

Nutrient Uptake and Distribution in Mycorrhizal Pistachio Seedlings under Drought Stress

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ABSTRACT

This study was conducted to determine the effects of arbuscular mycorrhizal fungi (*Glomus mosseae* and *Glomus intraradices*) symbiosis on mineral uptake of two pistachio cultivars (*Pistacia vera* cv. Qazvini and *Pistacia vera* cv. Badami-Riz-Zarand) grown in the greenhouse under different drought stress levels. Drought stress (DS) reduced the mycorrhizal colonization in both cultivars as well as nutrient uptake. The mycorrhizal plants had higher P, K, Zn and Mn concentrations than non-mycorrhizal plants regardless of soil moisture conditions while Cu and Fe concentrations were unchanged. Distribution of elements was affected by AMF treatments where all except P were accumulated more in leaves than in roots. Contrastingly, under drought conditions, the absorbed elements tended to remain in root tissue. In the case of P and Mn uptake, Qazvini was superior in comparison with Badami. In conclusion, it is suggested that AMF inoculation improves drought tolerance of pistachio cultivars at least in part through the enhanced uptake of slowly diffusing mineral ions such as PO_4^{2-} and Zn^{2+} . Moreover, arbuscular mycorrhizal (AM) colonization provides better osmotic adjustment which can be correlated with K^+ accumulation in top portions of inoculated plants. Results of this study also emphasized that 'Qazvini' cultivar may be more tolerant to drought than 'Badami'.

Keywords: Drought stress, Mycorrhizae, Nutrition, Pistachio.

INTRODUCTION

Pistachio (*Pistacia vera* L.) is one of the most important commercial trees grown in arid and semi-arid regions of Iran. One of the largest pistachio production centers in the world is Rafsanjan (FAOstat, 2006) which is located in the north-west of Kerman Province in the south-east of Iran. Increased establishment of irrigated pistachio orchards during the last two decades in this region has decreased the availability of underground water resources and prolonged drought periods is the major concern for the pistachio producers (Sheibani, 1994). However, competition for the limited water supply available for irrigation of pistachio orchards is

increasing. Although pistachio nut trees are drought tolerant, it does not mean that pistachio trees require less water for optimal performance.

Drought stress, in general, reduces nutrient availability in the soil, uptake by roots, transport from roots to shoots and partitioning in plants (Goicoechea *et al.*, 1997). In conditions of water deficit, some reports revealed disturbance in nutritional status of pistachio plants (Sepaskhah and Karimi-Goghary, 2003, 2005; Tajabadi Pour *et al.*, 2005). On the other hand, mycorrhizal fungi colonize plant roots and often enhance host plant growth and mineral nutrient acquisition, particularly for the plants grown under unfavorable soil conditions (Al-Karaki *et al.*,

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2004). An increase in the concentration of nutrients content of mycorrhizal plants under drought stress was reported by Wu and Zou (2009). The responses to AM fungi have been mainly attributed to enhanced uptake and translocation of the slowly diffusing nutrient ions such as PO_4^{2-} , NH_4^+ , Zn^{2+} and Cu^{2+} (Liu, 2000; Bi, 2003). The external hyphae of AM fungi play a vital role, especially in the host plant's P nutrition. The hyphae explore a soil volume extending beyond the depletion zone around the roots and thus provide access to P which is otherwise only transported by slow diffusion processes (Bi, 2003). A further hypothesis suggests that AM fungi modify rhizosphere pH thereby altering the availability of some nutrients (Ortas *et al.*, 2004). The degree of AM fungi response increases with decreasing soil fertility and with increasing the intensity of drought stress. Therefore, AM fungi, as an important factor in nutrient acquisition, may improve drought resistance under suboptimal plant growth conditions (Garcia, 2008).

There is a little information on the ability of pistachio to establish symbiotic relations with AMF. Ferguson (1998) reported that inoculation of three pistachio rootstocks (*P. atlantica*, *P. integerrima* and UCBI) at 4-5 leaf stage resulted in colonization percentages ranging from 39 to 80% with no significant difference in colonization extent between rootstocks. In this experiment, the maximum growth rate was obtained with mycorrhizal treatments. Kafkas and Ortas (2009) inoculated two genotypes from each of *P. vera* (cvs. 'Siirt' and 'Kirmizi'), *P. eurycarpa*, *P. atlantica* and *P. terebinthus* with ten different mycorrhizal species. They found significant differences between *Pistacia* species in growth, nutrient uptake and the percentage of mycorrhizal infection. Also mycorrhizal species were different in terms of enhancing plant growth and nutrient uptake. Mycorrhizal infection was 70–95% in *P. vera*, 56.7–95% in *P. eurycarpa*, 53.3–95% in *P. atlantica*, and 51.7–91.7% in *P. terebinthus*.

It is not clearly known whether the fungi have proper potential to establish symbiosis with pistachio under drought stress condition

and if so, whether the symbiosis benefits are large enough to have practical value. The present study was planned to address these questions determining the effects of two AM fungi, *Glomus mosseae* and *Glomus intraradices* on the nutrient uptake and distribution in different plant parts under different drought stress levels. However, both cultivars used in this experiment (Qazvini and Badami-Riz-Zarand) are well known in the region as drought tolerant rootstocks and we assumed that mycorrhizal symbiosis may improve this good trait.

MATERIALS AND METHODS

Experimental Site

A greenhouse experiment was conducted in 2009 at the Agri-College of Vali-e-Asr University of Rafsanjan $30^{\circ} 23' 06''$ N, $55^{\circ} 55' 30''$ E, at 1,523 m asl.

AM Inoculum Production

Glomus mosseae and *Glomus intraradices* [kindly supplied by Dr. E. Sedaghati (Plant Protection Department, Faculty of Agri-College, Vali-e-Asr University)] were propagated in a sterile potted soil cropped with wheat between February and May 2009. AM fungal inoculum consisted of a mixture of rhizospheric soil from trap cultures (*Triticum spp.*) containing spores, hyphae and mycorrhizal root fragments.

Soil Preparation and Seed Sowing

The soil used was an autoclaved sandy loam with the following characteristics: sand 72.2%, silt 14.2%, clay 13.6%, pH 7.6, P 16 mg kg^{-1} soil (Olsen *et al.*, 1954), K 76 mg kg^{-1} soil (Knudsen *et al.*, 1982), Fe 0.1 $\mu\text{g g}^{-1}$, Zn 1.29 $\mu\text{g g}^{-1}$, Mn 1.3 $\mu\text{g g}^{-1}$, Cu 1.35 $\mu\text{g g}^{-1}$ (Lindsay and Norvell, 1978) and cation exchange capacity 2.65 dS m^{-1} (Bouyoucos, 1951).

Seeds of two pistachio rootstocks, *P. vera* cv. Badami-Riz-Zarand and *P. vera* cv. Qazvini, were surface sterilized in 5% sodium hypochlorite and then incubated at 30°C on sterile moist cloth for one week. Five germinated seeds were sown in each pot containing 5 kg of autoclaved soil. The number of seedlings per pot was reduced to 3 within 21 days of germination.

