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Tolerance of Mycorrhiza infected Pistachio (*Pistacia vera* L.) seedling to drought stress under glasshouse conditions

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ABSTRACT

The influence of *Glomus etunicatum* colonization on plant growth and drought tolerance of 3-month-old *Pistacia vera* seedlings in potted culture was studied in two different water treatments. The arbuscular mycorrhiza (AM) inoculation and plant growth (including plant shoot and root weight, leaf area, and total chlorophyll) were higher for well-watered than for water-stressed plants. The growth of AM-treated seedlings was higher than non-AM-treatment regardless of water status. P, K, Zn and Cu contents in AM-treated shoots were greater than those in non-AM shoots under well-watered conditions and drought stress. N and Ca content were higher under drought stress, while AM symbiosis did not affect the Mg content. The contents of soluble sugars, proteins, flavonoid and proline were higher in mycorrhizal than non-mycorrhizal-treated plants under the whole water regime. AM colonization increased the activities of peroxidase enzyme in treatments, but did not affect the catalase activity in shoots and roots under well-watered conditions and drought stress. We conclude that AM colonization improved the drought tolerance of *P. vera* seedlings by increasing the accumulation of osmotic adjustment compounds, nutritional and antioxidant enzyme activity. It appears that AM formation enhanced the drought tolerance of pistachio plants, which increased host biomass and plant growth.

Introduction

In nature, plants are frequently exposed to adverse environmental conditions that have a negative effect on plant survival, development and productivity. Drought stress is considered to be one of the most important abiotic factors limiting plant growth and yield in many areas (Kramer and Boyer, 1997). The symptoms of drought are apparent by wilting of the plants, reductions in the net photosynthesis rate, stomatal conductance, water use efficiency, relative water content and gradually diminution in total chlorophyll content. Drought stress also impairs the electron transport system, leading to the formation of activated oxygen (Saraswathi and Paliwal, 2011; Smirnoff, 1993). Reactive oxygen species (ROS) such as H_2O_2, O_2^- and OH^- may accumulate during water deficit stress and damage the photosynthetic apparatus. These cytotoxic ROS can destroy normal metabolism through oxidative damage of lipids, proteins and nucleic acids. Plants under stress are well stocked with an array of protective and repair systems that minimize the occurrence of oxidative damage (Khalvati et al., 2010). According to Smirnoff (1993), these can be divided into two categories: systems that react with active forms of oxygen and keep them at a low level, i.e., superoxide dismutases, catalase (CAT), or peroxidases, and systems that regenerate oxidized antioxidants (glutathione, glutathione reductase, ascorbate and mono and dehydroascorbate reductases). CAT is considered to be the most important enzyme that eliminates H_2O_2 from cells (Abdel Latef, 2011). Peroxidase (POD) constitutes a class of heme-containing enzymes ubiquitously present in prokaryotic and eukaryotic organisms. This enzyme catalyzes the dehydrogenation of structurally diverse phenolic and endolic substances by H_2O_2 and is thus often regarded as an antioxidant enzyme, protecting cells from the destructive influence of H_2O_2 and derived oxygen species (Pandey et al., 2010; Shigeoka et al., 2002). The efficiency of the antioxidant defense system is correlated with tolerance to drought stress.

Plants can respond to drought stress at morphological, anatomical and cellular levels with modifications that allow the plant to avoid the stress or to increase its tolerance (Ruiz-Lozano, 2003). These morphological and anatomical adaptations can be of vital importance for some plant species, but they are not a general response of all plant species. In contrast, the cellular responses to drought stress seem to be conserved in the plant kingdom.

In addition to intrinsic protective systems of plants against stress, plants grow in association with a number of soil

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microorganisms that can alleviate stress symptoms. Arbuscular mycorrhizal (AM) fungi that associate with the roots of most plants not only stimulate the growth of plants, but also contribute in enhancing plant tolerance to abiotic stress such as salinity (Abbaspour, 2010), drought (Navarro et al., 2011) and temperature stress (Xiancan et al., 2010).

