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ORIGINAL ARTICLE

Effect of Zinc on the Growth of Two Pistachio (*Pistacia vera* L.) Rootstocks under Drought Stress

Alireza Kermani[1], Azam Jafari[*1], Soheil Karimi[2], Mostafa Shirmardi[1]

[1] *Department of Horticultural Sciences, Faculty of Agriculture & Natural Resources, Ardakan University, Ardakan, Iran*

[2] *Department of Horticulture, College of Aburaihan, University of Tehran, Tehran, Iran*

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ABSTRACT

In this research, the effect of two irrigation treatments (90 and 40% of available water) and four different levels of zinc (control, 5, 10, and 20 mg kg^{-1} soil) was investigated on pistachio rootstock seedlings including 'Akbari' and 'Badami' in a completely randomized design with four replications during 90 days. At the end of the experiment, plant growth characteristics including shoot height, stem diameter, number of leaves, leaf area, and dry matter accumulation in root, stem, and leaf were evaluated. Additionally, the relative water content, membrane stability index, photosynthetic pigment concentrations, and content of organic osmolytes were appraised as well. The results showed that drought stress reduced plant height, number of leaves, leaf area, and biomass of plants, and increased root dry weight. The zinc application increased plant growth under irrigation treatments. In addition, under drought stress, the application of 5 mg kg^{-1} zinc increased the membrane stability by16.76%, maintained chlorophyll a by 20.69%, b by 31.96%, and total chlorophyll of the leaf by 27.74%. However, the application of 20 mg kg^{-1} Zn in soil caused toxicity and exacerbated the effects of drought stress which led to the accumulation of proline and soluble carbohydrates in the pistachio rootstocks with more proline in 'Akbari' than 'Badami' rootstock, while Zinc could reduce the accumulation of soluble carbohydrates and proline. In general, 'Badami' was introduced as a tolerant rootstock for use underwater stress, as well as using 5 mg kg^{-1} Zn to improve the growth and efficiency of both rootstocks under drought stress or without stress.

Introduction

Water scarcity is increasing in the most important areas of the world (Zwart & Bastiaanssen, 2004), and restrictions on access to irrigation water require fundamental changes in irrigation management or the application of methods in which water resources are better preserved (Dagdelen *et al*., 2006). Drought and salinity stress have reduced the production of horticultural crops around the world more than any other factor. In some countries, such as Iran and Pakistan, more land is affected by abiotic stress with about 14.7% of the country area being located in saline conditions. Out of 165 million hectares in Iran, 25 million have

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[*] **Corresponding author**: Email address: ajafari@ardakan.ac.ir

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saline and alkaline soils, and the area of exploitable saline lands is estimated at 4.5 million hectares (Ahmadvand *et al*., 2012). In recent years, global climate change and warming have raised the drought stress and reduced rainfall making widespread droughts in the country. However, a decade ago, the International Climate Change Council (IPCC) predicted that by 2030, continuous, prolonged droughts would significantly reduce agricultural production (Bates *et al*., 2008). In such circumstances, identifying and introducing drought-tolerant plants or optimizing cultivation conditions, especially in arid and semi-arid regions, is not only a national necessity but also a global strategy for maintaining agricultural production in the country.