Mycorrhizal Inoculation

One hundred grams (fresh mass) of inoculum having an average of 75% of infected roots was placed on the soil surface immediately before planting and after placing the germinated seeds on it, seeds were covered with sterilized sand. Control plants received the same amount of an autoclaved mixture of both inocula. The growth of seedlings continued for a more 120 day period before the start of irrigation treatments, during which, the seedlings were watered every day up to the field capacity (FC) level with distilled water.

Irrigation Treatments

The four water regimes were 100% FC (as control), 75% FC (mild stress), 50% FC (moderate stress) and 25% FC (severe stress). To determine the water content of soil mixture at FC level, it was calculated based on the pot weight, soil dry weight and soil wet weight after watering and ceasing the runoff. Thereafter, for 50 days (from 1 July to 20 August 2009), soil water contents were determined by weighing the pots daily and water was added following the time of weighing to maintain the predetermined water content in each pot. During the experiment, the maximum temperature was 35±4°C, the minimum temperature was 21±3°C and the relative humidity was 55±5% with saturated light (without additional artificial lightening).

Root Sampling and Assessment of Arbuscular Mycorrhizal Colonization

Root samples with soil were excavated from individual plants and carefully rinsed with

running tap water and cut into 1 cm long fragments. Samples for mycorrhizal assessment were prepared according to the method of Philips and Hayman (1970). Mycorrhizal colonization was estimated after Giovannetti and Mosse (1980) at 100× magnification using 40 root segments of each sample.

Nutrient Analysis

Dried samples of roots, stems and leaves were weighed separately and ground to pass a 40-mesh sieve. The ground plant samples were dry-ashed at 500°C, the ashes dissolved in 10 cc HCl (2N) and made the volume to 100 cc with distilled water. Concentrations of Mn, Fe, Zn and Cu were determined by atomic absorption spectrometry. Potassium concentration was determined by flame photometry and Phosphorus concentration in the digest was determined by the ammonium molybdate blue method using spectrophotometry (Chapman *et al.*, 1982).

Statistical Analyses

A completely randomized design method was adopted in the experiment with three mycorrhizal treatments, four drought stress levels and two pistachio cultivars in three replications. The data were statistically analyzed using Analysis of Variance (ANOVA) and the means were separated by Duncan's multiple range test ($P < 0.05$) using MSTATC software.

RESULTS

Root Colonization

The rate of root colonization was similar between the two cultivars and both AM treatments. DS reduced the mycorrhizal colonization in both cultivars to the same degree but the difference was significant only with severe drought stress level. No

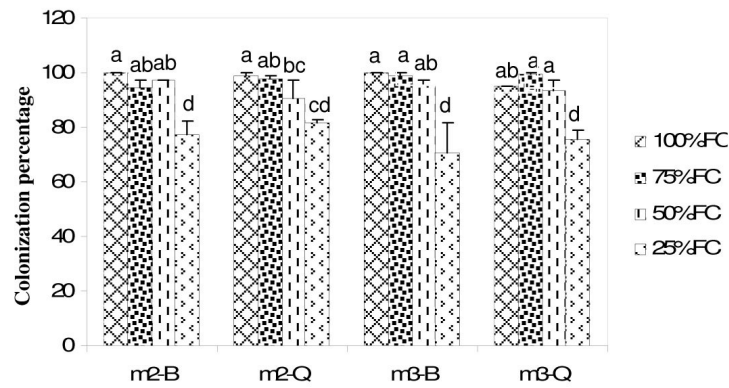


Figure 1. Effect of different water stress levels on mycorrhizal colonization percentage in ‘Badami’ (B) and ‘Qazvini’ (Q). Bars indicate the standard error. (M₂: *G. mosseae*, M₃: *G. intraradices*)

AM structures were noted in the roots of uninoculated seedlings (Figure 1).

Plant Nutrients Uptake and Distribution

Concentrations of selected nutrients in the pistachio plants are presented in Table 1. Nutrient concentrations of the elements in leaves, stems and roots and the corresponding distribution percentages in the same tissues are presented in Tables 2, 3 and 4 and Figures 2 and 3.

Regardless of DS treatments and cultivar,

the uptake of P by +M plants showed a significant increase. It is evident that P content of M₂ and M₃ treatments were 83.7 and 64.3% more than the control, -M plants (Table 1). The concentrations of P in different parts of +M plants were significantly higher than -M plants. The mean increased percentage was 37 and 46% in leaf, 151 and 148% in root and 125 and 56% in the stem of M₂ and M₃, respectively as compared to -M plants (Table 2). DS had no significant influence on P concentration irrespective of AM treatments and cultivar (Table 5) but it changed the partitioning pattern of P in plants body. As it is evident

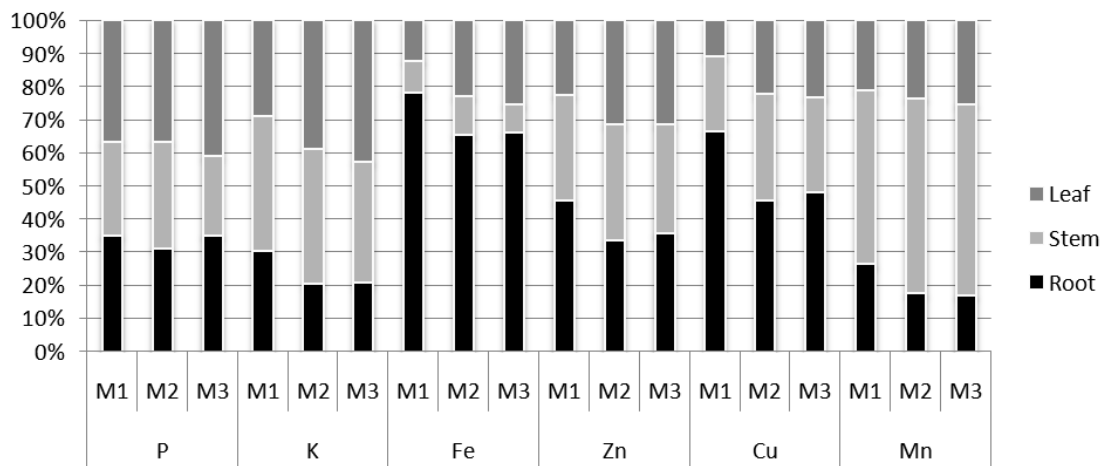


Figure 2. Effect of AMF treatments (M₁: Control; M₂: *G. mosseae*, M₃: *G. intraradices*) on P, K, Fe, Zn, Cu and Mn distribution percentage in different plant parts of pistachio seedlings regardless of drought stress level and cultivar.

