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Glycerol foliar application improves salt tolerance in three pistachio rootstocks

Ahmad Raoufi [a], Majid Rahemi [a,*], Mohammad Akbari [b]

[a] Department of Horticultural Sciences, Faculty of Agriculture, Shiraz University, Shiraz, Iran
[b] Department of Biological Sciences, College of Science and Technology, Florida A&M University, Tallahassee, FL 32307, USA

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ABSTRACT

Salinity is one of the most important abiotic stress factors that affect pistachio growth and productivity. Researches has indicated that the detrimental effects associated with salt stress could be overcome by the external application of Glycerol. In the present study, the effects of foliar Glycerol (in three levels: 0, 30 and 60 mM) on three pistachio rootstocks ('Ghazvini', 'Badami' and 'Italyayi') under different salinity stress (0, 4, 8 and 12 dSm^{-1}) were investigated. Salinity significantly decreased the rootstocks' biomass, number of grown leaves, leaf area, photosynthesis pigments, stomatal conductance, leaf water potential and K^+ content in the leaves, shoots and roots. The foliar application of Glycerol at the concentration of 60 mM alleviated growth indices of pistachio rootstocks under saline condition. Foliar Glycerol spray (60 mM) at 12 dSm^{-1} increased the dry weight of leaves and shoots, up to 176% and 57%, respectively, compared to the control. Leaf area and number of grown leaves increased up to 466% and 32%, respectively, under highest salinity level. Glycerol caused a slight increase in stomatal conductance at 40 and 75 days after imposing the salt stress while it did not have any effects on the leaf water potential. The highest increment percentage of chlorophyll *a* (51%), chlorophyll *b* (53%), and root K^+ content (40%) was observed in 60 mM Glycerol treated rootstocks at 12 dSm^{-1}. Glycerol application reduce the Na^+ concentration in the leaves, shoots and roots. Among three studied rootstocks, response to Glycerol under saline condition were different and was superior in 'Badami' In conclusion, Glycerol could be suggested as a cheap, safe and organic agrochemical for alleviating the negative impacts of salt stress in pistachio orchards cultivated in the saline soils.

1. Introduction

Pistachio (*Pistacia vera* L.) is a broadly cultivated and important tree nut crop. These nuts provide rich sources of health-promoting nutrients such as minerals, proteins, antioxidants, and phenols (Aliakbarkhani et al., 2017). The genus *Pistacia* L. belongs to the Anacardiaceae family comprising 83 genera and 860 species. The *Pistacia* genus comprises around 11 tree and shrub species. Among these species, the *P. atlantica*, *P. integerrima* and *P. terebinthus* are commonly used as rootstocks for cultivating *P. vera* (Sheikhi et al., 2019). *P. vera* L. is the only economic cultivated species, grafted on the mentioned pistachio species, however, in some pistachio cultivating areas in the world such as Iran, *P. vera* is the most applied rootstock in the pistachio orchards. More than 90 percent of the pistachio orchards in Iran are grafted on *P. vera* rootstocks. *P. vera* is cultivated in the dry and semi-dry areas of Iran and is called "green gold" due to its health-benefiting nutrients and economical importance (Aliakbarkhani et al., 2017; Talebi et al., 2016). Among all horticultural crops in Iran, pistachio has the most under cultivation area, which is the highest cultivation area of pistachio in the world (Taghizadeh-Alisaraei et al., 2017; Aliakbarkhani et al., 2015).

Salinity is a severe threat to crop productivity with devastating worldwide effects, assessed 50% of land loss by 2050 (Akbari et al., 2020). Salt and drought stress in Iran's pistachio orchards decrease the yield and productivity and affects billions of economic gain per annum (Raoufi et al., 2020; Goharrizi et al., 2020; Khoyerdi et al., 2016). Saline soils, normally recognized with electrical conductivity more than 4 dSm^{-1} and pH more than 8.2, are including about

* Corresponding author.
E-mail address: rahemi@shirazu.ac.ir (M. Rahemi).

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41 million hectares (25%) of Iran's lands (Mesgaran et al., 2017). In saline soils, low water potential along with the ion toxicity and subsequence nutritional imbalance, have been estimated to decrease the yield up to 50% (Stavridou et al., 2017). In Iran, most of the pistachio orchards are located in saline and alkaline soils, irrigated with salty and poor quality water. Although pistachios are considered semi-tolerant to saline soils, the best commercial production occurs in deep, well-drained, sandy loam soils that are not always available (Akbari et al., 2018). High salinity decreases the plants root growth, trunk diameter, leaf area, leaf number, total biomass, canopy height, and overall yield (Raoufi et al., 2019; Bastam et al., 2013; Ferguson et al., 2002).

To alleviate the salt stress and maintain acceptable yield, different chemical treatments and cultivation practices have been examined (Bastam et al., 2013). Glycerol is one the chemical treatments, which is a low cost byproduct of biodiesel production process, produced from vegetable oils through esterification with methanol (Baba et al., 2013; Tisserat and Stuff, 2011). Glycerol is a safe, edible, biodegradable sugar alcohol and ecofriendly agrochemical (Zhang et al., 2015; Tisserat and Stuff, 2011). Despite existing little amount of Glycerol in higher plants, it has been shown that the exogenous application of Glycerol could significantly affect the plant growth (Hu et al., 2014; Tisserat and Stuff, 2011).

Kaya et al (2013) reported that in corns treated with Glycerol a higher salinity tolerance were observed in comparison to untreated plants. In higher plants, Glycerol kinase is the key enzyme to utilize Glycerol and converting it to Glycerol-3-phosphate (G3P). Exogenous application of Glycerol on mutant *Arabidopsis* with lack of Glycerol kinase resulted in more tolerance to salinity and freezing (Eastmond, 2004). It also has been demonstrated that the foliar application of Glycerol on *Theobroma cacao* increased the disease resistant of the plant through the activation of defense system (Zhang et al., 2015).

The adverse effects of salt stress on pistachios are widely investigated and it is obvious that different pistachio genotypes exhibit various tolerance to salinity (Raoufi et al., 2020; Akbari et al., 2020). Among the studied pistachio rootstocks in the literatures; 'Badami', 'UCB1', 'Ghzvini' and 'Akbari' have been shown to have higher tolerance to salinity, while 'Italyayi' rootstock is considered as a moderately susceptible to salinity (Ahmadi Kouhbanani et al., 2016; Akbari et al., 2018; Momenpour and Imani, 2018; Raoufi et al., 2019).

Positive reports about the effects of Glycerol as an ecofriendly agrochemical on plants growth and tolerance to environmental stresses encouraged us to investigate the effects of Glycerol on pistachio rootstocks. To the best of our knowledge this is the first study to assess the effects of exogenous application of Glycerol on pistachio rootstocks ('Ghazvini', 'Badami' and 'Italyayi') under saline situation.

2. Material and methods

2.1. Plant material and growth condition

This experiment was carried out in research greenhouse of the Department of Horticultural Science, School of Agriculture, Shiraz University, Shiraz, Iran (52°32′E and 29°36′N, 1810 m) from March to November 2018. Three one-year-old uniform *P. vera* L. rootstocks ('Ghazvini', 'Badami' and 'Italyayi') were prepared for the study (Totally 144 pots, 48 for each rootstock). Rootstocks placed in 12-L pots filled with sieved 2-mm sandy-loam soil (Table 1). After 50 days of preculture, the examination started irrigating with 4 levels of saline water: 0.5 (tap water), 4, 12 and 18 dSm^{-1} for 75 days. Each time, plants were irrigated 0.5 l per pot in a three days interval. Salt treatments gradually increased within 14 days to avoid salt induced osmotic shock. Greenhouse conditions: 32/26 °C day/night temperature, average relative humidity of 70% and 14/10 h light/ dark and photosynthetic photon flux density of around 400–500 lmol m^{-2} s^{-1}.

2.2. Glycerol foliar application

Glycerol solutions were prepared using distilled water at three concentrations (0, 30 and 60 mM) using 85% Glycerol (EMSURE® Reag. Ph Eur, Merck, Germany). Pistachio rootstocks were sprayed by Glycerol treatments for 5 times (with 15 days' interval), from 10 days before starting salt stress up to day 50 of salinity period (100 ml/m^2). The spray continued until all leaves were completely covered by Glycerol and the solutions began to run off the leaves (around 20 ml/pot). At the end of the experiment, (75 days after imposing salt stress), plants were harvested and morphophysiological factors in different organs (root, shoot and leaves) were measured (Raoufi et al., 2019).

2.3. Growth parameters

Leaf area, number of new and abscised leaves, height, fresh and dry weight were measured. Leaf area of three expanded leaves were measured using a portable leaf area instrument (CRLA1 Devices Iran) at the end of the experiment. Number of new and abscised leaves were recorded during the 75 days of experiment. Growth height measured by subtracting primary height (cm) of plant (before introducing the treatments) from the final height (cm) at the end of the experiment. Fresh weight was immediately determined after harvesting different parts of the plants using a digital scale, then were dried in an oven at 65 °C for 7 days to measure the dry weight.

