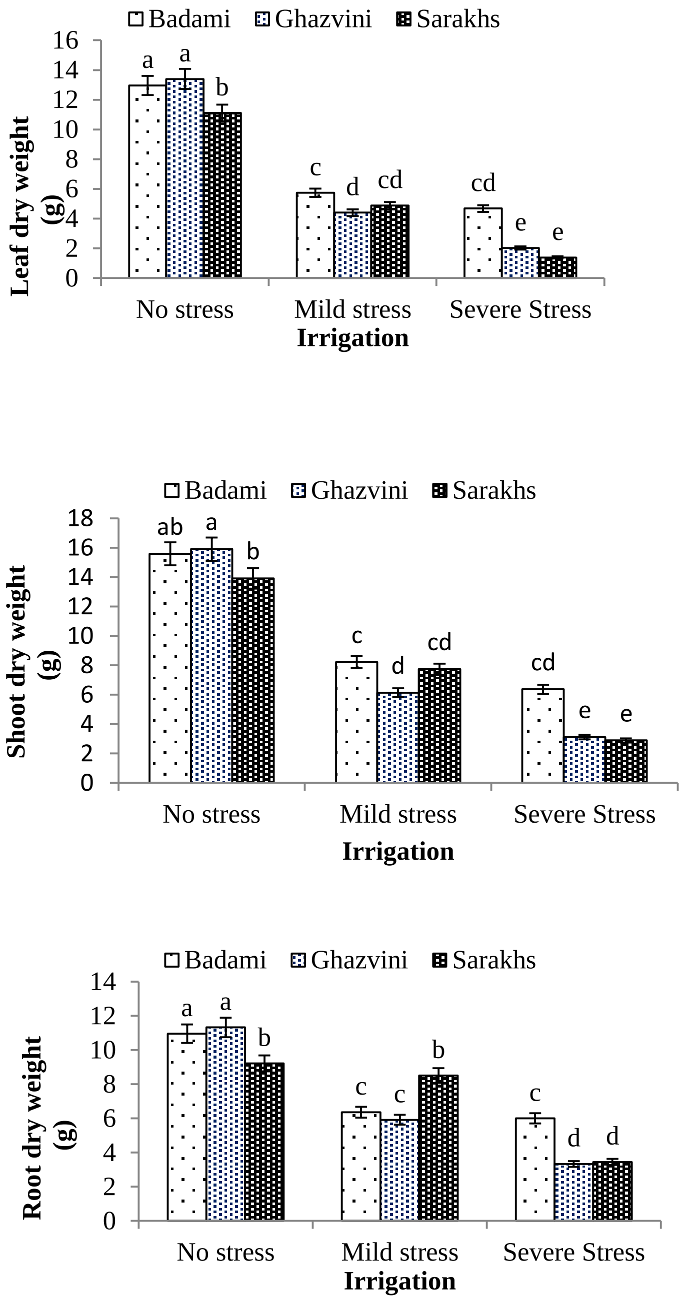

Fig. 3. The interaction effect of irrigation and genotype on shoot, leaf, and root dry weight of three pistachio seedling rootstocks grown under greenhouse conditions. Vertical bars indicate the standard error of the means (± SE).