Table 1. Means (n= 3) and standard errors (in parentheses) for P, K (%), and Fe, Zn, Cu, Mn ($\mu\text{g g}^{-1}$) concentrations of two pistachio cultivars after 50 days in drought - stressed or well-watered conditions with or without AMF colonization.

AMF status	Water status	<i>Pistacia Vera</i> Cv. Badami						<i>Pistacia Vera</i> Cv. Qazvini					
		P	K	Zn	Cu	Mn	Fe	P	K	Zn	Cu	Mn	Fe
Control	100%	0.12 ^{e-g*}	2.73 ^{b-e}	68 ^{efg}	49 ^d	53 ^{gh}	120 ^d	0.13 ^{dh}	2.83 ^{a-e}	68 ^{efg}	52 ^{bcd}	72 ^d	165 ^{acd}
	FC	(±0.00)	(±0.08)	(±2.60)	(±1.9)	(±2.80)	(±5.01)	(±0.00)	(±0.02)	(±1.60)	(±0.84)	(±5.50)	(±2.05)
	75%	0.10 ^{gh}	2.64 ^{cde}	68 ^{efg}	52 ^{bcd}	54 ^{gh}	168 ^{a-d}	0.11 ^{gh}	2.72 ^{b-e}	67 ^{fg}	51 ^{cd}	71 ^{de}	212 ^{a-d}
	FC	(±0.00)	(±0.15)	(±1.38)	(±1.10)	(±2.30)	(±26.50)	(±0.00)	(±0.25)	(±2.50)	(±3.30)	(±3.50)	(±32.50)
	50%	0.10 ^h	2.56 ^{de}	70 ^{c-g}	56 ^{cd}	51 ^{gh}	186 ^{a-d}	0.13 ^{dh}	2.88 ^{a-e}	65 ^g	53 ^{a-d}	60 ^{ef}	209 ^{acd}
	FC	(±0.01)	(±0.14)	(±2.50)	(±3.07)	(±7.20)	(±28.30)	(±0.00)	(±0.34)	(±4.10)	(±4.80)	(±5.90)	(±51.50)
<i>G. mosseae</i>	25%	0.17 ^{c-h}	2.40 ^e	78 ^{bc}	63 ^a	53 ^{gh}	236 ^{abc}	0.18 ^{ba}	2.60 ^{de}	68 ^{efg}	50 ^d	51 ^{fi}	180 ^{a-d}
	FC	(±0.03)	(±0.12)	(±1.26)	(±4.70)	(±2.60)	(±33.80)	(±0.03)	(±0.25)	(±4.40)	(±1.60)	(±5.20)	(±24.90)
	100%	0.21 ^{a-f}	3.26 ^a	77 ^{bcd}	56 ^{cd}	50 ^{fi}	198 ^{a-d}	0.26 ^{abc}	3.21 ^{ab}	76 ^{bcd}	54 ^{a-d}	97 ^a	152 ^{bcd}
	FC	(±0.03)	(±0.10)	(±1.28)	(±1.60)	(±2.90)	(±35.70)	(±0.04)	(±0.20)	(±3.60)	(±2.40)	(±3.30)	(±12.30)
	75%	0.19 ^{b-h}	2.86 ^{a-e}	80 ^b	56 ^{cd}	37 ^{ij}	152 ^{bcd}	0.30 ^a	3.03 ^{a-d}	75 ^{b-f}	54 ^{a-d}	94 ^{ab}	133 ^d
	FC	(±0.00)	(±0.22)	(±1.40)	(±0.93)	(±1.20)	(±26.50)	(±0.05)	(±0.04)	(±0.80)	(±0.80)	(±8.20)	(±11.20)
<i>G. intraradices</i>	50%	0.22 ^{a-d}	2.82 ^{a-e}	74 ^{b-g}	52 ^{bcd}	42 ^{hij}	186 ^{a-d}	0.28 ^{ab}	3.16 ^{abc}	77 ^{bcd}	61 ^{abc}	82 ^{bcd}	258 ^a
	FC	(±0.01)	(±0.02)	(±3.20)	(±5.20)	(±2.80)	(±30.90)	(±0.08)	(±0.15)	(±2.50)	(±3.01)	(±5.60)	(±25.80)
	25%	0.17 ^{c-h}	2.64 ^{cde}	75 ^{b-f}	54 ^{cd}	47 ^{fi}	195 ^{a-d}	0.26 ^{abc}	2.56 ^{de}	89 ^a	62 ^{ab}	56 ^{fg}	201 ^{a-d}
	FC	(±0.01)	(±0.14)	(±1.04)	(±2.3)	(±3.20)	(±29.10)	(±0.02)	(±0.19)	(±0.03)	(±1.10)	(±3.50)	(±10.80)
	100%	0.25 ^{abc}	3.20 ^{ab}	75 ^{b-f}	56 ^{cd}	45 ^{g-j}	199 ^{a-d}	0.21 ^{abg}	3.21 ^{ab}	77 ^{bcd}	57 ^{a-d}	80 ^{cd}	175 ^{acd}
	FC	(±0.03)	(±0.09)	(±3.20)	(±2.60)	(±4.20)	(±32.90)	(±0.01)	(±0.02)	(±1.50)	(±0.89)	(±1.50)	(±63.30)
Control	75%	0.18 ^{b-h}	2.91 ^{a-e}	71 ^{c-g}	55 ^{cd}	45 ^{g-j}	192 ^{a-d}	0.24 ^{abc}	3.13 ^{abc}	76 ^{b-e}	53 ^{a-d}	83 ^{bcd}	147 ^{bcd}
	FC	(±0.00)	(±0.06)	(±2.40)	(±5.20)	(±2.10)	(±43.80)	(±0.05)	(±0.02)	(±2.40)	(±1.60)	(±2.40)	(±5.90)
	50%	0.21 ^{a-f}	2.87 ^{a-e}	70 ^{c-g}	50 ^d	32 ⁱ	137 ^{cd}	0.22 ^{a-e}	2.96 ^{a-d}	74 ^{b-f}	56 ^{a-d}	89 ^{abc}	206 ^{acd}
	FC	(±0.02)	(±0.07)	(±1.16)	(±1.50)	(±3.10)	(±40.10)	(±0.02)	(±0.10)	(±2.14)	(±0.73)	(±3.70)	(±4.80)
	25%	0.16 ^{c-h}	2.73 ^{b-e}	74 ^{bcd}	63 ^a	50 ^{fi}	241 ^{ab}	0.22 ^{a-d}	2.53 ^{de}	69 ^{d-g}	54 ^{a-d}	93 ^{abc}	220 ^{a-d}
	FC	(±0.00)	(±0.03)	(±3.90)	(±6.70)	(±1.20)	(±4.90)	(±0.03)	(±0.14)	(±0.70)	(±2.60)	(±2.80)	(±20.40)

*Different letters within a column indicate significant differences ($P \leq 0.05$) using the Duncan's multiple-range test.