Previous studies have indicated that inoculation with AM fungi appeared to improve drought tolerance of host plants (Cuenca et al., 1997). Based on the research of Nelsen and Safir (1982) on onion, improved phosphorus nutrition is a crucial factor for increased drought tolerance of mycorrhizal plants. Exposure of plants to drought conditions led to increase in free proline, soluble sugar, peroxidate (POX) activities and malondialdehyde (MDA) concentration, and inhibitions of protein synthesis have been proved in many literatures (Dhindsa, 1991; Zhang and Kirkham, 1994; Arji and Arzani, 2008). However, there is very little research on improving drought tolerance of pistachio using AM associations.

Therefore, the purpose of this work was to test the effects of *Glomus etunicatum* on growth, nutrition, solute accumulation and antioxidant responses in leaves and roots of pistachio under drought stress, in order to further understand drought tolerance mechanisms in AM plants.

Materials and methods

Plant materials

Pistachio (*Pistacia vera* L. cv. Akbari) seeds were surface sterilized with 20% solution of sodium hypochlorite in distilled water and aseptically germinated on a moist mix of peat and sand in polystyrene trays. Twenty-three-day-old seedlings, uniform in size, were chosen for transplanting in order to homogenize the plant material used in the experiment. AM fungal inoculum, *Glomus etunicatum* Backer and Gerdemann (Gec) were used as the AM fungal inoculum. Pure starter cultures of *G. etunicatum* were provided by International Culture Collection of Arbuscular and Vesicular, Arbuscular mycorrhizal fungi (INVAM). *G. etunicatum* was multiplied in pot cultures with sterilized fine sand as a substrate. Maize (*Zea mays* L.) was used as a host and was cultured for 3 months in a greenhouse ($24 \pm 5\,^{\circ}C$) under natural light. Maize plants were harvested just prior to inoculation by excising and discarding shoots. Mycorrhizal inoculum consisted of soil, spores (the spore density was 10–12/g dry soil), mycelium of *G. etunicatum*, and infected root fragments with an infection level of 65% (Abbaspour, 2010).

Plant growth condition

Seedlings were transplanted in 20 cm × 15 cm plastic pots containing a mixture of salinity clay:sand (1:5, v/v) (four seedlings/pot). The characteristics of the soil after mixture with sand were: pH 6.7, EC 1.4 ds/m, 3.4% silt, 14.5% clay, 82.1% sand, 1.2% organic matter, 11.2 mg/kg P, 139 mg/kg K and 31 mg/kg N. The soil was collected from Damghan city, Iran, and P was not added to the soil in order to stimulate mycorrhiza formation. Potted plants were maintained in the greenhouse under an average maximum photosynthetic photon flux of 846 μmol m^{-2}/s. The Max/Min average temperatures were 29/18 °C and mean relative humidity was 48%. Twenty-three-day-old pistachio seedlings were inoculated with 100 g inoculum as the mycorrhizal treatment. All seedlings were irrigated twice a week until differential water treatments were initiated 90 days after transplanting. Well-watered irrigation treatment plants were watered to 100% water holding capacity, while deficit irrigation plants received 50% of the well-watered irrigation total, and for the drought treatment, irrigation was withheld for one month following transplanting (90–120 days after transplanting).

Experimental design

The experiment consisted of a randomized complete block design with two inoculation treatments: AMF and non-AMF. Six replicates of each treatment were performed for a total of 24 pots, so that half of them were cultivated under well-watered conditions throughout the entire experiment while the other half were drought stressed for one month before harvest.

Parameters measured

After 4 months, pistachio plants were harvested, and then shoots and roots were separated. Leaf area was determined using an AM-200 leaf area meter. Shoot and root dry weights were determined after over-drying at 70 °C for 48 h, and saved for mineral analysis.

The percentage of mycorrhizal root infection was estimated by the following procedures: roots from each plant were collected by gently washing out the sand under running tap water and rinsed three times in deionized water. A subsample of 0.5-g root segments was collected and cut into l-cm-long pieces. One hundred l-cm root segments per treatment were examined for the presence of arbuscules, vesicles, or hyphae. The root segments were cleared and stained for analysis of colonization by AMF using a modified Phillips and Hayman (1970) procedures. The roots were cleared for 50 min in a 10% KOH solution at 90 °C, rinsed, placed in 10 nk% HCl solution for 10 min and then stained with glycerol–trypan blue solution (0.05%) at 90 °C for 20 min. Infection units originating from entry points were counted, at 45–100× magnification. The results were expressed as infection units per root length. Root colonization by AMF was estimated by the gridline intersection method (Bierman and Linderman, 1981) and expressed as percentage of root length colonized.