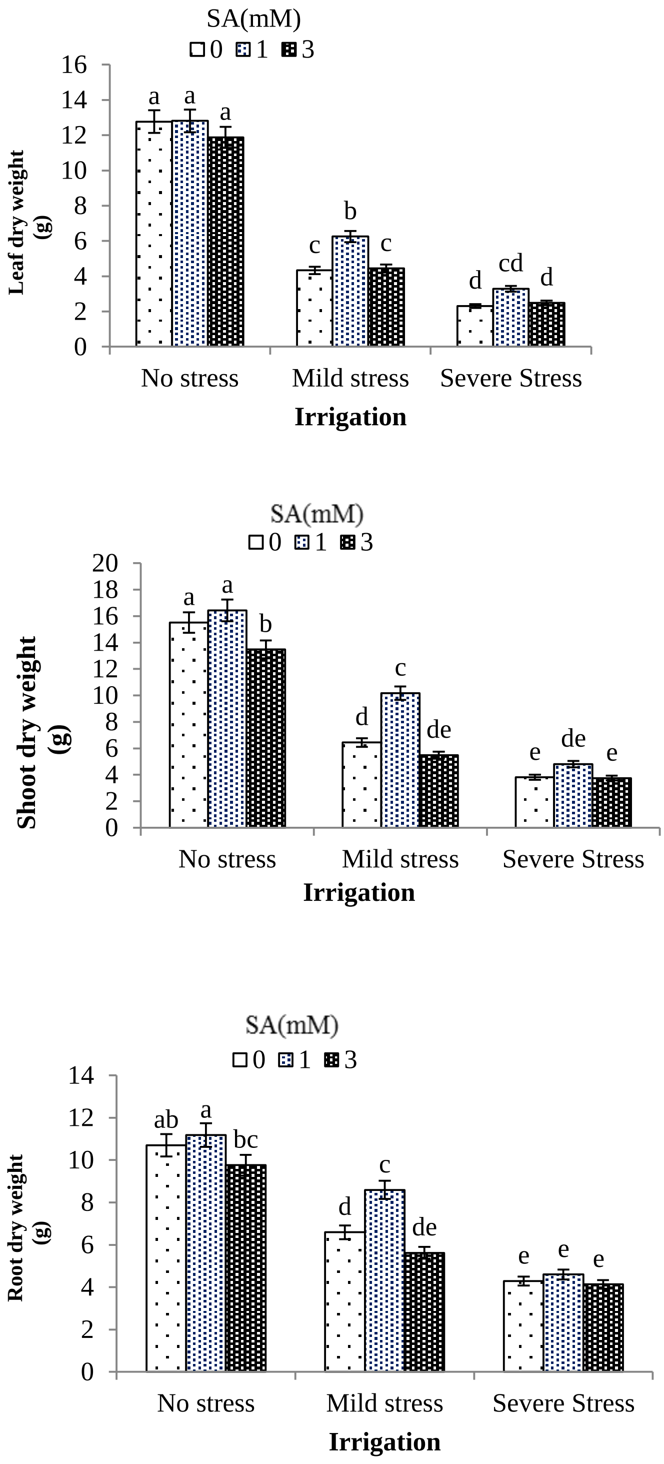

Fig. 4. The interaction effect of irrigation and salicylic acid treatments on shoot, leaf, and root dry weight of three pistachio seedling rootstocks grown under greenhouse conditions. Vertical bars indicate the standard error of the means (± SE).

Discussion

The reduction in photosynthesis under stress conditions is affected by stomata closure and limited CO_2 intake into the leaves (Bashir *et al*., 2015). In the present research, despite decreasing stomatal conductance and transpiration rate under drought, the negative impact of stress on photosynthesis was ameliorated by salicylic acid application. The positive effect of 1mM salicylic acid application on photosynthesis (Table 2) can be due to non-stomatal factors such as enzyme activity involved in the photosynthesis process (Khodary, 2004). Salicylic acid increases photosynthesis rate (Khan *et al*., 2003), regulates the activity of antioxidant enzymes, and improves plant tolerance to non-biotic stresses (He *et al*., 2002). The proper concentration of exogenous salicylic acid application is important, so under the condition of our experiment for pistachio rootstocks, one mM showed better performance than three mM foliar spray. Nazar *et al*. (2011) stated that concentrations of 0.1 and 0.5 mM salicylic acid stimulated, but one mM salicylic acid prevented growth and photosynthesis of treated plants under salinity stress.

Sun *et al*. (2016) suggested the efficiency of photosystem II as a diagnostic tool to detect the negative impact of environmental stresses on plants. Although the water deficit reduced Fv/Fm ratio, the negative effect of drought was greatly reduced with the application of salicylic acid (Table 2). Foliar application of salicylic acid has beneficial effects on photochemical efficiency of photosystem II, rubisco activity, and thereby, photosynthesis rate under water stress conditions (Nazar *et al*., 2015; Yousefzadeh Najafabadi and Ehsanzadeh, 2017).