Table 2. Means (n= 3) and standard errors (in parentheses) for P and K concentration (%) in roots, stems and leaves of two pistachio cultivars after 50 days in drought - stressed or well-watered conditions with or without AMF colonization.

AMF status	Water status	<i>Pistacia Vera Cv. Badami</i>						<i>Pistacia Vera Cv. Qazvini</i>					
		P			k			P			K		
		Leaf	Stem	Root	Leaf	Stem	Root	Leaf	Stem	Root	Leaf	Stem	Root
Control	100% FC	0.09 ^{def*} (±0.00)	0.05 ^c (±0.01)	0.02 ^{de} (±0.00)	1.72 ^{c-g} (±0.20)	1.80 ^{abc} (±0.07)	0.56 ^{abc} (±0.04)	0.10 ^{def} (±0.00)	0.047 ^c (±0.00)	0.03 ^{cde} (±0.00)	1.88 ^{a-f} (±0.03)	1.77 ^{abc} (±0.05)	0.58 ^{abc} (±0.02)
	75% FC	0.09 ^{def} (±0.00)	0.03 ^c (±0.00)	0.02 ^e (±0.00)	1.73 ^{c-g} (±0.17)	1.64 ^{bc} (±0.03)	0.56 ^{abc} (±0.03)	0.10 ^{def} (±0.00)	0.03 ^c (±0.00)	0.03 ^{de} (±0.00)	1.91 ^{a-f} (±0.07)	1.61 ^{bc} (±0.25)	0.54 ^{abc} (±0.05)
	50% FC	0.08 ^{ef} (±0.00)	0.04 ^c (±0.01)	0.02 ^{de} (±0.00)	1.64 ^{d-g} (±0.14)	1.60 ^{bc} (±0.09)	0.58 ^{abc} (±0.05)	0.12 ^{b-f} (±0.01)	0.04 ^c (±0.01)	0.02 ^{de} (±0.00)	1.89 ^{a-f} (±0.20)	1.78 ^{abc} (±0.25)	0.63 ^{abc} (±0.07)
	25% FC	0.08 ^{ef} (±0.00)	0.12 ^{abc} (±0.04)	0.04 ^{cde} (±0.00)	1.41 ^{fg} (±0.08)	1.62 ^{bc} (±0.11)	0.55 ^{abc} (±0.02)	0.12 ^{a-f} (±0.01)	0.10 ^{abc} (±0.04)	0.03 ^{cde} (±0.00)	1.86 ^{a-f} (±0.37)	1.52 ^c (±0.07)	0.50 ^{bc} (±0.02)
	100% FC	0.14 ^{a-f} (±0.01)	0.11 ^{abc} (±0.05)	0.05 ^{cde} (±0.00)	2.28 ^{ab} (±0.22)	1.95 ^{abc} (±0.05)	0.64 ^{ab} (±0.03)	0.18 ^a (±0.03)	0.13 ^{abc} (±0.04)	0.07 ^{b-c} (±0.01)	2.29 ^{ab} (±0.20)	1.98 ^{abc} (±0.07)	0.52 ^{bc} (±0.05)
	75% FC	0.14 ^{a-e} (±0.01)	0.06 ^{bc} (±0.00)	0.07 ^{b-c} (±0.00)	1.88 ^{a-f} (±0.03)	1.72 ^{abc} (±0.29)	0.67 ^a (±0.01)	0.15 ^{a-d} (±0.01)	0.22 ^a (±0.08)	0.07 ^{b-c} (±0.00)	2.03 ^{a-e} (±0.20)	1.97 ^{abc} (±0.13)	0.54 ^{abc} (±0.03)
<i>G. mosseae</i>	50% FC	0.13 ^{a-f} (±0.00)	0.11 ^{abc} (±0.04)	0.08 ^{a-d} (±0.03)	1.89 ^{a-f} (±0.06)	1.71 ^{abc} (±0.11)	0.61 ^{abc} (±0.04)	0.14 ^{a-d} (±0.02)	0.15 ^{abc} (±0.07)	0.12 ^{ab} (±0.04)	2.0 ^{a-e} (±0.17)	2.12 ^a (±0.08)	0.60 ^{abc} (±0.02)
	25% FC	0.07 ^f (±0.00)	0.07 ^{bc} (±0.00)	0.06 ^{cde} (±0.01)	1.75 ^{c-g} (±0.08)	1.66 ^{abc} (±0.16)	0.53 ^{abc} (±0.05)	0.11 ^{c-f} (±0.00)	0.20 ^{ab} (±0.04)	0.07 ^{b-c} (±0.01)	1.26 ^g (±0.02)	1.68 ^{abc} (±0.03)	0.56 ^{abc} (±0.05)
	100% FC	0.17 ^{abc} (±0.01)	0.12 ^{abc} (±0.05)	0.08 ^{b-e} (±0.01)	2.20 ^{abc} (±0.10)	1.91 ^{abc} (±0.14)	0.67 ^a (±0.06)	0.15 ^{a-d} (±0.00)	0.09 ^{abc} (±0.03)	0.06 ^{b-c} (±0.00)	2.37 ^a (±0.17)	1.86 ^{abc} (±0.06)	0.58 ^{abc} (±0.07)
	75% FC	0.12 ^{a-f} (±0.00)	0.07 ^{bc} (±0.00)	0.060 ^{cde} (±0.00)	2.01 ^{a-e} (±0.07)	1.72 ^{abc} (±0.10)	0.61 ^{abc} (±0.02)	0.12 ^{b-f} (±0.03)	0.14 ^{abc} (±0.06)	0.09 ^{abc} (±0.01)	2.22 ^{abc} (±0.18)	1.95 ^{abc} (±0.13)	0.50 ^{bc} (±0.03)
	50% FC	0.14 ^{a-d} (±0.00)	0.11 ^{abc} (±0.03)	0.06 ^{cde} (±0.00)	1.78 ^{b-f} (±0.05)	2.01 ^{ab} (±0.10)	0.49 ^{bc} (±0.03)	0.17 ^{ab} (±0.00)	0.09 ^{abc} (±0.02)	0.05 ^{cde} (±0.00)	2.16 ^{a-d} (±0.10)	1.78 ^{abc} (±0.10)	0.48 ^c (±0.01)
	25% FC	0.12 ^{b-f} (±0.00)	0.06 ^c (±0.00)	0.05 ^{cde} (±0.00)	1.82 ^{b-f} (±0.05)	1.71 ^{abc} (±0.09)	0.56 ^{abc} (±0.03)	0.14 ^{a-e} (±0.01)	0.05 ^c (±0.01)	0.14 ^a (±0.03)	1.60 ^{efg} (±0.03)	1.57 ^{bc} (±0.20)	0.60 ^{abc} (±0.02)

*Different letters within a column indicate significant differences ($P \leq 0.05$) using the Duncan's multiple-range test.

Table 3 Means (n= 3) and standard errors (in parentheses) for micro-nutrients concentration ($\mu\text{g.g}^{-1}$) in roots, stems and leaves of Qazvini cultivar after 50 days in drought-stressed or well-watered conditions with or without AMF colonization.