Pistachio (*Pistacia vera* L.) is one of the most important horticultural crops that is widely grown in Iran (Eslami *et al*., 2019; Sharifkhah *et al*., 2020). Most of the pistachio orchards in Iran, especially in Kerman province, where more than 70% of the country's pistachio cultivation area is located, are facing water shortage and drought stress. In these areas, the quantity and quality of water are the most important factors limiting the economic development of these regions (Hokmabadi, 2018). Although pistachio trees are considered drought-tolerant (Behboudian *et al*., 1986), they need enough water to produce the best yield (Goldhamer & Bideh, 2004). Moreover, irregular irrigation or waterscarcity decays fruit quality and increases the risk of fruit blackness (Hosseini *et al*., 2022). The identification of drought-resistant cultivars/rootstocks requires much research and implementation of long-term breeding programs or collection and evaluation of tolerant plants over many years. However, until the tolerant rootstocks and cultivars are identified, plant tolerance can be increased by optimizing cultivation conditions. Optimizing plant nutrition is considered one of the most practical techniques in this respect. The plant needs essential nutrients to grow and produce flowers and fruits the amount of which is usually recommended for stress-free conditions. However, some studies have shown that changing the nutrient amounts can improve plant tolerance to environmental stresses. Therefore, optimizing high-consumption elements such as nitrogen, potassium, and calcium has received more attention compared with low-consumption ones (Zhu, 2001; Farooq *et al*., 2008), the latest research, however, has shown that micro-nutrient can have significant effects on plant tolerance to environmental stresses. Zinc is an essential micro-nutrient that plays a fundamental role in crop resistance against drought stress, the activity of antioxidant enzymes, and limiting oxidative stress. Numerous studies have proven the positive effect of zinc (Norozi *et al*, 2019; Mohit Rabary *et al*., 2022; Maliha *et al*., 2022) and other mineral elements (Tabrizian *et al*., 2022) on improving plant physiological traits (Keshavarz *et al.*, 2011) and also on the induction of drought resistance, but little research has been done on the Zn effect on pistachio seedlings under stress (Tavallali *et al*., 2009, Tavallali *et al*., 2010). The purpose of this study was to evaluate the effect of different concentrations of Zn on the physicochemical properties of two pistachio rootstocks (Akbari and Badami) under drought stress.

Materials and Methods

This study was conducted during the successive seasons of 2018 and 2019 in a research greenhouse day/night at a temperature of 30/25 ± 4℃, relative humidity 35-40%, and photoperiod 16 hours, in the laboratory of Tehran University Abou Reihan Branch.

The plant materials used in this study were seedlings of two pistachio cultivars, 'Akbari' and 'Badami', fresh and uniform seeds of which were obtained from a commercial orchard in Ardakan. In August 2018, the seeds were disinfected with a 20% solution of sodium hypochlorite in distilled water and aseptically germinated and placed on a moist mixture of peat and sand. After germination, the seeds were sown in plastic black round pots of 20 × 16 cm size filled with the cockpit, perlite, and soil at a 2: 1: 1 ratio and irrigated with distilled water. The soil used was a silty loam with following traits: silt 31%; clay 13% and 56%; pH 7.5; electrical conductivity (ECE) 0.62 dS m^{-1}; organic matter 0.5%; phosphorus (P) 94/4%; nitrogen (N) 0.05%; potassium (K), zinc (Zn), iron (Fe), manganese (Mn), and copper (Cu) 2042, 0.48, 1.66, 8.79 and 1.3 mg kg^{-1}, respectively.

Three months after seed germination, the plants reached the 15-leaf stage with an average height of 25 cm. Four levels of zinc including 0, 5, 10, and 20 mg kg^{-1} as zinc sulfate ($ZnSO_4.7H_2O$, Merck, Pro Analyze) were added to the pots with irrigation water, and on the following day, the plants were irrigated under two irrigation regimes of 40 and 90 % of available water. These treatments were based on weighting the pots at two-day intervals and determining the amount of water in the soil. The water used in the culture medium was determined based on the difference in the weight of the culture medium in the pots at field capacity and permanent wilting point. By weighting the pots on both days, the water level of the pots was adjusted to the desired treatments.

Measured parameters

After 180 days of sowing seeds, plant growth and Physicochemical parameters were evaluated. Growth characteristics studied included plant height, leaves number, leaf area, shoot diameter, and shoot and root dry weight. Plant height was measured from the crown up to the highest point of the plants by a ruler, leaf area by a Leaf Area meter (ADC, England), and crown diameter by a caliper. The dry weight of the plant organs was investigated after 72 hours in an oven at 70°C (Raoufi *et al*., 2020).

The Relative Water Content (RWC) was investigated using Barrs and Weatherley's (1962) method, membrane stability index based on Sairam *et al*. (2002), chlorophyll and carotenoids concentration in leaf tissue using Arnon (1962), leaf proline concentration following Bates *et al*. (1973), and leaf soluble carbohydrate concentration using the Kochert (1978).