Phosphorus and nitrogen content were determined in dried shoots colorimetrically (Boltz and Lueck, 1958). K in plant shoots was determined by flam photometry. Other mineral nutrients (Zn, Cu, Fe, and Ca) were analyzed by atomic absorption.

Pigments were extracted using the method of Li (2000). Chlorophyll was extracted in 80% (v/v) acetone from 1 g of fresh leaf sample in the dark at room temperature. Absorbance was measured at 663 and 645 ηm in a UV/VIS spectrophotometer. Chlorophyll concentration was calculated using the equation:

$$Chl = 0.0202 \times A_{645} + 0.00802 \times A_{663}$$

For the determination of flavonoid content, 1 g of fresh leaf sample was homogenized with pure methanol and centrifuged at 3000 × *g* for 10 min. Chlorophylls and carotenoides were separated from flavonoid content with petroleum ether. Flavonoid concentration was calculated using the equation:

$$\text{Flavonoid} = A_{330} \times \frac{y}{E^{1\%}_{1\,cm}} \times 100$$

where *y* is the volume of dilution and $E^{1\%}_{1\,cm}$ is the coefficient of specific absorbance.

For the determination of free proline and total soluble sugar content, fresh roots and leaves were collected and proline content was assessed by spectrophotometric analysis at 515 ηm of the ninhydrin reaction using the protocol of Bates et al. (1973). Soluble sugar content was determined by 0.1 mL of the alcoholic extract reacting with 3 mL freshly prepared anthrone (200 mg anthrone + 100 mL of 72% H_2SO_4) and placed in a boiling water bath for 10 min according to Irigoyen et al. (1992). After cooling, the absorbance was read at 620 ηm.

To prepare the extraction of enzymes and soluble proteins, fresh leaves and roots were homogenized in 5 mL phosphate buffer (0.1 mol/L, pH 7.8), centrifuged at 10,000 × *g* for 20 min at 4 °C

Table 1
ANOVA considering root colonization with arbuscular mycorrhizal fungi (AMF), growth parameters, pigments content, shoot mineral content, soluble carbohydrate, proline, protein, CAT and POD activities in *Pistacia vera* grown under drought stress.•

Variable	AMF status	Drought	Interaction
AMF colonization	***	***	***
Shoot fresh weight	***	***	NS
Shoot dry weight	**	**	NS
Root fresh weight	***	**	NS
Root dry weight	**	**	NS
Leaf area	***	***	*
Total chlorophyll	***	***	NS
Flavonoids	***	**	NS
P content	**	**	NS
N content	**	**	NS
K content	*	NS	NS
Ca content	**	***	NS
Fe content	**	**	NS
Cu content	**	**	*
Mg content	NS	NS	NS
Zn content	**	**	*
Shoot soluble carbohydrate	**	***	*
Root soluble carbohydrate	NS	***	*
Shoot proline	**	**	NS
Root proline	**	NS	NS
Shoot protein	*	***	NS
Root protein	***	**	**
Shoot catalase	NS	***	NS
Root catalase	NS	NS	NS
Shoot POD	***	***	NS
Root POD	***	NS	NS

NS: non significant, according to Tukey multiple range test.
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.
• All parameters were positive impact at different P values.

and then the supernatant was used for assays. Soluble proteins were evaluated by the method of Bradford (1976). CAT activity was determined by the disappearance of H_2O_2 (Aebi, 1984). The reaction mixture (3 mL) contained 10.6 mM H_2O_2. The reaction was initiated by adding 25 μL of the extract and monitoring the change in absorbance at 240 ηm and 25 °C for 3 min. POD activity was measured using guaiacol oxidation (Bai et al., 1996) in a reaction mixture containing 50 mM phosphate buffer (pH 6.0), 20.1 mM guaiacol, 12.3 mM H_2O_2 and enzyme extract. The increase in absorbance was recorded by the addition of H_2O_2 at 470 ηm for 3 min.

Statistical analysis

All experimental data were statistically analyzed by analyses of variance (ANOVA) using the statistical software package SPSS 16.0. Tukey's test was used to compare means within and among treatments. All statements of significance were based on a probability of $P \leq 0.05$.