2.4. Leaf pigments

The content of chlorophylls and carotenoids were determined according to the method described by Arnon (1949) through measuring the absorbance of DMSO extract at 663, 645 and 470 nm. Leaf chlorophyll index (Fv/Fm) was measured on expanded leaves using fluorimeter (0S-30p model, USA). The youngest fully expanded leaves after 30 min dark adaptation were selected to determine the chlorophyll fluorescence parameters.

2.5. Leaf water potential & stomatal conductance

Leaf water potential and stomatal conductance were measured after 40 and 75 days. Leaf water potential was measured in the 4 youngest and well developed leaves between 10:00 and 12.00 am using a portable pressure chamber device (model 5100A, Santa barbara, CA, USA) according to the method described by Bhusal

Table 1
The physiochemical analysis of soil applied in the present study.

Sand %	Silt %	Clay %	pH	EC (dSm^{-1})	Na (mg/kg)	Ca (mg/kg)	Mg (mg/kg)	P (mg/kg)	K (mg/kg)	N (mg/kg)	FC
50	27	23	7.21	0.81	7.61	75.52	58.74	31.8	178.98	98.87	20%

et al (2019). Stomatal conductance was measured with a leaf porometer (SC-1; Decagan Devices Inc.) between 11:00 and 14:00.

2.6. Leaf mineral analysis and ST value

The sodium (Na^+) and potassium (K^+) contents in the leaves, shoots and roots were determined using a flame photometer (Model Jenway PFP7, UK). The oven dried samples (0.5 g) were ashed in a muffle oven for 6 h at 550 °C. The resulted ashes were extracted with HCl and diluted with deionized water before using flame photometer (Momenpour and Imani, 2018). Selective transport or ST value describes the plant ability to transport K^+ over Na^+ from roots to the leaves and is calculated by dividing the ratio of K^+/Na^+ in leaves to K^+/Na^+ ratio in the roots (Akbari et al., 2018).

2.7. Statistical analysis

This study was conducted as a factorial experiment with three factors based on completely randomized design (CRD) with four replications. The first factor was rootstock ('Ghazvini', 'Badami' and 'Italyayi'), the second factor was Glycerol in three concentrations (0, 30 and 60 mM) and the third factor was salinity in four levels (control (0.5), 4, 8 and 12 dSm^{-1}). Statistical analysis was performed using the SAS (version 9.01, SAS Institute, Cary, NC) and Duncan's multiple range test was applied for mean separation ($p \leq 0.05$). Principal component analysis (PCA) was performed using MINITAB software (version 19). Pearson correlation analysis was performed using R Project software (for Statistical Computing, 2014).

3. Results

3.1. Growth parameters

Salinity treatments significantly affected the fresh and dry weight of pistachio rootstocks (Tables 1a and 1b). Fresh and dry weight of leaves, shoots and roots were decreased by increasing the salinity level from 0.5 to 12 dSm^{-1}. Overall, maximum fresh and dry weight were observed in treatments with 30 or 60 mM Glycerol irrigated with control (0.5 dsm^{-1}) and the minimum fresh and dry weight observed in treatments with 0 mM Glycerol irrigated with 12 dSm^{-1} water. Based on the results, the maximum fresh weight in leaves and fresh and dry weight in the shoots and roots were belonged to 'Badami' rootstocks (30, 60 mM Glycerol, 0.5 dSm^{-1}). The minimum leaf and root dry weight were observed in 'Badami' while minimumshoot dry weight was observed in 'Italyayi' rootstock. Foliar application of Glycerol increased fresh and dry weight of pistachio rootstocks under salinity stress compared to control. 'Badami' showed the highest increase percentage regarding fresh and dry weight of leaf (195 and 176%, respectively) and shoot (66 and 57%, respectively) after exposing to 60 mM Glycerol compared to control (0 mM) at 12 dSm^{-1}. While the maximum increase of root fresh weight and dry weight was observed in 'Ghazvini' with 25 and 28% increase at 60 mM Glycerol and salinity of 12 dSm^{-1} in comparison to control (Tables 1a and 1b).

Salt stress significantly decreased the leaf area (Fig. 1a) and the number of grown leaves during stress (Fig. 1b). The minimum leaf area (18.5 cm^2) and number of grown leaves under salt stress were

Table 1a
The combined effects of glycerol and salinity on fresh and dry weight in different organs (leaf, shoot and root) of three studied pistachio rootstocks.

Rootstock	Gly (mM)	Salinity (dSm^{-1})	LFW (g)	LDW (g)	SFW (g)	SDW (g)	RFW (g)	RDW (g)
Gh.	0	0.5	17.75 ab	9.54 a	43.39 a-d	23.49 b-h	50.72 a-c	16.85 c-j
		4	7.43 h-n	4.38 d-h	31.61 e-j	23.65 b-h	39.84 e-k	18.60 b-g
		8	7.22 h-n	4.47 d-h	36.68 d-g	24.84 b-f	46.97 b-f	19.91 b-e
		12	6.8275 i-n	3.86.e-i	22.24 jk	17.85 e-k	33.61 k	12.47 kl
	30	0.5	17.26 a-c	9.32 a	49.25 a-c	28.22 a-c	53.31 ab	21.48 ab
		4	8.53 g-l	4.42 d-h	36.67 d-g	20.05 d-k	41.51 d-k	18.04 b-h
		8	7.13 h-n	3.76 e-i	37.37 d-g	22.59 b-j	40.05 e-k	17.38 b-i
		12	5.02 m-n	2.75 hi	21.80 jk	15.74 h-k	41.27 d-k	14.13 h-l
	60	0.5	18.73 a	9.81 a	49.90 ab	30.65 ab	47.23 b-e	20.20 a-d
		4	9.99 e-k	5.35 c-f	36.80 d-g	19.64 d-k	36.81 h-k	18.94 b-f
		8	10.56 e-i	5.71 c-e	38.10 d-g	23.15 b-i	48.36 a-d	20.31 a-c
		12	8.34 g-l	4.46 d-h	25.26 h-k	16.81 f-k	42.27 d-j	16.01 e-k
Ba.	0	0.5	19.93 a	10.26 a	43.91 a-d	25.88 a-e	43.86 c-h	17.64 b-i
		4	8.33 g-l	4.42 d-h	34.46 d-h	18.98 e-k	42.22 d-j	16.47 c-k
		8	6.15 k-n	3.20 g-i	40.91 a-f	20.23 c-k	23.08 l	11.17 l
		12	3.99 n	2.21 i	21.21 jk	13.85 k	34.69 jk	12.36 kl
	30	0.5	17.27 a-c	8.47 ab	38.92 c-g	30.47 ab	41.76 d-j	16.03 e-k
		4	9.76 e-k	4.95 c-g	50.48 a	27.28 a-d	43.12 c-h	18.23 b-h
		8	7.28 h-l	4.45 d-h	41.79 a-e	22.04 c-j	43.10 c-i	15.40 f-k
		12	6.82 i-n	3.71 e-i	30.14 f-k	20.64 c-k	38.52 j-k	14.66 g-l
	60	0.5	20.32 a	10.48 a	49.24 a-c	33.90 a	56.25 a	24.23 a
		4	9.36 f-l	4.95 c-g	38.37 c-g	24.05 b-g	45.00 c-g	19.21 b-f
		8	10.33 e-j	5.44 c-f	37.31 d-g	20.21 c-k	38.45 g-k	16.27 c-k
		12	11.79 e-g	6.16 cd	35.15 d-h	21.39 c-k	36.26 h-k	15.41 f-k
It.	0	0.5	18.90 a	9.36 a	39.44 b-g	22.33 c-j	34.74 jk	13.72 i-l
		4	13.45 c-e	6.69 c	29.93 g-k	18.09 e-k	39.00 f-k	13.02 j-l
		8	5.41 l-n	3.26 g-i	31.51 e-j	17.24 f-k	42.97 c-i	15.34 f-k
		12	3.88 n	2.41 hi	20.35 k	13.64 k	34.52 jk	13.62 i-l
	30	0.5	20.10 a	10.54 a	38.76 c-g	20.65 c-k	34.99 i-k	13.90 i-l
		4	13.05 d-f	6.70 c	36.87 d-g	23.42 b-h	48.23 a-d	17.10 c-j
		8	10.95 e-h	5.69 c-e	30.35 f-k	16.07 g-k	39.72 e-k	14.77 g-l
		12	6.50 j-n	3.45 f-i	23.50 i-k	15.31 i-k	36.54 h-k	14.50 g-l
	60	0.5	19.75 a	9.85 a	38.90 c-g	17.94 e-k	40.76 d-k	14.38 h-l
		4	14.07 b-c	7.00 bc	33.55 d-i	17.28 f-k	43.41 c-h	17.46 b-i
		8	13.63 c-e	6.80 bc	28.70 g-k	19.85 d-k	39.74 e-k	16.14 d-k
		12	8.35 g-l	4.36 d-g	22.42 jk	14.88 jk	42.47 d-j	15.50 f-k

Gh: 'Ghazvini'; Ba: 'Badami'; It: 'Italyayi'; Gly: glycerol; LFW: leaf fresh weight; LDW: leaf dry weight; SFW: shoot fresh weight; RFW: root fresh weight; RDW: root dry weight.