The present study was conducted as a factorial experiment (rootstock effects, drought stress, and zinc concentrations) in a completely randomized design with four replications. Statistical calculations were performed using SPSS 23 software. A comparison of mean traits was performed using the Duncan test at a 5% significance level.

Results

Plant height

Based on the results under drought stress, the plant height was significantly reduced compared to control. Further, Zinc application in the soil increased plant height compared to the control treatment (Table 1), however, little difference was observed between 5, 10, and 20 mg kg^{-1} zinc concentrations.

As shown in Fig. 1, evaluation of the interaction of zinc and rootstock suggested that zinc treatment increased plant height and the highest of which (47.5 cm) was observed in ‘Badami’ at 5 mg kg^{-1} zinc treatment. In ‘Akbari’ seedlings, the highest plant height with 47.5 and 45 cm was observed in 10 and 20 mg kg^{-1} zinc treatments, respectively which were significantly higher than 5 mg kg^{-1} zinc and control treatments.

Table 1. Effect of drought stress and Zn application on leaf number, leaf area, and plant height

Treatment	Leaf number	Leaf area (cm^2)	Plant height (cm)
		Irrigation	
90%	20 a	320.4 a	49.4 a
40%	13.3 b	170.1 b	38.2 b
		Zn (mg kg^{-1} soil)	
Control	13.2 c	202.9 c	8.6 b
5	17.5 b	306.7 a	45.5 a
10	20.3 a	250.2 b	44.2 a
20	18.6 ab	192.2 c	46.0 a

Different letters in each column show significant differences at $p<0.05$ (Duncan test).

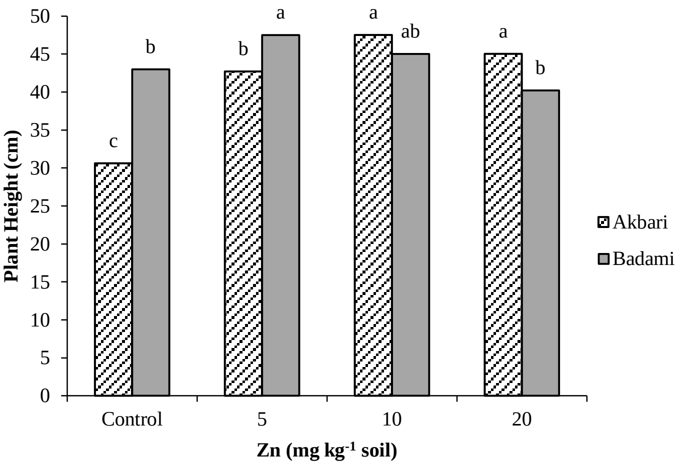

Fig. 1. Interaction effect of Zn treatment and rootstock on plant height.

Leaf number and area

The number of leaves under drought stress conditions was significantly lower than the control treatment. Zinc application also significantly increased the number of leaves compared to the control treatment. Among zinc treatments, the 10 mg kg^{-1} treatment had the highest effect (53.79%) on increasing leaf number (Table 1).

Under drought stress, leaf area decreased significantly compared to control. The use of 5 and 10mg kg^{-1} Zn significantly increased leaf area by 306.7 and 250.2 cm^2, respectively compared to the control by 202.9 cm^2 (Table 1).

The interaction of zinc and drought treatments showed that using zinc up to 10 mg kg^{-1} soil, increased the leaf area of pistachio rootstocks by 280.7 cm^2, and using 5mg kg^{-1} zinc, increased the leaf area by 408.9 cm^2 which was the highest leaf area (Fig. 2). But the application of 20 mg kg^{-1} zinc significantly decreased the leaf area of the plants compared to the control, while under drought stress; 5 to 10 mg kg^{-1} Zn increased the leaf area of the plant.