Results

After 97 d under mycorrhizal association, morphological and physiological characteristic of AM were observed in *Pistacia vera* roots inoculated with *G. etunicatum*. Extraradical hyphae were associated with the roots, arbuscules and vesicles and were observed in cortical cells. The symptom of drought injury appeared and shoots became wilted. Moreover, plant injury was more severe in NM than M-treated plants.

ANOVA (Table 1) showed that drought stress had significant negative effects on all parameters except K and Mg content, root proline, CAT and POD activities. However, AMF treatments had a significant positive effect on all parameters except Mg content, root soluble carbohydrates, and root and shoot CAT activity. The interactions between drought stress and AMF were significant for AMF colonization, leaf area, Cu and Zn content, shoot and root soluble carbohydrate and root soluble proteins in plants.

The mycorrhizal infection rates of the seedlings under different treatments are presented in Table 2. No mycorrhizal colonization was observed in the plants that did not receive the AM inoculum. However, in AM-treated seedlings that were infected by AMF, the root length colonization percentage (RLC %) was reduced by drought stress (Table 2). The shoot and root weights, leaf area, total chlorophyll and flavonoid content increased significantly ($P < 0.05$) in mycorrhizal more than in nonmycorrhizal plants under drought and non-drought conditions (Table 2). With the exception of flavonoid content, both M and NM parameters decreased with drought stress.

The drought treatment resulted in a significant decrease in P, Zn and Ca uptake by the shoots of M and NM plants (Table 3). Moreover, Fe content in M plants, and N and Cu content in NM plants were reduced significantly under drought stress (Table 3). Under drought stress and well-watered conditions in M plants, significantly high contents of P, K and Cu were observed compared to NM plant. Although N, Fe, Zn and Ca contents in shoots were higher in AM than NM seedlings, the differences were only significant under drought stress conditions for N and Ca content and for Fe and Zn contents under the well-watered condition. However, Mg contents in shoots were not affected by AM symbiosis and drought stress.

The data presented in Table 4 show that water stress increased proline content in both leaves and roots of AM-inoculated and non-AM inoculated plants compared with the well-watered conditions. Proline content in the leaves was markedly lower in mycorrhizal than in nonmycorrhizal plants during drought stress. Moreover, there were no significant differences in proline contents in roots of M and NM plants under well irrigated and drought stress conditions. Also, drought stress increased the accumulation of soluble sugar in both leaves and roots of M and NM plants compared with those under well-watered conditions. In addition, AM seedlings consistently had a higher content of soluble sugars in leaves than non-AM under water stress conditions. However, AMF colonization significantly increased the soluble sugar contents in roots under well irrigation conditions.

Under water stress, soluble proteins in shoots showed a significant decrease in non-AM but not in M plants. However, it was increased significantly in roots in AM and nonsignificantly reduced in NM plants (Table 5). AM-treated plants showed a higher content of soluble proteins in shoots and roots compared with NM during the whole water treatment. The differences for shoots and roots were significant under water stressed conditions (Table 5). Water stress also showed an opposite effect on CAT and POD activity in roots and shoots and significantly increased the activity of CAT and POD in both AM and non-AM leaves. In roots, water stress resulted in a nonsignificant decrease in the activity of CAT and POD in both AM and non-AM plants (Table 5). Mycorrhizal inoculation showed a different effect on CAT and POD activity in roots and leaves. On the other hand, CAT activity in leaves and roots was not affected significantly by mycorrhizal inoculation during the whole water period (Table 5). POD activity in leaves and roots of AM plant was higher than in non-AM plant under well-water and drought stress but it did not significantly affected in roots of well-watered (Table 5).

Discussion

In the present study, physiological and biochemical aspects related to water relations and drought tolerance in AM and non-AM-treated plants subjected to the drought stress were

Table 2
Effects of inoculation with *G. etunicatum* and water regime on root length colonization (RLC), growth variables and total chlorophyll and flavonoids content in seedlings of *P. vera*.