Table 1b
The effects of glycerol and salinity on fresh and dry weight; in different organs (leaf, shoot and root) of pistachio rootstocks.

		LFW (g)	LDW (g)	SFW (g)	SDW (g)	RFW (g)	RDW (g)
Rootstock	Gh	10.40 B	5.65 B	35.75 A	22.22 A	43.50 A	17.86 A
	Ba	10.94 B	5.75 AB	38.49 A	23.24 A	40.53 B	16.42 B
	It	12.33 A	6.34 A	31.19 B	18.06 B	39.76 B	14.95 C
Gly (mM)	0	9.94 B	5.34 B	32.97 B	20.00 A	38.85 B	15.10 C
	30	10.80 B	5.72 B	36.32 A	21.87 A	41.84 A	16.30 B
	60	12.93 A	6.70 A	36.14 A	21.64 A	43.08 A	17.84 A
Salinity (dSm^{-1})	0.5	18.89 A	9.78 A	43.52 A	25.95 A	44.89 A	17.60 A
	4	10.44 B	5.43 B	36.53 B	21.38 B	42.13 B	17.45 A
	8	8.74 C	4.75 B	35.86 B	20.69 B	40.27 BC	16.30 A
	12	6.83 D	3.71 C	24.67 C	16.68 C	37.79 C	14.30 B

Gh: 'Ghazvini'; Ba: 'Badami'; It: 'Italyayi'; Gly: glycerol; LFW: leaf fresh weight; LDW: leaf dry weight; SFW: shoot fresh weight; RFW: root fresh weight; RDW: root dry weight.

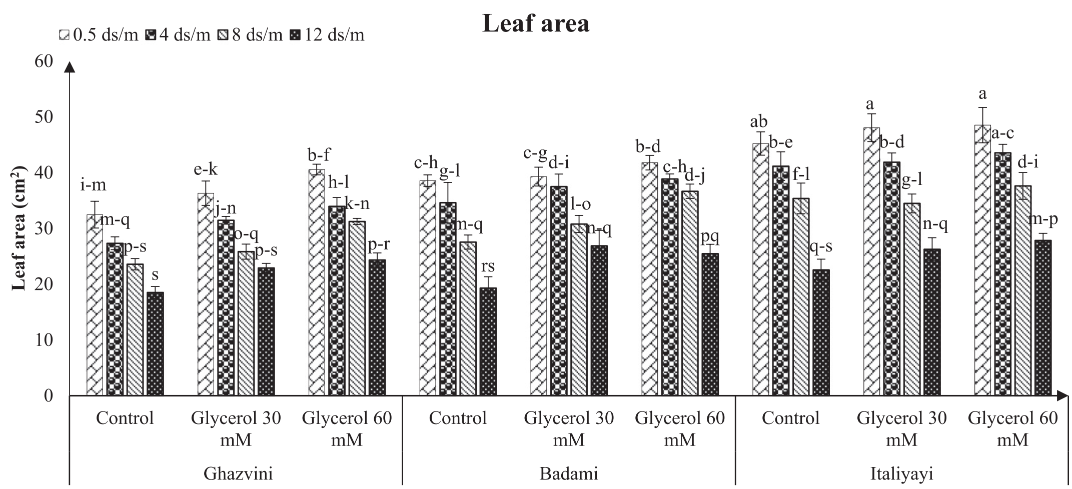

Fig. 1a. The effects of Glycerol and salinity on leaf area in three studied pistachio rootstocks.

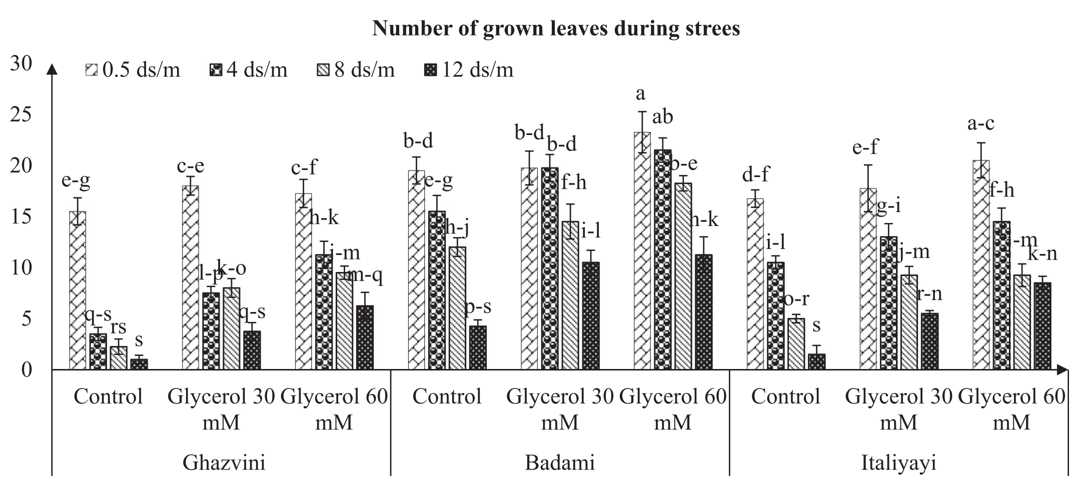

Fig. 1b. The effects of Glycerol and salinity on number of grown leaves during stress in three studied pistachio rootstocks.

observed in 'Ghazvini' at control Glycerol treatment and 12 dSm^{-1} salinity level. However, the application of Glycerol to the salt stressed pistachio rootstocks significantly increased the leaf area and the number of grown leaves in all studied rootstocks. The highest leaf area increase percentage and number of grown leaves were observed in 'Italyayi' (46.6%) and 'Badami' (32%) rootstocks, respectively, exposed to 60 mM Glycerol treatment and salinity level of 12 dSm^{-1} compared to their control at the same salinity level (Figs. 1a and 1b). The number of abscised leaves significantly increased by increasing salinity stress from 0.5 to 12 dSm^{-1} in all studied rootstocks (Fig. 1c). Maximum abscised leaves were observed in 'Ghazvini' in control Glycerol treatment and 12 dSm^{-1} salinity level. However, Glycerol application significantly decreased the number of fallen leaves in under salt stress pistachio

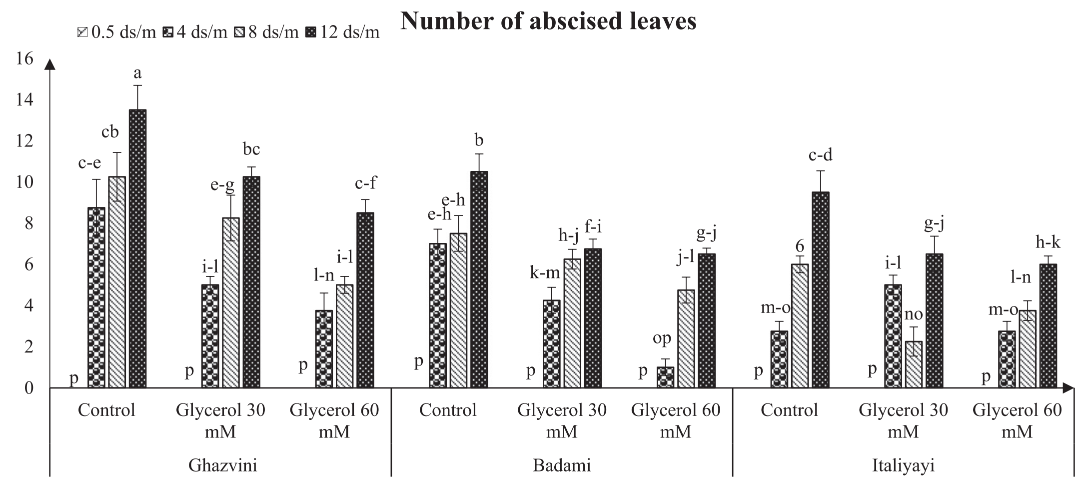

Fig. 1c. The effects of Glycerol and salinity on number of abscised leaves in three studied pistachio rootstocks.

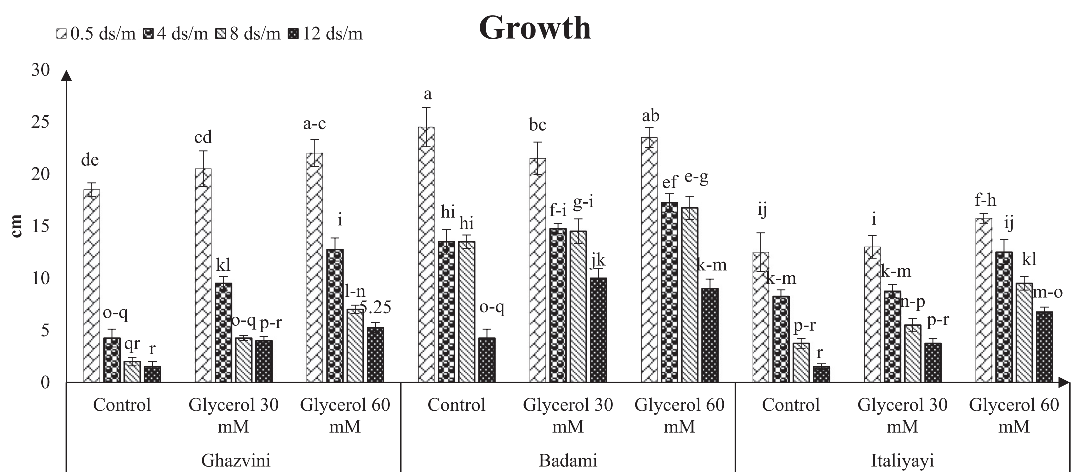

Fig. 2. The effects of Glycerol and salinity on growth (height) in three studied pistachio rootstocks.

rootstocks. The maximum abscised leaves were observed in 'Badami' at 60 mM (12 dSm^{-1}) compared to the control at the same salinity level.