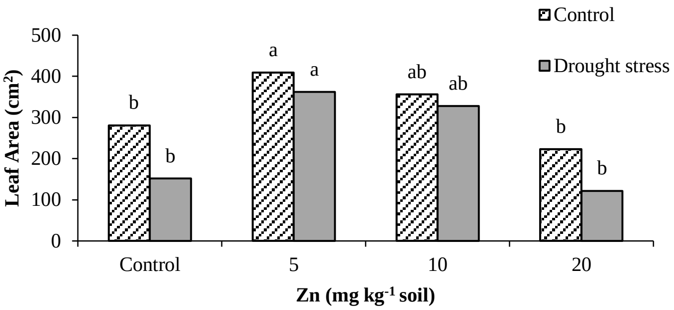

Fig. 2. Interaction effect of Zn and drought treatments on leaf area.

Dry weight

As Table 2 displays, the dry weight of stem, leaf, root, and total plant under drought stress was significantly reduced compared to the control treatment.

Table 2. Effect of drought stress, Zn application, and rootstock on the dry weight of shoot, root, leaves, and total dry weight.

Treatment	Dry weight of shoot (g)	Dry weight of root (g)	Dry weight of leaves (g)	Total Dry weight (g)
		Irrigation		
90%	4.43 a	2.17 a	3.11 a	9.7 a
40%	3.23 b	1.85 b	1.28 b	6.35 b
		Zn (mg kg^{-1} soil)		
Control	4.15 b	1.99 b	1.85 c	7.99 c
5	5.93 a	2.56 a	3.28 a	11.78 a
10	3.37 bc	2.20 b	2.56 b	8.13 b
20	2.41 c	1.43 c	1.52 d	5.37 d
		Rootstock		
Akbari	3.32 b	1.57 b	2.08 a	6.97 b
Badami	4.34 a	2.42 a	2.37 a	9.14 a

Different letters in each column show significant differences at $p<0.05$ (Duncan test).

Application of 5 mg kg^{-1} zinc made the highest accumulation of dry matter in stem by 42.89%, root by 28.64%, leaf by 77.3%, and total biomass of plants by 47.43% compared to the control treatment. The lowest dry matter accumulation was observed in plants treated with 20 mg kg^{-1} zinc. Although dry matter accumulation in leaves of pistachio rootstocks was similar, the stem and root of ‘Akbari’ were significantly lower compared to ‘Badami’. The total biomass of ‘Akbari’ was lower than that of ‘Badami’ as well.

Drought stress (40% irrigation) decreased shoot dry weight by 3.23 g compared to 90 % irrigation treatments by 4.43 g. Zinc usage in soil prevented shoot dry weight loss and the highest shoot dry weight of 5.93 g was observed in 5 mg kg^{-1} Zn.

The root dry weight was increased significantly by 2.56 g using 5 mg kg^{-1} Zn in soil compared to control which increased by 1.99 g. Although 10 mg kg^{-1} zinc treatment had an insignificant effect on root dry weight which was decreased in 20 mg kg^{-1} treatment. Drought stress condition, however, increased root dry weight in control and 5 mg kg^{-1} zinc treatment, which was reduced in 10 and 20 mg kg^{-1} zinc treatments significantly (Table 2).

Based on the results, 5 and 10 mg kg^{-1} Zn increased leaf dry weight, the highest of which was 3.28 g in 5 mg zinc treatment. Although 20 mg kg^{-1} zinc reduced leaf dry weight by 1.52g compared to the control by 1.85g (Table 2).

The results showed that using 5 mg kg^{-1} zinc significantly increased the dry weight of the plant and drought stress reduced plant weight. However, as Table 2 shows, 20 mg kg^{-1} Zn in soil significantly decreased plant dry weight under drought stress.

Physiological characteristics

RWC

Relative water content was only affected by drought stress, and Zn treatments and rootstock had a negligible effect on RWC. According to Fig. 3, the relative water content of leaf at 40% irrigation treatment decreased by 14.65% and changed significantly from 70.5% at 40% irrigation treatment to 82.6 % in the control treatment.