AMF status	Water Status	Cu			Zn			Mn			Fe		
		Leaf	Stem	Root	Leaf	Stem	Root	Leaf	Stem	Root	Leaf	Stem	Root
Control	100% FC	20 ^{b*} (±0.81)	26 ^{c-f} (±1.90)	33 ^{bc} (±1.33)	37 ^{d-g} (±1.82)	25 ^{c-f} (±0.70)	36 ^a (±2.10)	63 ^d (±7.05)	11 ^{bc} (±0.70)	71 ^{ef} (±2.09)	30 ^{c-g} (±0.81)	147 ^{abc} (±2.26)	
	75% FC	17 ^b (±1.01)	24 ^{ef} (±1.87)	36 ^{bc} (±3.55)	36 ^{fg} (±0.99)	26 ^{b-f} (±2.20)	32 ^{ab} (±7.72)	64 ^d (±3.62)	11 ^{bc} (±1.47)	122 ^{a-d} (±25.57)	35 ^{c-g} (±1.20)	161 ^{abc} (±28.75)	
	50% FC	17 ^b (±0.80)	26 ^{b-f} (±1.09)	36 ^{bc} (±5.62)	37 ^{efg} (±1.66)	25 ^{c-f} (±1.23)	24 ^{abc} (±5.97)	24 ^{abc} (±7.22)	55 ^{de} (±7.22)	10 ^c (±2.05)	92 ^{b-f} (±20.43)	53 ^b (±8.42)	169 ^{abc} (±49.78)
	25% FC	19 ^b (±2.28)	22 ^f (±1.22)	34 ^{bc} (±1.53)	36 ^g (±1.67)	23 ^{def} (±0.08)	24 ^{abc} (±3.50)	44 ^{cd} (±4.01)	9 ^c (±1.10)	86 ^{b-f} (±25.61)	42 ^{b-c} (±4.51)	151 ^{abc} (±27.43)	
	100% FC	22 ^b (±1.73)	29 ^{a-e} (±0.37)	30 ^{bc} (±1.83)	44 ^{abc} (±2.33)	29 ^{a-d} (±1.41)	26 ^{abc} (±3.93)	110 ^a (±7.21)	10 ^c (±0.93)	70 ^{ef} (±5.52)	25 ^{fg} (±1.45)	134 ^{abc} (±14.55)	
	75% FC	22 ^b (±0.62)	31 ^{abc} (±1.19)	28 ^{bc} (±1.60)	46 ^{ab} (±1.24)	29 ^{a-d} (±1.55)	32 ^{ab} (±15.54)	102 ^{ab} (±3.55)	6 ^c (±0.42)	66 ^{ef} (±7.53)	30 ^{c-g} (±2.41)	103 ^{bc} (±7.42)	
<i>G. mosseae</i>	50% FC	23 ^b (±1.39)	32 ^{ab} (±0.73)	36 ^{bc} (±5.88)	46 ^{ab} (±0.35)	32 ^{ab} (±0.03)	21 ^{abc} (±2.69)	85 ^c (±11.43)	17 ^{ab} (±5.80)	105 ^{a-e} (±18.28)	52 ^b (±2.83)	230 ^{abc} (±40.15)	
	25% FC	34 ^a (±1.47)	28 ^{a-e} (±2.20)	29 ^{bc} (±1.37)	41 ^{a-g} (±0.35)	27 ^{a-f} (±1.85)	20 ^{bc} (±2.22)	54 ^{de} (±3.70)	10 ^c (±1.05)	108 ^{a-e} (±6.10)	46 ^{bc} (±7.43)	147 ^{abc} (±8.78)	
	100% FC	25 ^{ab} (±1.20)	30 ^{a-e} (±3.81)	34 ^{bc} (±3.57)	42 ^{a-f} (±3.13)	33 ^a (±1.74)	22 ^{abc} (±1.09)	89 ^{bc} (±1.00)	10 ^{bc} (±2.35)	77 ^{b-f} (±11.98)	28 ^{d-g} (±1.44)	157 ^{abc} (±82.95)	
	75% FC	22 ^b (±1.70)	30 ^{a-e} (±1.48)	28 ^{bc} (±1.35)	46 ^{ab} (±2.85)	30 ^{abc} (±2.43)	22 ^{abc} (±2.89)	94 ^{abc} (±1.58)	7 ^c (±0.85)	96 ^{b-f} (±18.16)	32 ^{c-g} (±2.03)	93 ^c (±9.45)	
	50% FC	24 ^b (±1.26)	30 ^{a-e} (±1.39)	30 ^{bc} (±1.42)	43 ^{a-d} (±1.11)	28 ^{a-e} (±1.03)	29 ^{abc} (±4.74)	99 ^{abc} (±1.13)	9 ^c (±0.31)	125 ^{abc} (±24.68)	39 ^{b-f} (±2.14)	145 ^{abc} (±25.80)	
	25% FC	34 ^a (±0.89)	29 ^{a-e} (±3.33)	30 ^{bc} (±0.14)	41 ^{b-g} (±2.13)	26 ^{c-f} (±0.97)	30 ^{abc} (±4.50)	99 ^{abc} (±3.43)	10 ^c (±0.58)	146 ^a (±29.41)	43 ^{bcd} (±6.71)	140 ^{abc} (±6.70)	
<i>G. intraradices</i>	100% FC	25 ^{ab} (±1.20)	30 ^{a-e} (±3.81)	34 ^{bc} (±3.57)	42 ^{a-f} (±3.13)	33 ^a (±1.74)	22 ^{abc} (±1.09)	89 ^{bc} (±1.00)	10 ^{bc} (±2.35)	77 ^{b-f} (±11.98)	28 ^{d-g} (±1.44)	157 ^{abc} (±82.95)	
	75% FC	22 ^b (±1.70)	30 ^{a-e} (±1.48)	28 ^{bc} (±1.35)	46 ^{ab} (±2.85)	30 ^{abc} (±2.43)	22 ^{abc} (±2.89)	94 ^{abc} (±1.58)	7 ^c (±0.85)	96 ^{b-f} (±18.16)	32 ^{c-g} (±2.03)	93 ^c (±9.45)	
	50% FC	24 ^b (±1.26)	30 ^{a-e} (±1.39)	30 ^{bc} (±1.42)	43 ^{a-d} (±1.11)	28 ^{a-e} (±1.03)	29 ^{abc} (±4.74)	99 ^{abc} (±1.13)	9 ^c (±0.31)	125 ^{abc} (±24.68)	39 ^{b-f} (±2.14)	145 ^{abc} (±25.80)	
	25% FC	34 ^a (±0.89)	29 ^{a-e} (±3.33)	30 ^{bc} (±0.14)	41 ^{b-g} (±2.13)	26 ^{c-f} (±0.97)	30 ^{abc} (±4.50)	99 ^{abc} (±3.43)	10 ^c (±0.58)	146 ^a (±29.41)	43 ^{bcd} (±6.71)	140 ^{abc} (±6.70)	

*Different letters within a column indicate significant differences ($P \leq 0.05$) using the Duncan's multiple-range test.