Overall, Glycerol showed higher effects on leaf area than number of leaves (Fig. 1a–c). Fig. 2 demonstrates the result of salinity and Glycerol foliar application on the growth of studied rootstocks as is obvious in the Fig. 2, salt stress significantly decreased the growth of pistachio rootstocks. However, exogenous application of Glycerol has significantly alleviated the growth of stressed pistachio rootstocks. At highest salinity level (12 dSm^{-1}), the growth of 'Ghazvini' rootstocks treated with 60 mM Glycerol increased 4.5-fold compared to control (Fig. 2).

3.2. Chlorophylls, carotenoid and chlorophyll index

The highest content of chlorophyll *a*, chlorophyll *b*, and total chlorophyll observed in control plants and increasing salinity reduced the chlorophyll content of the studied rootstocks (Tables 2a and 2b). Glycerol application significantly increased the chlorophyll content under saline conditions. The maximum increase percentage in chlorophyll *a* (51%), chlorophyll *b* (53%), and total chlorophyll (36%) were observed in 'Ghazvini' and 'Italyayi', in 60 mM Glycerol under 12 dSm^{-1} salinity. Salinity stress decreased the carotenoid content of the studied rootstocks and applying Glycerol did not have any significant effects on carotenoid content. It worth noting that the maximum chlorophyll and carotenoid content was observed in 'Badami' rootstock exposed to 30, 60 mM Glycerol (Tables 2a and 2b). Salinity stress reduced the chlorophyll content at 12 dSm^{-1}. However, foliar application of Glycerol at 30 and 60 mM slightly increased the chlorophyll content compared to the control. The chlorophyll index in 'Italyayi' exposed to 60 mM Glycerol and salinity of 12 dSm^{-1} was higher than other rootstocks at the same condition (Fig. 3a). As is shown in Fig. 4b, the maximum damage due to salt toxicity was observed non-treated Glycerol plants. Whereas application of Glycerol (especially at the concentration of 60 mM) reduced the typical symptoms of salt toxicity (Fig. 3b).

3.3. Stomatal conductance and leaf water potential

Based on the obtained results, by increasing the salinity levels, stomatal conductance reduced in all studied rootstocks at both 40 and 75 days after starting the salt stress treatments (Tables 3a and 3b. Glycerol application at the rate of 60 mM could increase stomatal conductance in salinized plants, this increment was different among rootstocks and measurement time.

At the first time of measurement (40 days), the stomatal conductance of 'Ghazvini' and 'Badami' increased in response to

Table 2a
The combined effects of glycerol and salinity on chlorophylls and carotenoid content in three studied pistachio rootstocks.

Rootstock	Gly (mM)	Salinity (dSm^{-1})	Chl a (mg/gFw)	Chl b (mg/gFw)	Chl t (mg/gFw)	Car (mg/gFw)
Gh	0	0.5	1.568 b-j	0.261 b-e	1.830 c-i	3.386 b-h
		4	1.372 g-l	0.245 c-h	1.617 h-m	3.540 b-h
		8	1.004 l-o	0.240 d-h	1.244 no	3.114 f-h
		12	0.689 o	0.162 i	0.851 p	2.889 h
	30	0.5	1.561 b-j	0.273 a-e	1.835 c-i	3.421 b-h
		4	1.457 f-k	0.270 a-e	1.727 f-k	3.566 b-h
		8	1.196 j-n	0.255 b-g	1.452 j-n	3.227 e-h
		12	0.846 no	0.182 f-i	1.028 op	3.234 d-h
	60	0.5	1.595 b-i	0.339 a	1.935 b-h	3.937 a-c
		4	1.503 d-k	0.306 a-d	1.810 c-j	3.572 a-g
		8	1.225 i-m	0.265 a-e	1.490 i-n	3.469 b-h
		12	1.042 l-o	0.196 e-i	1.238 no	2.921 gh
Ba	0	0.5	1.550 b-j	0.297 a-d	1.847 b-i	3.639 a-f
		4	1.510 c-k	0.286 a-d	1.797 d-j	3.910 a-d
		8	1.153 k-n	0.261 b-e	1.414 k-n	3.596 a-g
		12	0.853 m-o	0.150 i	1.003 op	3.652 a-f
	30	0.5	1.497 e-k	0.271 a-e	1.768 e-k	3.691 a-f
		4	1.666 a-h	0.262 a-e	1.928 b-h	3.971 ab
		8	1.368 h-l	0.298 a-d	1.667 g-l	3.466 b-h
		12	1.139 k-n	0.156 i	1.296 m-o	3.614 b-f
	60	0.5	1.874 a-d	0.293 a-d	2.168 a-c	3.879 a-e
		4	1.724 a-h	0.289 a-d	2.014 a-g	3.538 b-h
		8	1.483 e-k	0.257 d-e	1.740 f-k	3.523 b-h
		12	1.164 k-n	0.179 g-i	1.343 l-o	3.736 b-f
It	0	0.5	1.725 a-h	0.295 a-d	2.020 a-g	3.667 a-f
		4	1.649 a-h	0.314 a-d	1.963 b-h	3.698 a-f
		8	1.578 b-i	0.264 a-e	1.843 c-i	3.941 a-c
		12	1.023 l-o	0.171 hi	1.195 n-p	3.402 b-h
	30	0.5	1.915 ab	0.300 a-d	2.216 ab	3.561 b-h
		4	1.843 a-e	0.321 a-c	2.164 a-d	3.691 a-f
		8	1.766 a-f	0.271 a-e	2.037 a-f	4.251 a
		12	1.236 i-l	0.246 c-h	1.438 i-n	3.084 f-h
	60	0.5	2.011 a	0.332 ab	2.341 a	3.744 a-f
		4	1.883 a-c	0.249 c-g	2.133 a-e	3.658 a-f
		8	1.748 a-g	0.269 a-e	2.018 a-g	3.320 b-h
		12	1.371 h-l	0.263 a-e	1.634 h-m	3.274 c-h

Gh: 'Ghazvini'; Ba: 'Badami'; It: 'Italyayi'; Gly: glycerol; Chl: Chlorophyll; Car: carotenoid.

Table 2b
The effects of Glycerol and salinity on chlorophylls and carotenoid content in three different studied pistachio rootstocks.

		Ch a (mg/gFw)	Ch b (mg/gFw)	Ch t (mg/gFw)	Car (mg/gFw)
Rootstock	Gh	1.25 C	0.250 B	1.505 C	3.356 B
	Ba	1.41 B	0.250 B	1.665 B	3.685 A
	It	1.64 A	0.274 A	1.921 A	3.607 A
Gly (mM)	0	1.306 B	0.245 B	1.552 B	3.536 A
	30	1.458 A	0.259 AB	1.717 A	3.565 A
	60	1.552 A	0.270 A	1.822 A	3.548 A
Salinity (dSm^{-1})	0.5	1.700 A	0.296 A	1.996 A	3.658 A
	4	1.623 A	0.282 AB	1.906 A	3.683 A
	8	1.391 B	0.265 B	1.656 B	3.545 A
	12	1.040 C	0.189 C	1.230 C	3.312 B

Gh: 'Ghazvini'; Ba: 'Badami'; It: 'Italyayi'; Gly: glycerol; Chl: Chlorophyll; Car: carotenoid.

60 mM Glycerol at the salinity level of 12 dSm^{-1} while at the second time (75 days), Glycerol application (60 mM) could increase stomatal conductance of all rootstocks (Tables 3a and 3b). By increasing salinity level, leaf water potential decreased, however this reduction was only significant at 12 dSm^{-1} salinity level and Glycerol application had no significant effects at both measured times (Tables 4a and 4b).

3.4. Mineral concentration

According to the results, salinity significantly increased the Na^+ content in the leaves, shoots and roots (Tables 4a and 4b). At the highest salinity level with no Glycerol application, the maximum concentration of Na^+ in leaves were observed in 'Ghazvini' rootstock, whereas the maximum Na^+ concentration in the shoots and roots were observed in 'Italyayi'. Foliar Glycerol application significantly decreased the Na^+ concentration under salinity stress. Glycerol application at 60 mM and 12 dSm^{-1} could significantly reduce the Na^+ concentration in the leaves, shoots and roots of 'Ghazvini' and 'Badami' rootstocks. The effects of Glycerol application on reducing Na^+ concentration in the leaves were higher than shoots and roots.