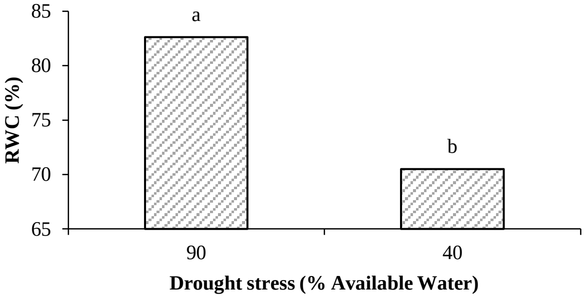

Fig. 3. Effect of drought stress on RWC.

Membrane stability index

Membrane stability index was significantly affected by drought stress and zinc application in soil (Table 3). Application of 5 mg kg^{-1} of zinc increased the membrane stability index up to 16.76% compared to the control treatment, while using 20 mg kg^{-1} Zn in soil significantly decreased the membrane stability index in leaves of plants.

Table 3. Effect of drought stress and Zn application on membrane stability index, chlorophyll a, b, and total chlorophyll

Treatment	Membrane stability index (%)	Chlorophyll a (mg g^{-1})	Chlorophyll b (mg g^{-1})	Total Chlorophyll (mg g^{-1})
		Irrigation		
90%	61.8 a	6.9 a	13.5 a	20.4 a
40%	50.7 b	5.5 b	10.1 b	15.6 b
		Zn (mg kg^{-1} soil)		
Control	54.3 c	5.8 c	9.7 b	15.5 b
5	63.4 a	7 a	12.8 a	19.8 a
10	61 bc	6.4 bc	11.4 a	17.8 ab
20	43.8 d	4.9 c	7.4 c	12.3 c

Different letters in each column show significant differences at p<0.05 (Duncan test).

Photosynthetic pigment concentrations

Results showed that chlorophyll a and b concentrations in the leaf of pistachio rootstocks under drought stress were significantly lower in comparison with the control treatment. The use of 5mg kg^{-1} Zn in soil increased chlorophyll-a concentration up to 20.69% in the leaf of pistachio rootstocks, as well as 5 and 10mg kg^{-1} zinc in soil significantly increased chlorophyll b compared to the control treatment.

However, chlorophyll b in the 20mg kg^{-1} zinc treatment was significantly decreased compared to the control. In addition, chlorophyll b in plant leaves was also affected by the rootstock type (Table 3) and in the 'Akbari' leaf was significantly lower than in the 'Badami' leaf (Fig. 4).

Drought stress, based on Table 3, significantly decreased total chlorophyll concentration in leaves, while5 mg kg^{-1} Zn increased by 27.74%. On the other hand, 20 mg kg^{-1} Zn significantly reduced the total chlorophyll concentration in the leaves of plants. In 'Badami', chlorophyll concentration was higher than in 'Akbari' (Fig. 5), while the experimental treatments had little effect on carotenoid concentration in leaves of pistachio rootstocks (Table 3).

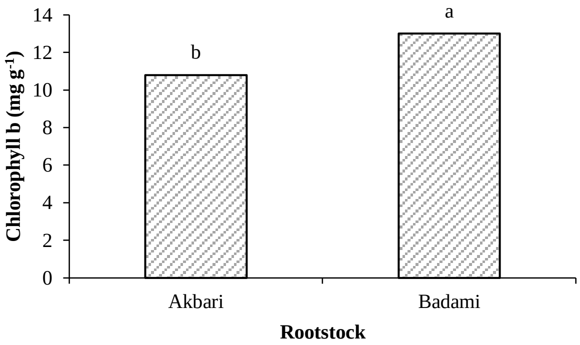

Fig. 4. Effect of rootstock on chlorophyll b.

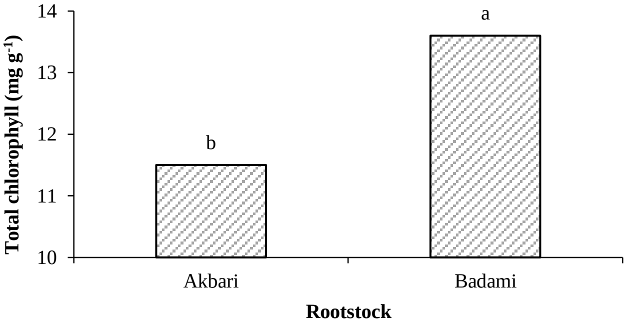

Fig. 5. Effect of rootstock on total chlorophyll.