Water regime	AMF status	RLC percent	Shoot weight (g/plant)		Root weight (g/plant)		Leaf area (mm^2)	Total chlorophyll (mg/g fresh wt)	Flavonoids content (mg/g fresh wt)
			Fresh	Dry	Fresh	Dry			
Well irrigated	M	49.6^{a}	5.7^{a}	1.97^{a}	1.93^{a}	0.4^{a}	928^{a}	5.03^{a}	6.1^{ad}
	NM	0.0^{c}	4.6^{b}	1.46^{b}	1.23^{b}	0.29^{b}	752^{b}	3.76^{b}	5.03^{b}
Drought stressed	M	32^{b}	3.53^{c}	1.54^{b}	1.31^{b}	0.33^{ab}	703^{c}	3.29^{b}	7.04^{c}
	NM	0.0^{c}	2.36^{d}	1.04^{c}	0.69^{c}	0.19^{c}	513^{d}	3.24^{c}	5.9^{bd}

Within each column, means superscript with different letters are significantly difference at $P < 0.05$.

Table 3
P, K, Ca, Mg, N (mg/g dry wt) Zn, Fe and Cu (μg/g dry wt) concentrations in M and NM *P. vera* seedlings grown under well irrigated or drought stressed conditions.

Water regime	AMF status	P	N	K	Mg	Ca	Fe	Zn	Cu
Well irrigated	M	20.3^{a}	275^{a}	1272^{a}	152^{a}	729^{a}	2869^{a}	732^{a}	68^{a}
	NM	15.2^{b}	256^{a}	1075b^{c}	160^{a}	704^{ab}	1932^{b}	623^{b}	50^{b}
Drought stressed	M	12.93^{b}	258^{a}	1234^{ab}	157^{a}	672^{b}	1988^{b}	589^{bc}	61^{a}
	NM	9.74^{c}	221^{b}	1013^{c}	166^{a}	613^{c}	1469^{b}	534^{c}	36^{c}

Within each column, means superscript with different letters are significantly difference at $P < 0.05$.

Table 4
Soluble sugar and proline content in shoots and roots of AM or NM pistachio plants under well irrigated or drought stressed conditions.

Water regime	AMF status	Soluble sugar (mg/g fr wt)		Proline content (mg/g fr wt)	
		Leaf	Root	Leaf	Root
Well irrigated	AM	3.28^{ab}	1.39^{a}	0.21^{a}	0.17^{a}
	NM	2.89^{b}	0.96^{b}	0.24^{a}	0.19^{a}
Drought stressed	AM	4.78^{c}	1.83^{c}	0.39^{b}	0.28^{b}
	NM	3.71^{ad}	1.94^{c}	0.51^{c}	0.31^{b}

Within each column, means superscript with different letters are significantly difference at $P < 0.05$.

investigated. Mycorrhiza enhanced plant weight, leaf area and plant pigments under both well-irrigated and water stressed conditions. This increase in plant growth has been attributed to the improvement of water uptake resulting in the enhancement of P nutrition (Safir et al., 1972; Davies et al., 1992; Ruiz-Lozano, 2003; Roldán et al., 2008) and direct water uptake and transport via external hyphae (Ruiz-Lozano and Azcón, 1995; Augé et al., 2003). Moreover, increasing leaf area and chlorophyll content in M-treated plants may be attributed directly to the enhancement of photosynthesis associated with the increase of P uptake in plant (Dietz and Foyer, 1986). On the other hand, the enhancement of plant weight related to root AMF colonization was decreased under water stressed conditions. This may have occurred due to the reduced AMF-root colonization under water stressed conditions, and consequently, reduced the effects of AMF on plant growth. Similar results were reported by Ryan and Ash (1996) and Al-Karaki and Al-Raddad (1997a).

In the current study, the formation of AM fungi induced a significant improvement in the content of leaves pigment under drought stress which is consistent with the results in previous research (Demir, 2004; Wu et al., 2006; Zhao and He, 2007). These results suggest that the formation of mycorrhiza could increase the rate of leaf transpiration, reduce leaf temperature and restrain the decomposition of chlorophyll.