On the contrary, by increasing salinity levels, K^+ content significantly decreased. The highest content of K^+ was observed in the

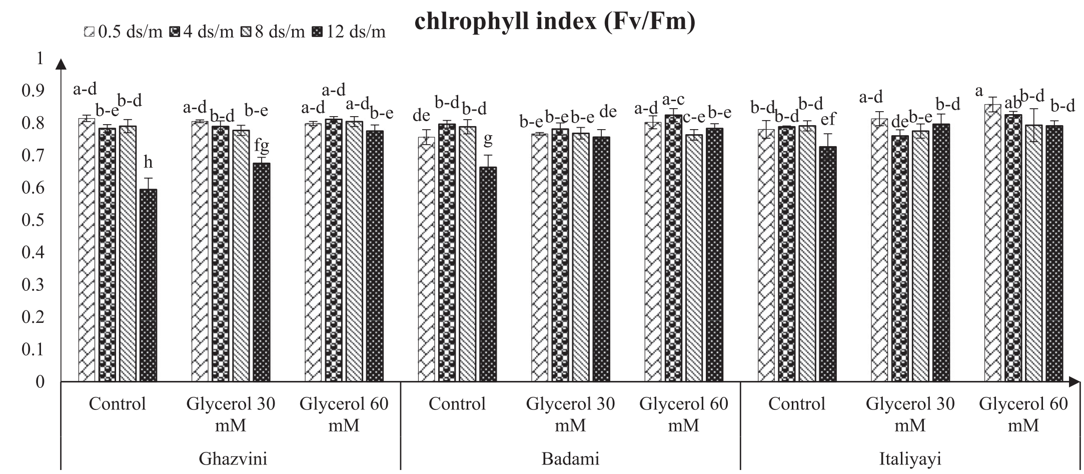

Fig. 3a. The effects of Glycerol and salinity on chlorophyll index in three studied pistachio rootstocks.

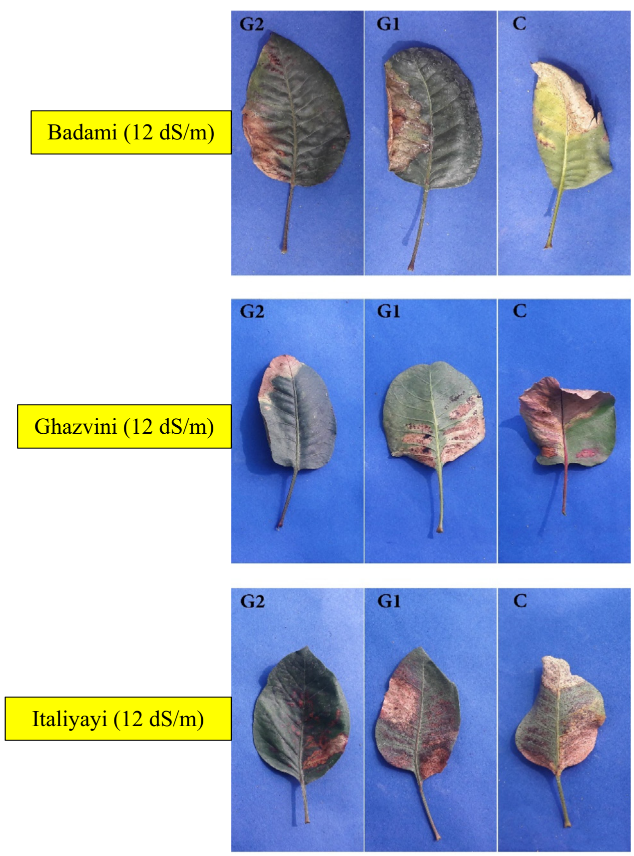

Fig. 3b. The effects of different Glycerol concentrations (C: control, G1: 30 mM, G2:60 mM) on leaf chlorophyll in three studied pistachio rootstocks at 12 dSm⁻.

shoots and roots of 'Italyayi' rootstocks. Glycerol application maintained higher K^+ in comparison to control plants at the same salinity levels. The maximum K^+ content in the leaves were observed in 'Italyayi' whereas the maximum K^+ content in the shoots and roots were observed in 'Ghazvini'. Overall, K^+ concentration in the roots were much more affected by Glycerol application than leaves and shoots (Tables 4a and 4b).

As stated before, ST-value is the ability of plants in transmitting K^+ over Na^+ from the plants' root to the leaf. According to Fig. 4, exogenous application of Glycerol did not have any significant effects on ST-value of pistachio rootstocks under saline condition. However, at control salinity level, the ST value of 'Badami' significantly increased by applying 60 mM Glycerol (Fig. 4).

3.5. PCA and correlation analysis

Principal components analysis (PCA) was performed to visualize the grouping of Glycerol-salinity treatments (Fig. 5a and b) and rootstocks-salinity treatments (Fig. 5c and d) as well as the relation of the measured factors with principal components. Eigenvalues greater than 1 was selected to extract the main principal components. PCA score plot (Fig. 5a) and biplot (Fig. 5b) of Glycerol-salinity treatments generated the first two component using 26 variables where the first and second component explained, 79.2 and 7.7% of the total variance (86.9%) respectively. Salt stressed plants at the level of 0.5 and 4 dSm^{-1} were located at the right side of the plot (black and green ellipse, respectively), while those at 8 and 12 dSm^{-1} were located at the left side (red and blue ellipse, respectively) of the plot. The first component had strong, positive correlation with the treatment of 60 mM Glycerol at the salinity level of 0.5 dSm^{-1} may be explained by the high values of shoot and root fresh and dry weight, K^+ and pigments concentration and other variables that are co-located in this region of the PC space while it was negatively correlated with the control treatment (0 mM) at 12 dSm^{-1}. Moreover, this treatment is the only one located at the lower left side (blue ellipse) which shows that Glycerol treatments at higher salinity level (8 and 12 dSm^{-1}) had a better performance than control at the same salinity levels (Fig. 5a and b). PCA score plots (Fig. 5c) and biplot (Fig. 5d) of rootstock-salinity treatments generated the first two components using 26 variables where the first and second component explained, respectively, 65.6 and 10.6% of the total variance (76.2%). According to the salinity levels, they were divided into 4 groups including 0.5 dSm^{-1} (black ellipse), 4 dSm^{-1} (green), 8 dSm^{-1} (red) and 12 dSm^{-1}(blue). 'Badami' rootstock at 0.5 dSm^{-1} had higher positive correlation with the first component and at the salinity level of 12 dSm^{-1} had a lower negative correlation with the first component (Fig. 5c). On the other hand, the first component was positively associated with leaf, root and shoot dry weight, leaf water potential, stomatal conductance, K^+ and pigments content (Fig. 5d). which is indicating the higher performance of 'Badami' under saline condition than the other two rootstocks.

Pearson correlation analysis was performed to analyze the correlation between the measured factors (Fig. 6). Based on the

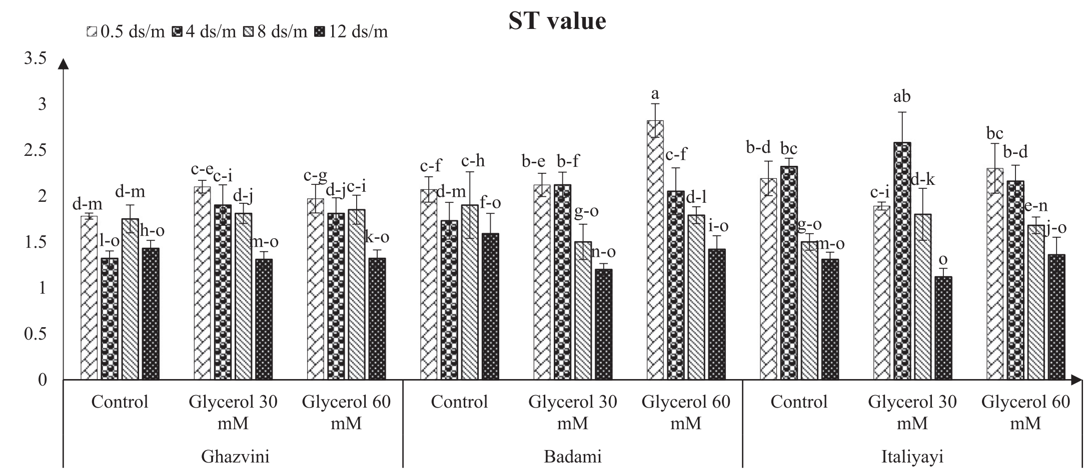

Fig. 4. The effects of Glycerol and salinity on ST value in three studied pistachio rootstocks.

Table 3a
The combined effects of Glycerol and salinity on stomatal conductance and leaf water potential in three studied pistachio rootstocks at days 40 and 75 after imposing salt stress.