Proline

Proline concentration in drought stress significantly increased in leaves. Based on the data in Table 4, All Zn treatments significantly decreased proline concentration in leaf tissue by 308.8 µmol g^{-1} compared to the control treatment. Evaluation of pistachio rootstocks showed that 'Akbari' had less proline content (208 µmol g^{-1}) than 'Badami' (267.2 µmol g^{-1}) (Fig. 6).

Table 4. Effect of drought stress and Zn application on proline and soluble carbohydrates

Treatment	Proline (µmol g^{-1})	Soluble carbohydrates (µg g^{-1})
	Irrigation	
90%	175.2 b	478.9 b
40%	314a	530 a
	Zn (mg kg^{-1} soil)	
Control	308.8 a	764 a
5	225.2 bc	372.1 d
10	196.3 c	424.1 c
20	257.9 b	500.2 b

Different letters in each column show significant differences at $p<0.05$ (Duncan test).

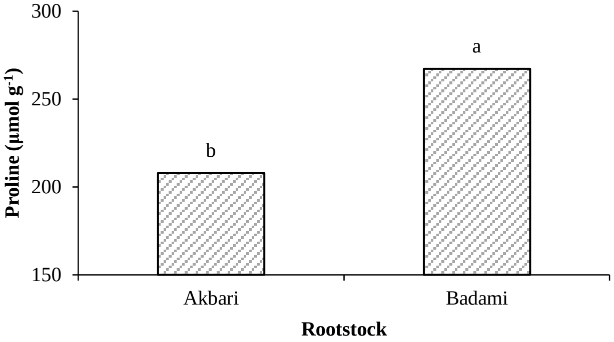

Fig. 6. Effect of rootstock on proline content.

Under non-stress conditions, proline content in leaf tissue of 'Akbari' (111.6 µmol g^{-1}) was significantly less than that of 'Badami' (230.3 µmol g^{-1}), whereas, under drought stress, little difference was seen between the two plants (Table 5).

Soluble carbohydrates

Under drought stress, the soluble carbohydrates in the leaf of pistachio rootstocks significantly increased compared to the control treatment. Zinc application in soil decreased the concentration of soluble carbohydrates in leaves compared to the control treatment. The lowest concentrations of soluble carbohydrates (372.1µg g^{-1}) were observed in leaves treated with 5 mg kg^{-1} zinc (Table 4). Under drought stress and normal irrigation conditions, differences between the two rootstocks were negligible (Table 5).

Table 5. Effect of rootstock and drought stress on proline and soluble carbohydrates.

Rootstock	Proline (µmol g^{-1})	Soluble carbohydrates (µg g^{-1})
	Irrigation 90%	
Akbari	111.6 c	435.3 a
Badami	230.3 b	491.4 ab
	Irrigation 40%	
Akbari	538.4 a	318.2 a
Badami	521 a	309.5 a

Different letters in each column show significant differences at $p<0.05$ (Duncan test).

Discussion

In this study, drought stress reduced plant growth characteristics including plant height, leaf area, leaves number, and plant dry weight. In agreement with our results, previous studies have shown a decrease in the growth of plants under drought stress conditions. Results of an experiment performed on *Pistacia atlantica* under greenhouse conditions showed that drought stress significantly reduced the stem height and diameter growth and leaf area of *Pistacia atlantica* seedlings as well as photosynthetic activity and gas exchange rate in this plant (Mirzaei and Karamshahi, 2014). A study by Bagheri *et al*. (2011) on the effect of drought stress on the two pistachio cultivars (Badami Zarand and Qazvini) revealed a significant reduction in vegetative characteristics such as stem height and diameter, leaf area, stem, leaf, and root dry weight with increasing severity of drought stress. In addition, results manifested that the relative water content of leaves and water use efficiency were strongly affected by drought stress and decreased. An experiment showed that vegetative growth and dry matter content of olive seedlings under drought stress decreased with increasing stress intensity due to decreased nutrient uptake and transport (Chartzoulakis *et al*., 2000). Moreover, according to Rucker *et al*. (1995), the decrease in leaf area is due to the decrease in cell elongation under dehydration. The extent of leaf area and stem elongation depends on temperature and nutrient leaf supply. Many studies indicate the adverse effects of drought stress on plant growth characteristics. Salisbury and Ross (1992) identified cell growth as the most sensitive response to water stress. Since cellular dimension expansion requires turgor pressure, decreasing cell turgescence due to drought stress reduces stem and leaf cell proliferation (Syversten, 1985). In addition, under drought stress limiting nutrient uptake inhibits the growth and development of plant organs as well. Hsiao (1973) stated that water shortage leads to reduced shoot growth, leaf loss, and then reduced plant total photosynthetic capacity (Manivannan *et al*., 2007). Decreasing leaf area is a defense mechanism against drought stress conditions and helps to maintain the water content of the plant by transpiration restriction (Levitt, 1980).