Several studies have indicated that AM contributes to plant growth via assimilation of immobile soil nutrients (Al-Karaki and Al-Raddad, 1997a). In the current study, colonized pistachio plants had considerably higher mineral nutrient contents (P, N, K, Ca, Zn, and Cu) than nonmycorrhizal plants under both well irrigated and water stressed conditions. This probably resulted from the greater absorption of the surface area provided by extensive fungal hyphae (Raju et al., 1990; Ruiz-Lozano et al., 1995; Al-Karaki and Al-Raddad, 1997b; Marulanda et al., 2007; Navarro et al., 2011). The changes in plant growth were associated with root colonization by *G. etunicatum* and have frequently been correlated with an increase in phosphorus nutrition of the host plant. The extra P in M-treated plants could be due either to an indirect mycorrhizal effect on the root structure or physiology or to direct uptake by hyphae with subsequent transfer to the root or both. The enhancements of plant growth attributed to AMF root colonization were decreased

Table 5
Soluble proteins, CAT and POD activities in shoots and roots of AM and non-AM pistachio plants grown under well irrigated or drought stressed conditions.

Water regime	AMF status	Soluble proteins (mg/g fr wt)		CAT activity (mg protein/min)		POD activity (mg protein/min)	
		Shoot	Root	Shoot	Root	Shoot	Root
Well irrigated	M	0.66^{a}	0.50^{a}	1.37^{ab}	0.86^{a}	3.26^{a}	2.94^{a}
	NM	0.63^{a}	0.46^{a}	1.22^{a}	0.83^{a}	2.73^{b}	2.7^{ab}
Drought stressed	M	0.64^{a}	0.69^{b}	1.94^{c}	0.78^{a}	4.72^{c}	2.85^{a}
	NM	0.51^{b}	0.43^{a}	1.72^{bc}	0.81^{a}	3.9^{d}	2.56^{b}

Within each column, means superscript with different letters are significantly difference at $P < 0.05$.

under drought stress. This may be due to the decrease of hyphal P transport into roots and the uptake by the plant under these conditions. The mobility of Cu, Zn, P and Fe in soils is consequently low; mycorrhizal plants could take up more metal nutrients via extraradical hyphae, which provide larger surface areas than the roots alone, reduce the distance for diffusion and enhance the absorption of immobile metal nutrients (Jaleel et al., 2007). The higher density of extraradical hyphae in soil, the greater absorption surface and the more effectively mycorrhizal plants to absorb these low-mobility metal nutrients (Azevedo-Neto et al., 2006). Enhanced acquisition of P, Zn, Cu and Fe by mycorrhizal plants has been reported (Kafkas and Ortas, 2009; Navarro et al., 2011). On the other hand, potassium plays an important role in the drought tolerance of plants and is related to stomatal movement in responses to the changes produced in leaf water status (Ruiz-Lozano and Azcón, 1995). Thus, the protection of M-treated plants against water stress is partially related to K uptake.

In the current study, mycorrhizal colonization improved nutritional status in pistachio plants exposed to drought stress. These results are consistent with those reported by Frey and Schüepp (1993) and Tobar et al. (1994), who indicated that the external mycelium of AM fungi activity assists the host plants to enhance N uptake and translocation when water availability is limited. Moreover, Ca content increased in M and NM-treated plants under well-irrigated and drought conditions, which is consistent with other reports (Pai et al., 1994). Indirectly, these increments may have a positive impact on host plant drought resistance. Shoot concentrations of magnesium do not appear to be affected by AM symbiosis and drought conditions, since Mg is usually abundant in most soils and its deficiency is rare. This finding agrees with a previous report in other plants (Wu et al., 2007a). Extraradicular hyphae are less likely to contribute to uptake of Mg when this element is in a good supply in the soil (Liu et al., 2002). Thus, the effects of AM on host growth during drought are often related to increase the uptake of other nutrients such as P, Zn, Cu and N (Thangadurai et al., 2010).

In order to tolerate drought stress, plants accumulate a high concentration of low molecular-mass organic solutes such as soluble sugars, proline or other amino acids to regulate the osmotic potential of cells aiming at improving absorption of water under drought stress (Zhang et al., 2010). Our data indicated that the concentrations of soluble sugars and proline in leaves and roots increased during water stress in both AM and non-AM treated plants. These results are in agreement with previous reports (Porcel and Ruiz-Lozano, 2004; Wu and Xia, 2004, 2009; Zhang et al., 2010). The content of soluble sugar in the leaves of AM-treated plants was higher than that in non-AM treated leaves during water treatments, confirming the earlier findings of Subramanian and Charest (1995) and suggest that natural physiological metabolism of non-AM treated leaves was less than in AM-treated leaves during well-watered and water stress conditions. On the other hand, these results also suggest that AM inoculation was propitious to the accumulation of carbohydrates, especially soluble sugars, in adversity conditions, resulting in a decrease of osmotic potentials in host cells (Wu et al., 2006).