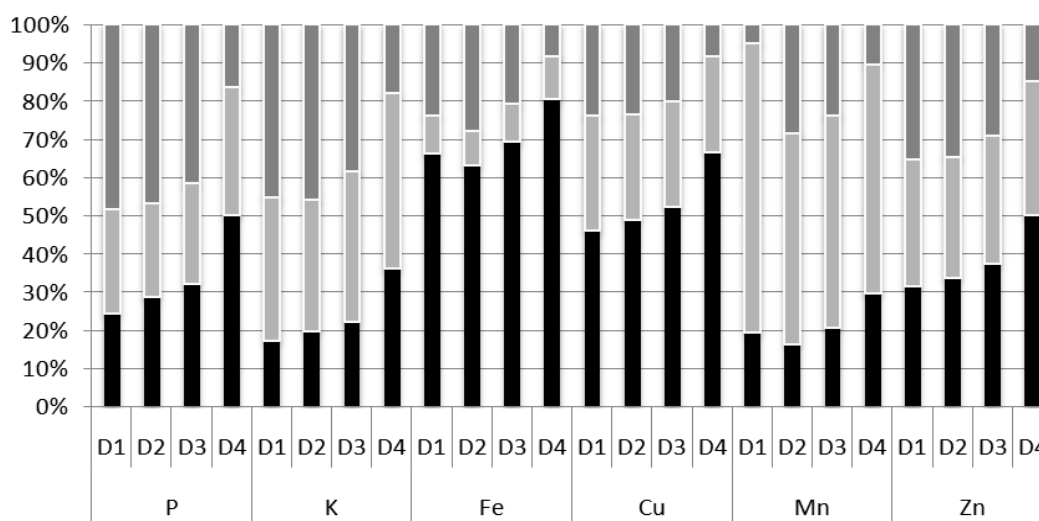


Figure 3. Effect of drought stress levels (D₁: 100% FC; D₂: 75% FC; D₃: 50%FC, D₄: 25%FC) on P, K, Fe, Zn, Cu and Mn distribution percentage in different plant parts of pistachio seedlings regardless of mycorrhizal treatments and cultivar.

from Figure 3, DS intensity caused a remarkable increase in P concentration of root and decrease in leaf P concentration whereas P concentration of stem was unchanged. AM treatments did not change P distribution pattern in root, stem and leaf of treated plants (Figure 2). On average, Qazvini absorbed and accumulated 22.4% more P compared with Badami cultivar (Table 1). No interaction effect was found between AM treatments and DS levels on P uptake (Table 4).

Regardless of DS treatments, AMF notably increased K⁺ levels in whole plant by about 10% in comparison with -M plants (Table 1). The rates of K⁺ accumulation in leaf were 9 and 15% more than control in M₂ and M₃, respectively while it was 10 and 9% more in stem with no significant increase in K⁺ content observed in root of +M plants compared with corresponding controls (Table 2). K⁺ concentration of -M plants was not influenced by DS levels while K⁺ concentration in +M plants was decreased. However, the differences were significant only with severe DS level (Table 1). Furthermore, severe DS resulted in 24 and 14% reduction in mean content of K⁺ in leaf and stem, respectively (Table 2). Both cultivars followed the same pattern in K⁺

uptake and accumulation (Table 5). Data presented in Figure 2 revealed that K⁺ accumulation percentage in root tissue was decreased to 20 and 21% in M₂ and M₃ respectively compared with the control of 30%. At the same time, K⁺ movement toward leaf tissue was increased in M₂ and M₃ by 39 and 43%, respectively in comparison with 29% in the control. DS had a negative effect on K⁺ accumulation in root and leaf tissues irrespective of AM treatments. It caused an increase of root K⁺ content from 17% in the control, well-watered plants to 36% under severe DS and a decrease of leaf K⁺ from 45% in the control to 18% in plants exposed to severe DS (Figure 3).

Mycorrhizal inoculation had a remarkable effect on micro-nutrient contents of *Pistacia vera* seedlings 170 days after seed germination and 50 days after the onset of DS (Table 5). Regardless of DS levels and cultivar, the Zn and Mn concentrations were increased in AM treatments. The Zn concentration was significantly increased by 13.3 and 6.8% in M₂ and M₃, respectively while it was 7.8 and 10.9% for Mn concentration (Table 1). The concentrations of Fe and Cu were not influenced by AMF treatments (Table 5). The Cu, Zn and Fe

Table 4. Means (n=3) and standard errors (in parentheses) for micro-nutrients concentration ($\mu\text{g g}^{-1}$) in roots, stems and leaves of Badami cultivars after 50 days in drought-stressed or well-watered conditions with or without AMF colonization.