Rootstock	Gly (mM)	Salinity (dSm^{-1})	Stomatal conductance (40) ($mmolm^{-2}\ s^{-1}$) 1	Stomatal conductance (75) ($mmolm^{-2}\ s^{-1}$) 2	Leaf water potential (40) (MPa)	Leaf water potential (75) (MPa)
Gh	0	0.5	390.90 bc	375.27 a	−0.925 g-i	−0.925 b-f
		4	318.50 c-g	291.95 b-d	−0.875 e-i	−0.705 a
		8	203.85 j-q	225.20 e-h	−0.900 f-i	−0.875 a-d
		12	161.25 n-q	140.72 i-k	−0.800 b-g	−0.900 a-e
	30	0.5	328.65 c-f	346.12 ab	−1.10 j	−1.000 d-i
		4	277.80 f-k	284.60 b-d	−0.950 hi	−0.975 c-h
		8	153.60 o-q	239.95 e-g	−0.950 hi	−1.075 f-k
		12	227.52 h-o	120.15 k	−0.850 a-e	−0.950 b-g
	60	0.5	470.70 a	322.22 a-c	−1.000 ij	−1.125 h-k
		4	195.12 l-q	195.27 e-k	−0.925 j-i	−1.050 e-j
		8	190.20 l-q	152.80 h-k	−0.775 a-f	−1.000 d-i
		12	245.75 g-m	176.35 e-k	−0.775 a-f	−1.000 d-i
Ba	0	0.5	395.12 a-c	284.85 b-d	−0.825 c-h	−0.900 a-e
		4	327.20 h-n	226.87 e-h	−0.800 b-g	−1.075 f-k
		8	167.72 m-q	162.30 g-k	−0.825 c-h	−1.050 e-j
		12	126.20 q	169.80 f-k	−0.675 ab	−0.825 a-c
	30	0.5	356.67 b-e	251.15 c-e	−0.675 ab	−1.150 i-k
		4	220.40 i-p	162.27 g-k	−0.850 d-h	−0.900 a-e
		8	190.22 l-q	219.42 e-i	−0.675 ab	−1.225 k
		12	141.22 q	133.12 jk	−0.800 b-g	−0.975 c-h
	60	0.5	251.82 f-l	213.50 e-i	−0.700 ac	−1.025 d-i
		4	234.15 h-n	166.12 f-k	−0.825 c-h	−1.025 d-i
		8	137.02 q	222.87 e-h	−0.725 a-d	−1.025 d-i
		12	147.30 pq	202.25 e-j	−0.850 d-h	−1.125 h-k
It	0	0.5	432.35 ab	243.75 c-f	−0.825 c-h	−0.800 ab
		4	294.07 d-i	153.10 h-k	−0.950 hi	−0.975 c-h
		8	277.35 f-k	229.75 e-h	−0.650 a	−0.975 c-h
		12	265.20 f-l	158.60 h-k	−0.850 d-h	−1.200 jk
	30	0.5	364.02 b-d	180.07 e-k	−0.875 e-i	−0.925 b-f
		4	226.10 i-o	225.40 e-h	−0.775 a-f	−0.875 a-d
		8	279.72 e-j	222.05 e-h	−0.671 ab	−1.100 g-k
		12	199.52 k-q	151.80 h-k	−0.700 a-c	−1.125 h-k
	60	0.5	419.42 ab	191.17 e-k	−0.850 d-h	−0.875 a-d
		4	304.70 d-h	140.42 i-k	−0.725 a-d	−1.125 h-k
		8	267.40 f-l	182.55 e-k	−0.725 a-d	−1.200 jk
		12	173.07 m-q	178.05 e-k	−0.800 b-g	−1.450 l

Gh: 'Ghazvini'; Ba: 'Badami'; It: 'Italyayi'; Gly: Glycerol.

results, number of abscised leaf had a positive correlation with Na^+ content in the leaves and shoots and had a highly negative correlation with chlorophyll *a* and total chlorophyll. Stomatal conductance had a negative correlation with shoot Na^+. ST value was negatively correlated with Na^+ content of shoot and leaf. However, no significant correlation was observed between leaf water potential and carotenoid content with other factors (Fig. 6).

4. Discussion

According to the obtained results in this study, salt stress significantly reduced the growth and growth related characteristics in studied pistachio rootstocks. In accordance to our findings Decreasing biomass, leaf area, leaf number, growth and increasing abscised leaf during salt stress in *Pistacia* sp. have been previously

Table 3b
The effects of Glycerol and salinity on stomatal conductance and leaf water potential on three studied pistachio rootstocks at days 40 and 75 after imposing salt stress.

		Stomatal conductance (40) (mmolm^{-2} s^{-1}) 1	Stomatal conductance (75) (mmolm^{-2} s^{-1})	Leaf water potential (40) (MPa)	Leaf water potential (75) (MPa)
rootstock	Gh	263.65 B	239.22 A	−0.893 B	−0.968 A
	Ba	217.09 C	201.21 B	−0.768 A	−1.025 B
	It	291.91 A	188.06 B	−0.783 A	−1.052 B
Gly (mM)	0	272.48 A	221.85 A	−0.825 A	−0.937 A
	30	247.12 B	211.34 AB	−0.814 A	−1.022 B
	60	253.06 AB	195.30 B	−0.806 A	−1.085 C
Salinity (dSm^{-1})	0.5	378.85 A	267.57 A	−0.863 B	−0.969 A
	4	256.45 B	205.11 B	−0.852 B	−0.972 A
	8	207.46 C	206.32 B	−0.766 A	−1.058 B
	12	187.45 C	158.98 C	−0.777 A	−1.061 B

Gh: 'Ghazvini'; Ba: 'Badami'; It: 'Italyayi'; Gly: Glycerol.

Table 4a
The combined effects of Glycerol and salinity on Na^+ and K^+ content in different organs (leaf, shoot and root) of three studied pistachio rootstocks.

Rootstock	Gly (mM)	Salinity (dSm^{-1})	Na^+ (leaf) %	Na^+ (shoot) %	Na^+ (root) %	K^+ (leaf) %	K^+ (shoot) %	K^+ (root) %
Gh	0	0.5	0.353 n-q	0.321 j	0.440 r	0.756 b-g	0.620 a-c	0.528 a-c
		4	0.650 f-i	0.507 h	0.499 l-p	0.735 b-h	0.585 b-h	0.429 g-k
		8	0.649 f-j	0.611 e-g	0.634 c-e	0.730 c-h	0.524 j-l	0.413 i-m
		12	0.824 a	0.649 c-f	0.680 a-c	0.523 p	0.460 m	0.302 o
	30	0.5	0.350 o-q	0.336 ij	0.483 n-r	0.719 d-j	0.580 b-i	0.472 c-h
		4	0.465 lm	0.507 h	0.516 l-n	0.789 a-d	0.611 a-c	0.474 c-h
		8	0.577 jk	0.606 fg	0.621 d-g	0.777 a-e	0.540 f-l	0.464 d-i
		12	0.743 b-d	0.672 b-d	0.593 e-h	0.629 k-h	0.545 e-k	0.387 k-m
	60	0.5	0.335 p-r	0.356 ij	0.465 o-r	0.787 a-d	0.587 b-h	0.561 a
		4	0.409 l-o	0.476 h	0.499 l-p	0.747 b-g	0.599 a-f	0.509 a-e
		8	0.478 l	0.588 g	0.585 f-i	0.709 d-k	0.578 b-j	0.472 c-h
		12	0.684 d-g	0.662 b-d	0.590 e-h	0.646 i-m	0.550 d-k	0.424 h-l
Ba	0	0.5	0.299 q-s	0.369 i	0.504 l-o	0.681 g-l	0.618 a-c	0.559 a
		4	0.424 l-n	0.489 h	0.529 k-n	0.589 m-p	0.561 c-k	0.431 g-k
		8	0.366 e-h	0.611 e-g	0.685 ab	0.714 d-j	0.521 j-l	0.410 i-m
		12	0.791 ab	0.695 a-c	0.672 a-c	0.561 n-p	0.505 k-m	0.309 no
	30	0.5	0.274 rs	0.359 ij	0.440 r	0.721 d-i	0.620 a-c	0.552 ab
		4	0.343 o-r	0.484 h	0.534 j-m	0.709 d-k	0.576 b-j	0.521 a-d
		8	0.631 g-k	0.576 g	0.499 l-p	0.749 b-g	0.564 c-k	0.401 j-m
		12	0.659 e-i	0.678 b-d	0.578 f-j	0.608 l-o	0.524 j-l	0.441 f-k
	60	0.5	0.256 s	0.374 i	0.440 r	0.713 a-c	0.627 ab	0.497 b-f
		4	0.365 n-q	0.474 h	0.511 l-o	0.693 f-k	0.597 a-g	0.481 c-h
		8	0.574 k	0.614 e-g	0.539 i-l	0.858 a	0.524 i-l	0.450 f-j
		12	0.659 e-i	0.639 d-f	0.529 k-n	0.636 j-n	0.569 b-j	0.366 mn
It	0	0.5	0.340 o-r	0.366 ij	0.453 p-r	0.787 a-d	0.602 a-e	0.483 c-g
		4	0.470 lm	0.479 h	0.624 d-f	0.813 a-c	0.546 e-k	0.462 e-i
		8	0.715 c-f	0.614 e-g	0.649 b-d	0.695 e-k	0.519 j-m	0.427 g-l
		12	0.773 a-c	0.731 a	0.703 a	0.528 op	0.482 lm	0.370 lm
	30	0.5	0.325 q-s	0.372 i	0.445 qr	0.768 b-f	0.609 a-d	0.559 a
		4	0.406 l-p	0.502 h	0.616 d-h	0.817 ab	0.599 a-f	0.497 b-f
		8	0.590 i-k	0.581 g	0.573 h-k	0.813 a-c	0.580 b-i	0.450 b-j
		12	0.725 b-f	0.655 b-f	0.649 b-d	0.596 m-p	0.538 g-l	0.483 c-g
	60	0.5	0.317 q-s	0.356 ij	0.488 m-q	0.808 a-c	0.653 a	0.552 ab
		4	0.401 m-p	0.476 h	0.575 g-k	0.662 h-m	0.613 a-c	0.446 f-j
		8	0.605 h-k	0.606 fg	0.575 g-k	0.730 c-h	0.564 c-k	0.413 i-m
		12	0.728 b-f	0.698 ab	0.654 b-d	0.660 h-m	0.533 h-l	0.448 f-j

Gh: 'Ghazvini'; Ba: 'Badami'; It: 'Italyayi'; Gly: Glycerol.

reported by other authors (Karimi and Sadeghi-Seresht, 2018; Raoufi et al., 2019).