In the present study, zinc application improved plant growth parameters under non-drought conditions (Tables 1-4). Zinc deficiency shortens the internodes and prevents stem and leaf growth (Saravanan *et al*., 2007; Hong and Jin, 2007) due to the role of zinc in the tryptophan biosynthesis and auxin in the plants (Rout and Das, 2009); therefore, zinc deficiency results in auxin deficiency and growth restriction. Thus, plant growth is expected to improve after zinc application in alkaline calcareous soil (Marschner, 1995). The improvement of plant growth by application of zinc in control conditions is due to the improvement of plant photosynthesis (Hosseini *et al*., 2007) since zinc increases chlorophyll concentration in leaf tissue and improves leaf photosynthetic capacity. Furthermore, in our study, an increase was observed in chlorophyll concentration in the leaves of pistachio rootstocks, especially under 5 mg zinc treatment (Marschner, 2012). Zn, because of its role in the biosynthesis of antioxidant enzymes such as superoxide dismutase and catalase, strongly protects the tissue against oxidative damage under drought stress conditions (Sohla *et al*., 1995). Moreover, Zn use causes an appreciable increase in leaf area, the content of chlorophyll and other photosynthetic pigments, as well as stomatal conductance, thus leading to improved growth, yield of winter wheat, and common bean (Karim *et al*., 2012; Ibrahim *et al*., 2017).

The current research showed that Zn application improves seed germination, plant water relations, cell membrane stability, osmolyte accumulation, stomatal regulation, water use efficiency, and photosynthesis, thus significantly improving plant yield (Table 2). The enhancing effect of zinc on seed germination and other physiological traits has been also shown in a study on bell pepper (Hosseinifarahi et al., 2022)

The extent of membrane injury can be estimated by measuring electrolyte leakage from the cell. The leakage rate can be correlated with different physiological and biochemical processes including the synthesis of antioxidant enzymes, WUE, osmotic potential, and stomatal regulation (Liu and Huang, 2000; Sreenivasulu *et al*., 2000; Lotfi *et al.*, 2019). Thus, low electrolyte leakage can be recommended as a criterion to identify stress resistance in different crop species and genotypes (Ghanepour *et al*., 2015). The result of our study confirms the role of Zn in increasing leaf antioxidant capacity, reducing leaf tissue damage and leaf loss under drought stress. In this research, increasing Zn content by20 mg kg^{-1} soil led to a reduction in growth characteristics, chlorophyll content, and membrane stability (Table 1). Accumulation of zinc in the leaves caused plant toxicity and reduced leaf area, and then reduced stem and root biomass (Rout and Das, 2009; Sagardoy *et al*., 2009).

Another mechanism that maintains tissue water under drought stress is the accumulation of metabolically compatible substances and osmotic regulation (Patakas *et al*., 2002). Accumulating these soluble substances also called osmotic or osmolyte protectors are used by many plants to counteract drought, modulate osmotic regulation, and protect subcellular structures (Pinheiro *et al*., 2004; Behzadi Rad *et al.*, 2021). Evidence suggests that free proline is the most important factor in accumulating plants in the face of water scarcity and helps the plant to increase its cellular osmolality during water deficit stress (Verslues *et al*., 2006). Mostajeran and Rahimi's (2009) results showed that free proline and soluble sugars can be used as a criterion for the selection of drought-tolerant plants. Other researchers have also studied the effects of water stress on some pistachio rootstocks and demonstrated the importance of free proline as an indicator of stress (Karimi and Tavallali, 2017).