The enhanced sugar content in AM roots under well-irrigated conditions may be due to the sink effect of the mycorrhizal fungus demanding sugars from shoot tissues. Under drought stress, the soluble sugar in roots was similar in both AM and non-AM treated plants and confirmed the earlier finding (Porcel and Ruiz-Lozano, 2004). Proline, the other osmoregulator measured in this study, was lower in the leaves of AM-treated plants than non-AM treated leaves when exposed to drought stress conditions. These results may be attributed to either greater drought resistance of AM seedlings or less injury in AM seedlings grown under drought stress conditions (Subramanian and Charest, 1995; Porcel and Ruiz-Lozano, 2004; Wu et al., 2006, 2007b; Wu and Xia, 2009). Under certain conditions, some plants produce a large amount of proline to enhance osmosis and prevent dehydration (Lu et al., 2007). Thus, the accumulation of proline and soluble sugar in roots could provide the plant with an osmotic mechanism to maintain a favorable potential gradient for water entrance into the roots (Irigoyen et al., 1992) leading to a lower stress injury in the plant (Porcel and Ruiz-Lozano, 2004).

It is well documented that water deficit in plants increases the concentration of free radicals in cells, resulting in oxidative stress (Ruiz-Lozano, 2003). Plants possess a number of antioxidant mechanisms (including enzymatic and nonenzymatic antioxidant) to protect themselves against the production of reactive oxygen species (ROS). In the present study, AM-treated pistachio had a higher content of soluble proteins in the leaves and roots under drought stress, indicating that AM infection might alleviate or decrease RNA disassembly and might enhance the ability of the nonenzymatic antioxidant defense system by means of soluble proteins (Zhang and He, 2007; Baozhong et al., 2010). Huang et al. (2011) reported that AM symbiosis may increase the drought resistance of plants by promoting antioxidant enzymes such as POD and CAT in plants under water stress.

Previous reports have indicated that the formation of AM could reduce the activity of POD, and CAT reached its peak in the middle period of drought stress (Liu et al., 2007; Zhao and He, 2007). In the present study, POD activities of *P. vera* seedlings inoculated with *G. etunicatum* were increased significantly compared to uninoculated seedlings, whether the host plants were under well-watered or drought conditions. These results suggest that the activation of enzymatic activity of the antioxidant system by AMF does not always occur under adverse conditions. In contrast to POD activities, CAT activities did not vary significantly among the treatments under AM colonization. Drought stress induced an increase of CAT activities in AM and NM-treated seedling shoots, resulting in great drought tolerance of pistachio plants regardless of AMF colonization.

Overall, two possibilities can be suggested to explain the low oxidative damage found in the AMF-treated plants: (1) either they suffered less drought stress due to a primary drought avoidance effect by the symbiosis (e.g. by direct water uptake by fungal hyphae and transfer to the host plant) which kept plants protected against the generation of ROS or (2) mycorrhizal infection increased the activities of a set of defense enzymes, especially PODs, involved in the elimination of ROS.

The current study revealed that AM seedlings tended to have a higher content of flavonoids during irrigation water and water stress induced increment of flavonoids content in AM seedlings. Flavonoids are the most common secondary metabolites in vascular plants (Stafford, 1994). In contrast to pharmacological properties of flavonoid pigments, numerous *in vitro* studies have indicated that flavonoids can directly scavenge molecular species of active oxygen, such as superoxide (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical (•OH), singlet oxygen ($^1O_2$), or peroxyl radical (Bors et al., 1990; Yamasaki et al., 1996). These results suggest that flavonoids may contribute to the overall mechanism for protecting cells from oxidative damage in addition to their actions as optical filters (Gould et al., 1995).

In conclusion, the results of the current study suggest that the presence AM fungi inside the roots of *P. vera* increased plant drought tolerance by means of drought avoidance and drought tolerance mechanisms. It appears that the AM symbiosis enhances antioxidant enzymes, adjusting osmotic and nutrient acquisition under drought stress. These findings contribute to our knowledge of AM- induced drought stress tolerance.

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