Reducing biomass under saline condition could be attributed to the reduction of photosynthesis activity. In addition, decreased water uptake due to the enhanced osmotic potential increases the negative effects on growth (Bastam et al., 2013). Decreasing leaf area under saline condition is generally attributable to the limited cell expansion and division, which is a result from reduced available water (Karimi and Sadeghi-Seresht, 2018; Raoufi et al., 2019).

Adish et al (2010) demonstrated that accumulation of toxic ions such as Cl^- and Na^+ in addition to nutrient imbalance occurred under saline condition, causes leaf senescence, abscission and deceases the leaf number in pistachio rootstocks.

In the current study, a highly negative correlation between Na^+ content and leaf number were observed in pistachio rootstocks. Foliar application of Glycerol significantly increased the growth and growth related parameters under salinity condition. These results are in line with Tisserat and Stuff (2011) who reported that the foliar application of Glycerol stimulated the growth parameters in corn, carrot and spearmint. In another study, Kaya et al (2013) stated that foliar application of Glycerol improved the growth of salt-stressed maize plants under greenhouse condition. This might be due to the effect of Glycerol on photosynthesis activity by

Table 4b
The effects of Glycerol and salinity on Na^{+} and K^{+} content of different organs (leaf, shoot and root) in three studied pistachio rootstocks.

		Na^{+} (leaf) %	Na^{+} (shoot) %	Na^{+} (root) %	K^{+} (leaf) %	K^{+} (shoot) %	K^{+} (root) %
Rootstock	Gh	0.543 A	0.524 A	0.550 B	0.712 AB	0.565 A	0.453 A
	Ba	0.495 B	0.530 A	0.538 B	0.694 B	0.567 A	0.451 A
	It	0.533 A	0.536 A	0.584 A	0.726 A	0.570 A	0.466 A
Gly (mM)	0	0.579 A	0.537 A	0.589 A	0.676 B	0.546 B	0.427 B
	30	0.507 B	0.527 A	0.546 B	0.725 A	0.574 A	0.475 A
	60	0.484 C	0.527 A	0.538 B	0.729 A	0.583 A	0.468 A
Salinity (dSm^{-1})	0.5	0.316 D	0.357 D	0.462 D	0.760 A	0.613 A	0.529 A
	4	0.437 C	0.488 C	0.545 C	0.753 AB	0.588 B	0.472 B
	8	0.609 B	0.601 B	0.595 B	0.728 B	0.546 C	0.433 C
	12	0.732 A	0.676 A	0.627 A	0.599 C	0.523 D	0.392 D

Gh: 'Ghazvini'; Ba: 'Badami'; It: 'Italyayi'; Gly: Glycerol.

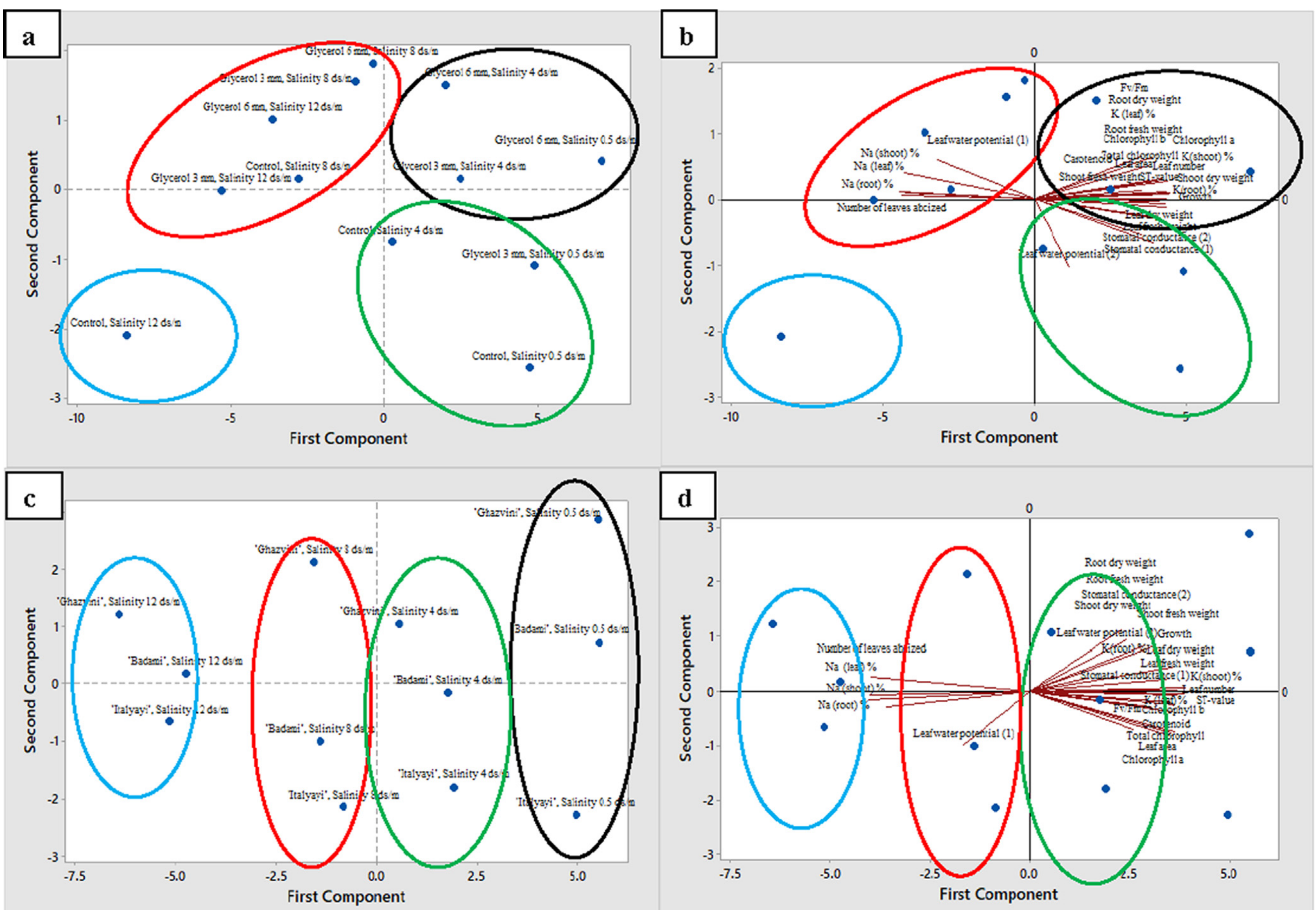

Fig. 5. Principal components analysis (PCA) of Glycerol-salinity treatments (figure (a) [score plot] and (b) [biplot]) and Rootstock-salinity treatments (figure (c) [score plot] and d [biplot]) in three studied pistachio rootstocks.

protecting chlorophylls from the negative impact of salinity stress such as reactive oxygen species (ROS) (Eastmond, 2004). In the current study, chlorophylls content of salinized pistachio rootstocks significantly increased by Glycerol application particularly at the rate of 60 mM. Biosynthesis of new chlorophylls during stress situation might cause a higher growth, cell division and expansion during this condition. Moreover, exogenous application of 60 mM Glycerol significantly decreased the sodium concentration of areal parts of plants resulted in remaining higher leaf number as well as decreasing the number of abscised leaves. The increment of chlorophylls pigment along with the reduction of sodium concentration in pistachio suggested the potential role of Glycerol in increasing leaf number and area, biomass and growth of salinized pistachio rootstocks. In addition, increasing root biomass under saline condition as a result of Glycerol application, could assist the plants to absorb more water which in turn increases the growth rate.

Consistent with our results, Kaya et al (2013) observed a notable increase in chlorophylls content and a significant decrease in leaf sodium concentration in salinized maize plants sprayed by Glycerol.