AMF status	Water Status	Cu			Zn			Mn			Fe		
		Leaf	Stem	Root	Leaf	Stem	Root	Leaf	Stem	Root	Leaf	Stem	Root
Control	100%	19 ^{b*}	27 ^{a-f}	29 ^{bc}	41 ^{bc}	39 ^{c-g}	22 ^f	29 ^{abc}	44 ^{ef}	7 ^c	53 ^f	32 ^{c-g}	95 ^c
	FC	(±0.26)	(±0.90)	(±2.02)	(±1.08)	(±2.69)	(±1.82)	(±2.06)	(±6.78)	(±0.50)	(±2.53)	(±6.41)	(±39.22)
	75%	18 ^b	25 ^{def}	36 ^{bc}	42 ^{bc}	37 ^{d-g}	23 ^{ef}	27 ^{abc}	42 ^{ef}	11 ^{bc}	60 ^{ef}	32 ^{c-g}	160 ^{abc}
	FC	(±0.80)	(±1.61)	(±1.09)	(±2.09)	(±0.86)	(±1.56)	(±4.16)	(±7.72)	(±1.35)	(±5.67)	(±2.40)	(±47.16)
	50%	18 ^b	28 ^{a-e}	39 ^{ab}	38 ^c	39 ^{c-g}	27 ^{a-f}	26 ^{abc}	39 ^{ef}	12 ^{bc}	59 ^{ef}	44 ^{bc}	175 ^{abc}
	FC	(±0.87)	(±2.40)	(±6.08)	(±2.16)	(±3.27)	(±1.40)	(±2.25)	(±10.21)	(±3.01)	(±9.05)	(±12.56)	(±47.16)
25%	18 ^b	29 ^{a-e}	47 ^a	38 ^c	47 ^a	32 ^{ab}	18 ^{bc}	44 ^{ef}	19 ^a	48 ^f	51 ^b	255 ^a	
FC	(±0.41)	(±1.30)	(±5.67)	(±0.65)	(±1.15)	(±1.79)	(±0.25)	(±4.99)	(±3.06)	(±6.73)	(±4.16)	(±47.86)	
<i>G. mosseae</i>	100%	21 ^b	33 ^a	30 ^{bc}	43 ^{bc}	44 ^{abc}	30 ^{a-d}	27 ^{abc}	36 ^f	12 ^{bc}	71 ^{ef}	26 ^{efg}	200 ^{abc}
	FC	(±1.73)	(±2.19)	(±1.34)	(±3.02)	(±1.14)	(±0.58)	(±3.87)	(±1.57)	(±2.38)	(±16.6)	(±0.81)	(±61.28)
	75%	21 ^b	31 ^{abc}	32 ^{bc}	44 ^{bc}	47 ^a	29 ^{a-d}	17 ^{bc}	30 ^f	9 ^c	63 ^{ef}	21 ^g	145 ^{abc}
	FC	(±0.57)	(±1.21)	(±2.31)	(±1.53)	(±1.61)	(±0.51)	(±1.38)	(±1.73)	(±1.49)	(±7.47)	(±3.09)	(±35.05)
	50%	22 ^b	29 ^{a-e}	27 ^c	42 ^{bc}	45 ^{abc}	24 ^{c-f}	19 ^{bc}	34 ^f	10 ^c	81 ^{b-f}	32 ^{c-g}	166 ^{abc}
	FC	(±2.38)	(±0.95)	(±4.60)	(±1.84)	(±0.95)	(±2.08)	(±3.02)	(±2.65)	(±1.43)	(±15.57)	(±5.02)	(±28.82)
25%	21 ^b	30 ^{a-d}	31 ^{bc}	43 ^{bc}	43 ^{a-e}	26 ^{b-f}	22 ^{abc}	39 ^{ef}	9 ^c	75 ^{c-f}	68 ^a	151 ^{abc}	
FC	(±0.52)	(±1.04)	(±4.18)	(±1.51)	(±0.56)	(±2.52)	(±1.76)	(±3.58)	(±1.56)	(±6.68)	(±2.27)	(±50.31)	
<i>G. intraradices</i>	100%	20 ^b	28 ^{a-e}	35 ^{bc}	38 ^c	42 ^{a-f}	33 ^a	26 ^{abc}	30 ^f	12 ^{bc}	47 ^f	19 ^g	234 ^{ab}
	FC	(±0.69)	(±1.44)	(±3.99)	(±1.68)	(±1.81)	(±3.70)	(±3.28)	(±4.79)	(±1.54)	(±2.93)	(±0.99)	(±51.26)
	75%	21 ^b	31 ^{abc}	31 ^{bc}	37 ^c	43 ^{a-e}	26 ^{b-f}	20 ^{abc}	37 ^{ef}	10 ^c	73 ^{def}	28 ^{d-g}	187 ^{abc}
	FC	(±1.17)	(±1.38)	(±5.85)	(±0.57)	(±1.06)	(±2.14)	(±1.26)	(±5.09)	(±1.96)	(±10.54)	(±1.17)	(±58.61)
	50%	21 ^b	29 ^{a-e}	25 ^c	37 ^c	44 ^{a-d}	24 ^{c-f}	16 ^c	26 ^f	8 ^c	51 ^f	19 ^g	137 ^{abc}
	FC	(±0.52)	(±1.78)	(±1.31)	(±1.38)	(±1.41)	(±0.99)	(±0.48)	(±3.07)	(±2.01)	(±8.44)	(±2.38)	(±50.66)
25%	33 ^a	29 ^{a-e}	33 ^{bc}	48 ^b	41 ^{a-g}	25 ^{c-f}	22 ^{abc}	43 ^{ef}	11 ^{bc}	112 ^{ab}	38 ^{b-f}	211 ^{abc}	
FC	(±10.02)	(±1.28)	(±1.21)	(±6.95)	(±2.69)	(±0.81)	(±1.36)	(±3.61)	(±1.55)	(±20.78)	(±8.04)	(±28.86)	

*Different letters within a column indicate significant differences ($P \leq 0.05$) using the Duncan's multiple-range test.

**Table 5.** Three-factor ANOVA statistics for P, K, Fe, Zn, Cu and Mn concentrations of two pistachio cultivars (C) colonized by different AMF treatments (M) under drought stress (D).

	P	K	Fe	Zn	Cu	Mn
Cultivars(C)	**	ns	ns	ns	ns	***
Drought (D)	ns	***	*	*	ns	**
Mycorrhizae (M)	***	***	ns	***	ns	***
CxD	ns	ns	ns	ns	ns	***
CxM	ns	ns	ns	*	ns	***
DxM	ns	ns	ns	ns	ns	***
CxDxM	ns	ns	ns	***	ns	***

$P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$, ns: Not significant.

concentrations of leaf-tissues were significantly higher in +M as compared to -M plants (Tables 3 and 4, Figure 2). However, leaf Mn concentration was unchanged in AM treatments.

Concentrations of Cu, Mn and Zn in the stem of +M plants were increased whereas Fe concentration of the same tissue was remained unchanged in comparison with -M plants. In root of +M plants, Mn and Fe concentrations remained un-changed while Zn content was increased and Cu content was decreased significantly compare with -M plants (Tables 3 and 4).

Irrespective of AM treatments, DS conditions caused a significant reduction in Fe and Zn uptake while there was a significant increase in Mn uptake and no change was observed in Cu content of plants (Table 1). The leaf contents of Cu, Fe and Zn were increased with increasing DS level whereas Mn concentration remained unchanged. Stem-tissues received more Fe when DS intensity increased while under the same condition the content of Mn decreased and Cu and Zn concentrations were unchanged (Tables 3 and 4).

In most of the cases, there were no significant interactions between AMF and DS levels (Table 5). The rates of Zn and Mn uptake were higher in Qazvini while the same uptake rates were observed for Cu and Fe between the two cultivars. Movement of Cu, Zn and Fe was increased toward leaf tissue and decreased in roots of +M plants while Mn contents of root and leaf were almost unchanged (Figure 2). With

increasing DS intensity, all measured micro-nutrients were preferentially retained in the root tissues and their accumulations were decreased in leaf tissue (Figure 3).