The higher relative water content (66.16%) observed in `Badami' rootstock than the other studied rootstocks (Fig. 1) may be due to the better regulation of osmotic adjustment in its leaves under water stress conditions. Osmotic adjustment is an important adaptive mechanism against stress conditions that helps plants absorb higher water by the roots under the water stress and maintain cell turgor (Arzani, 1994; Yousefzadeh Najafabadi and Ehsanzadeh, 2017).

Water stress increases the production of toxic and destructive groups of reactive oxygen species (ROS). The ROS damage cell membrane lipids, proteins, and nucleic acids, so the membrane stability is lost and increased electrolyte leakage (Inze and Montagu, 1995; Huang *et al*., 2014). Maintaining membrane stability under water stress condition is considered one of the best physiological indicators of drought tolerance and can be used for screening drought-tolerant genotypes (Bajji *et al*., 2002; Chowdhury *et al*., 2017). The lower ion leakage in the `Badami' rootstock may be due to the higher drought resistance of this genotype. Pistachio `Sarakhs' rootstock with the highest ion leakage may lead to more water stress susceptibility. The one mM salicylic acid application reduced the ionic leakage, but this decrease was not statistically significant. The reduction of the negative impact of salinity stress on pistachio leaf ion leakage with 0.50 mM salicylic acid application was observed by Bastam *et al*. (2013). It has been reported that salicylic acid activates the plant's antioxidant system (Ignatenko *et al*., 2019) and may alleviate the risk of increasing the amount of ROS during the period when the plant is exposed to stress. Consequently, the damage to the cell membrane structure and the change in its permeability under stress conditions can be prevented (Rao *et al*., 1997).

Yordanov *et al*. (2000) demonstrated that drought induces osmotic stress, leading to the senescence of older leaves and stomatal closure, loss of leaf chlorophyll pigments, reduced photosynthesis, and finally led to the decline in plant growth (Yordanov *et al.*, 2000). The amount of dry biomass produced during drought conditions is a good indicator for stress tolerance evaluation in many crops (Munns, 2002).

Esmaeilpour *et al.* (2016) applied different degrees or levels of water stress on three young pistachio rootstocks seedlings, demonstrated that with increasing the degree of water stress, the production of dry plant biomass was decreased. In addition, the significant decrease in dry biomass was in agreement with results reported by Ranjbarfordoei *et al*. (2000) and Abbaspour *et al.* (2012) in pistachio trees.

It has been reported that an increase in growth parameters under stress conditions by proper salicylic acid concentration may be due to improving the absorption of nutrients, photosynthesis, cellular activity, improving cell wall structure, or improving root development (Misra and Misra, 2012). Husen *et al.* (2019) found that 1mM SA application significantly increased shoot length, leaves, and leaf width of Niger (*Guizotia abyssinica*) plant under salinity stress. In addition, the amelioration effect of lead stress on the subjected plant growth through the mechanism of the reduction in free amino acids and increase of protein synthesis using the salicylic acid application was reported by Zanganeh *et al.* (2019). In this research, better results in ameliorating the negative effects of water stress on pistachio rootstock seedlings were observed using a lower salicylic acid concentration (Fig. 3). In our research, the degree of effectiveness of salicylic acid was concentration-dependent, so the higher three mM salicylic acid showed a negative effect on pistachio growth. Shafiei *et al*. (2019) reported that the use of salicylic acid at a concentration of 2 mM alleviated the adverse effects of drought stress (66 and 33 % ETcrop) in young olive trees (*Olea europaea* 'Konservalia') through the improvement of chlorophyll content, enhancing leaf total soluble carbohydrate and proline content which can lead to osmotic adjustment.

Conclusions

According to the results, *P. vera* 'Badami Zarand' showed a better response to salicylic acid under stress conditions because of the highest photosynthesis, relative leaf water content, dry weight of organs (leaves, shoots, roots), and the lowest ion leakage under drought stress belonged to this rootstock. In addition, the shoot, leaf, and root dry weights were higher in the one mM concentration of salicylic acid. Therefore, it can be concluded that the use of *P. vera* 'Badami Zarand' as a drought-tolerant rootstock is recommended for pistachios, and foliar application of 1 mM salicylic acid in the early stages of seedling growth can improve physiological, biochemical, and growth parameters of this rootstock and the thus better establishment and growth.

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Conflict of interest

The authors have no conflict of interest.

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