According to the obtained results, chlorophylls, carotenoid and chlorophyll index in pistachio rootstocks decreased with increasing salinity levels. In keeping to our finding, Mirfattahi et al (2017) and Karimi and Nasrolahpour-Moghadam (2016) also reported chlorophyll and carotenoid decrease in 'Ghazvini' and 'Badami' rootstocks under saline situation. Decreased photosyn-

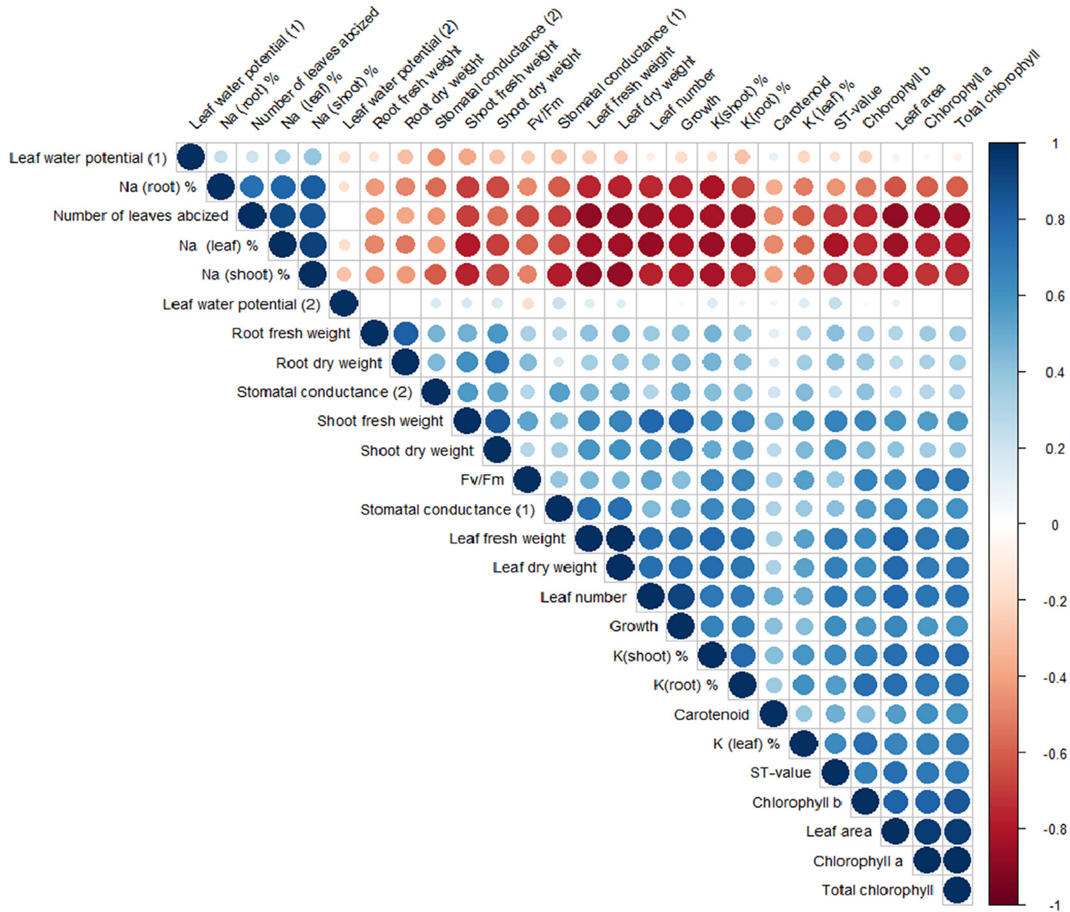

Fig. 6. Correlation analysis between all measured values.

thesis pigments under salinity condition could be attributed to the structural damage to the photosynthetic device and chlorophyll degradation by ROS, produced during stress condition (Akbari et al., 2018; Raoufi et al., 2020). Based on the obtained results, pistachio plants treated with 60 mM Glycerol had a higher content of chlorophylls pigments and index as well as carotenoid in comparison to controls plants. The higher content of pigments in Glycerol treated pistachios might be result of lower ROS content. Kaya et al (2013) observed the substantial decrease in antioxidant enzymes activity in treated plants with Glycerol under salt stress. They suggested that Glycerol might act as an alternative radical scavenger, so that in the presence of antioxidant enzymes its antioxidative activity decrease. In addition, Mohan et al (2000) stated that the higher stability of chlorophyll during salt stress could be used as an index in determining salt tolerant rootstocks. Accordingly, Glycerol application by protecting chlorophylls could help the pistachio rootstocks to better tolerate the saline condition.

In this study, salt stress had a negative impact on stomatal conductance and leaf water potential and both factors significantly reduced by increasing salinity levels. Ahmadi Kouhbanani et al (2016) and Alipour (2018) also reported the same result of salinity stress on stomatal conductance of pistachio rootstocks. Mirfattahi et al (2017) and Karimi et al (2018) reported decreased leaf water potential in 'Ghazvini' and 'Badami' under salinity condition, which is in line with our results. Under saline condition, reduction in stomatal conductance is generally due to the stomata closure which is an early physiological response of plants under desiccation. This response is part of the defense mechanism in plants in order to reserve more water and prevent water loss through the transpiration (Momenpour and Imani, 2018; Alipour, 2018). Reduced leaf water potential is a direct effect of decreased soil water potential due to the higher salt concentration. Bhusal et al (2019) stated that there is a high positive correlation between stomatal conductance and mid-day leaf water potential under the stress condition. Exogenous application of Glycerol caused a slight increase in stomatal conductance at 40 and 75 days after imposing the salt stress while it did not have any effects on the leaf water potential in studied pistachio rootstocks. Although Glycerol application did not have significant effect on stomatal conductance and leaf water potential, it has been demonstrated that Glycerol could act as an osmoprotectant and cell membrane stabilizer which is able to protect cell against environmental stress during desiccation and freezing (Hasegawa et al., 2000). Moreover, it also has been reported that exogenous Glycerol under saline condition could decrease electrolyte leakage (stabilizer role) and increase the proline content (osmoregulation role) in maize (Kaya et al., 2013).

Based on the present results, by increasing salinity level, the Na^+ content in the leaves, stem, and roots of pistachio rootstocks significantly increased, whereas, K^+ content in different organs significantly decreased. These results are parallel with the results reported by Karimi and Sadeghi-Seresht (2018) and Karimi and Nasrolahpour-Moghadam (2016) *Pistacia atlantica* and 'Badami' rootstocks under salt stress, respectively. Under saline condition, due to high NaCl concentration in root environment, Na^+ strongly compete with K^+ because they have similar binding site on root cells membrane. Therefore, the concentration of Na^+ in plant's organs increase while the content of K^+ decrease and leads to K^+ deficiency in plants. Potassium has a crucial role in osmotic adjustment, activation of metabolic enzymes, protein synthesis and stomata movements. Therefore, K^+ deficiency result in limiting growth and tolerance to stress. Plants with a higher ability to uptake and transfer K^+ to the areal parts over Na^+ can better mitigate the adverse effects of salinity (Alipour, 2018; Momenpour and Imani, 2018; Akbari et al., 2018; Raoufi et al., 2020). In the current study, salinized plants treated with Glycerol had a higher K^+ and lower content of Na^+ than control (0 mM). Similarly, Kaya et al (2013) observed that foliar application of Glycerol on salt induced pistachio caused a significant decrease in Na^+ and increase in K^+ content of leaves, shoots and roots in maize. The higher growth rate of Glycerol treated plants in this study could also be related to higher content of K^+ in their organs. It has been shown that foliar application of Glycerol increases the level of Glycerol-3-phosphate through the phosphorylation reaction mediated by Glycerol kinase in plants (Eastmond, 2004). In this regard, it has been proved that Glycerol-3-phosphate has a significant role in activation of plant immune systems under biotic and abiotic stress. It also may act as mobile signaling molecule under stress condition (Zhang et al., 2015; Chanda et al., 2011).

Our results demonstrated that the effects of salt stress and foliar Glycerol application is rootstock dependent and is different among studied rootstocks. However, Glycerol application caused a significant increase in growth parameters in all studied rootstocks under control condition (0.5 dSm^{-1}). Based on the PCA analysis, 'Badami' had a better performance under no saline condition (0.5 dSm^{-1}) as well as highest salinity (12 dSm^{-1}) than two other rootstocks. Moreover, 'Badami' also showed the best response to exogenous Glycerol application. Although 'Italyayi' showed lower growth than 'Ghazvini' at control (0.5 dSm^{-1}) condition, however it had a higher performance than 'Ghazvini' at 12 dSm^{-1}.

5. Conclusion

The data presented here indicate that the salt stress significantly decrease growth indices, photosynthesis pigments, stomatal conductivity, leaf water potential, and K^+ concentration in pistachio rootstocks ('Ghazvini', 'Badami' and 'Italyayi'). However, the foliar application of Glycerol at the rate of 60 mM could improve the growth and enhance the tolerance of pistachio rootstocks by increasing biomass, number of grown leaves during stress, chlorophyll *a*, b and total chlorophyll, leaf area, growth and K^+ concentration in leaves, shoots and roots and decreasing Na^+ concentration. Therefore, Glycerol could be recommended as a cheap, safe and organic agrochemical for alleviating the negative effects of salt stress in pistachio orchards. Among studied rootstocks in the present study, 'Badami' showed the best response to Glycerol application. The authors suggest more future investigation regarding the effects of foliar Glycerol application on pistachio cultivars and the suitable time for its application.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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