In addition, the present study identified that under stress conditions the amount of free proline increases (Table 4). Drought stress was found to increase the number of osmolytes such as free proline, glycine betaine, and soluble sugars as well which is in agreement with Lotfi *et al.* (2010). Moreover, increasing stress levels in this study decreased shoot and root dry weight, leaf area, relative humidity content, total chlorophyll, carotenoids, and total soluble proteins. The researchers further stated that different rootstocks respond differently to drought stress and that the cultivars which produce more free proline are more susceptible to drought (Khoyerdi *et al*., 2016). In our study, the Badami cultivar with a higher free proline level showed more ability to maintain growth and leaf area under drought stress. Therefore, it can be concluded that the proline accumulation of pistachio cultivars can be related to their tolerance to drought stress.

Furthermore, soluble sugars such as sucrose are considered adaptive osmolytes in the plant and play an important role in osmotic regulation (Sairam and Tyagi, 2004). The accumulation of soluble sugars in drought conditions was additionally observed in this research (Table 4). This was more due to the reduced plant growth and in ‘Akbari’ which grew less under drought stress, sugar accumulation was higher than that of ‘Badami ‘with higher growth. Hence, it seems that accumulating soluble sugars under drought stress is an inactive response to stress and such substances cannot, like proline, contribute to the increase in drought tolerance of pistachios.

This study proved that zinc application decreases proline concentration in leaf tissue of plants (Table 4) which can be due to the reduction of stress on the plant (Karimi and Tavallali, 2017). Additionally, proline depletion in pistachio root tissues under drought stress can be due to reduced oxidative stress in leaves. On the other hand, zinc application increased plant growth and then caused proline dilution. The lowest proline content was observed in 5 and 10 mg kg^{-1} Zn treatments which had lower drought stress according to plant biomass data. In 20 mg kg^{-1} zinc treatment, because of the effects of zinc toxicity and restriction of plant growth, more proline accumulation was observed than in the 5 and 10 mg kg^{-1} treatments, (Rout and Das, 2009).

Zinc use reduced the concentration of soluble carbohydrates in leaf tissue as well (Table 4). Zinc deficiency, however, reduces plant growth and accumulates sugars in the leaves. As in plants under severe zinc deficiency, sugar secretion from the leaf surface was seen (Marschner and Marschner, 2012). Accordingly, the reason for the reduction of sugar accumulation in zinc application treatments can be the growth recovery and increased zinc intake by the plant, as well as dilution of these substances in larger volumes of plant biomass.

Conclusions

In this study, growth reduction was observed under drought stress conditions. Based on the results, preserving the vegetative growth strength of drought-tolerant cultivars indicates their high ability to absorb and use water under drought stress conditions. Under water stress, the Badami cultivar had higher roots and stems dry weight as well as total biomass than those of the Akbari cultivar suggesting the greater efficiency of the 'Badami' against drought stress. The higher proline accumulation in Badami cultivar was suggestive of the drought tolerance of this plant, whereas the accumulation of soluble sugars was higher in the Akbari cultivar, which was associated with reduced plant growth. These results indicated the importance of proline accumulation in drought tolerance in pistachios. Further, using 5mg kg^{-1} zinc under irrigations and drought conditions improved plant growth and biomass which was done to maintain leaf area, improve plant photosynthetic capacity, and help tolerate oxidative damage. Furthermore, 20 mg kg^{-1} zinc use caused plant toxicity and intensified the adverse effects of drought on the plant. Overall, 5 mg kg^{-1} zinc was suggested to improve pistachio growth under drought conditions.

Conflict of interests

The authors confirm that there are no conflicts of interest and there is no source of funding regarding the manuscript Effect of zinc on the growth of two pistachio (*Pistacia vera* L.) rootstocks under drought stress.

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