DISCUSSION

Water and different nutrients exist together in plant tissues in close association, because nutrient ions are dissolved in the soil solution and nutrient uptake by plants depends on water flow through the soil-root-shoot continuum (Keller, 2005). Leaf transpiration creates the tension necessary for the roots to absorb the soil solution containing essential nutrients (Keller, 2005). Water stress has at least three adverse effects on plant nutrition. First, it reduces growth rates, particularly leaf expansion rates (Granier and Tardieu, 1999) and is expected to magnify the down-regulation of uptake. Second, it reduces nutrient availability in the root zone and thus decreases uptake rates. Third, water stress reduces stomatal conductance. This induces a lowering of leaf-internal CO_2 (C_i) and thus of photosynthesis rate and decreases demand for nutrient uptake as well as reduction in transpiration rate and mass flow of nutrients. Our experiment results showed that under DS condition, P and K^+ contents of leaves were reduced whereas these elements in roots were not changed revealing a significant decrease in transporting from roots to leaves under DS condition. Increase in Cu, Fe and Zn contents of leaves under

DS could be attributed to dilution effect due to reduction in leaf growth (Data not shown). However, distribution percentage of P and K in plant body under DS showed an increased accumulation of both elements in roots and decrease of their movement toward leaves (Figure 3). The responses of cytosolic and vacuolar compartments to K^+ deprivation have revealed that cytosolic K^+ is held constant at the expense of vacuolar K^+ . As discussed in Fernando *et al.* (1992), the first responses of plant roots to K^+ deprivation is the net transfer of K^+ from the vacuole to the cytosol and then to the shoot via the xylem. As K^+ stress intensifies, there is a more reduction of the transfer of K^+ from roots to shoots. It has also been demonstrated previously (Asher and Loneragan, 1967) that at the whole-plant level, a wide range of plant species partitioned greater proportions of absorbed P to roots than to shoots under conditions of low available P. This is similar to the reduction of K^+ transport to the shoot. As was the cases for K^+ , the maintenance of a constant value for root cytosolic P must involve adjustments of fluxes at the plasma membrane, at the tonoplast, and to and from the stele.

AM symbiosis is widely believed to protect host plants from the detrimental effects of drought. The potential mechanisms proposed to explain this tolerance include a stomatal regulation (Wu *et al.*, 2007), a greater osmotic adjustment (Wu and Xia, 2006) and enhanced activity of enzymes involved in antioxidant defense and nitrate assimilation (Wu *et al.*, 2007). Furthermore, the contribution of the AM symbiosis to plant drought tolerance can be resulted from the nutritional effects. Inoculation of pistachio cultivars with the AMF had a beneficial effect on plant nutrition. AMF may improve nutrient uptake by improving the exploration of the soil pore space (Augé, 2004). Davies (1992) found that external hyphal development and soil aggregation of mycorrhizal plants were enhanced by drought acclimation. External hyphae adhere to soil particles through a

special glycoprotein glomalin, which would improve contact with the soil solution (Wu *et al.*, 2008). These hyphae access smaller pore spaces than plant roots and root hairs (Augé, 2004). As soil water content decreases, the relative importance of these factors will increase. In our study, AM colonization increased pistachio P content under drought conditions. Numerous greenhouse and field experiments have conclusively demonstrated that plants colonized by AM fungi are much more efficient in taking up soil P than -M plants leading to improvement in plant growth (Fitter and Hay, 2002). Drought may be relieved by an increased rate of root growth and more efficient extraction of water from the soil as a consequence of increased P uptake (Desnos, 2008). Our results indicated that K^+ is translocated to roots through mycorrhizal hyphae efficiently and then moved toward leaves. Potassium plays a key role in plant water stress and has been found to be the cationic solute responsible for stomatal movement in response to changes in bulk leaf water status (Ruiz-Lozano *et al.*, 1995). Results of the present study illustrated the positive role of AM symbiosis in Zn and Mn uptake while Fe and Cu were not affected. The effects of AMF on acquisition of immobile metal nutrients by the host plant are still unclear and factors responsible for the variable results reported by researchers in this field need to be understood. Inconsistent responses of mycorrhizal plants in micronutrient uptake may be related to highly variable soil conditions. One such variable soil condition is the level of available micronutrients. It has been proved that under conditions of low micronutrients level, uptake by AMF hyphae is increased (Liu *et al.*, 2000).

Distribution of elements were affected by AMF treatments and all of them except P were accumulated more in leaves (Figure 2) exhibiting the positive role of AMF on water relations of colonized plants (Augé, 2001, 2004).

Based on the results of previous researches, 'Qazvini' exhibited higher



adaptive potential under salinity stress when compared to 'Badami' (Karimi *et al.*, 2009). The results of the present study also indicated that +M Qazvini plants may tolerate higher DS intensities in terms of nutritional status.

In conclusion, the results of this study suggest that AM inoculation improves drought tolerance of pistachio cultivars at least in part through the enhanced uptake of slowly diffusing mineral ions such as PO_4^{2-} and Zn^{2+} and provides more osmotic adjustment which can be correlated with K^+ accumulation in top portions of treated plants. However, AMF-mediated improvement of both drought resistance and growth rate (data not shown) in pistachio seedlings is not merely a nutritional process and some other mechanisms are also involved.

Abbreviations

AM: Arbuscular mycorrhizal; AMF: Arbuscular mycorrhizal fungi; DS: Drought stress; +M: Mycorrhizal; -M: Non-mycorrhizal; Badami: Badami-Riz-Zarand, FC: Field capacity.

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جذب و توزیع عناصر در نهال‌های پسته میکوریزی در شرایط تنش خشکی

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چکیده

این پژوهش به منظور تعیین اثرات همزیستی قارچ‌های میکوریز آربسکولار (*Glomus mosseae*)، *Glomus intraradices*) بر میزان جذب عناصر معدنی توسط دو رقم پسته (*Pistacia vera*)، رشد یافته در شرایط گلخانه‌ای (*Pistacia vera* cv. Badami-Riz-Zarand و cv. Qazvini) در سطوح مختلف از تنش خشکی موجب کاهش آلودگی میکوریزایی و همچنین جذب عناصر در هر دو رقم گردید. گیاهان آلوده به میکوریز صرف نظر از شرایط رطوبتی خاک در مقایسه با گیاهان غیر میکوریز دارای غلظت بالاتر فسفر، پتاسیم، روی و منگنز بودند در حالی که محتوای مس و آهن آنها بدون تغییر باقی ماند. توزیع عناصر تحت تاثیر تیمار قارچ‌های میکوریز آربسکولار قرار گرفت و تجمع تمامی آنها بجز فسفر در برگ‌ها بیشتر از ریشه بود در حالی که در شرایط تنش خشکی عناصر جذب شده تمایل به باقی ماندن در ریشه داشتند. رقم قزوینی از لحاظ جذب فسفر و منگنز برتر از بادامی بود. در مجموع نتایج این پژوهش نشان می‌دهند که مایه کوبی ارقام پسته با قارچ‌های میکوریز آربسکولار سبب افزایش مقاومت به خشکی در آنها می‌گردد که حداقل بخشی از آن به افزایش در جذب برخی از یون‌های معدنی کم تحرک از قبیل فسفات و روی و فراهم نمودن تنظیم اسمزی بیشتر که می‌تواند به تجمع بیشتر یون پتاسیم در بخش‌های هوایی گیاهان تیمار شده با میکوریز منجر گردد، مربوط باشد. نتایج این پژوهش تاکید می‌کند بر این که رقم قزوینی ممکن است مقاوم‌تر از رقم بادامی در برابر تنش خشکی باشد.