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Response of Petunia Plants (*Petunia hybrida cv.* Mix) Inoculated with *Glomus mosseae* and *Glomus intraradices* to Phosphorous and Drought Stress

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ABSTRACT

In order to assess drought tolerance of mycorrhizal petunia plants (*Petunia hybrida cv.* Mix), a greenhouse experiment was carried out with two different mycorrhizae species (*Glomus mosseae* and *Glomus intraradices*) applied singly or in combination at two phosphorous (P) levels (0 and 100 mg kg^{-1} dry soil) and three irrigation regimes (100% field capacity (FC) as control, 75% FC producing moderate water stress and 50% FC producing severe water stress). Both mycorrhizal endophytes established well on roots of the petunia plants with higher colonization values at lower P concentration and lower colonization rate at increasing water stress. Mycorrhizal colonization generally enhanced plant vegetative and reproductive growth, both under full and reduced field capacities and with and without P fertilization. The content of soluble sugar in AMF-inoculated leaves was higher than that in non-AMF-inoculated plant leaves in response to drought treatments but proline level did not show any significant increase in mycorrhizal treatments at the same conditions. This study confirms that mycorrhizal colonization can mitigate the adverse effects of water stress on treated plants restoring most of the key growth parameters to levels similar or close to those in unstressed plants.

Keywords: Drought stress, *Glomus intraradices*, *Glomus mosseae*, Petunia, Phosphorous.

INTRODUCTION

The most important limitations of landscaping in arid regions with alkaline soils are the lack of water and available nutrients, especially phosphorous (P). The limitation of both resources impacts the establishment and maintenance of landscaped areas. Rafsanjan is located in the North-west of Kerman province in the South-east of Iran and is one of the largest pistachio production centers in the world (FAOstat, 2006). The climate of Rafsanjan is characterized as arid subtropical with less than 100 mm mean annual rainfall. Increased production of irrigated pistachio orchards during the last two decades in the region has decreased the availability of ground water resources. Under these water-limited conditions, a key component for improving survival of landscape plants is the introduction and cultivation of drought resistant species and the development of methods for increasing their water use efficiency.

Arbuscular mycorrhizal fungi (AMF) associated with plant roots can enhance crop productivity under drought conditions by improving the mineral nutrition (mainly of P) (Al-Karaki *et al.,* 2004; Al-Karaki and Al-Raddad 1997; Marshner and Dell 1994;

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Michelsen and Rosendahl 1990; Trimble and Knowles 1995) and enhancing water uptake (Davies *et al.,* 1992; Ruiz-Lozano *et al.,* 1995). Compared to non-Mycorrhizal plants, mycorrhizal plants have higher water uptake due to hyphal extraction of soil water (Davies *et al.,* 1992; Faber *et al.,* 1991; Ruiz-Lozano *et al.,* 1995) and larger root hydraulic conductivity (Auge and Stodola 1990).

Petunia (*Petunia hybrida*) is one of the most popular plant species used in landscaping in Rafsanjan because of its ability to thrive under deficit irrigation and at high summer temperatures. Its fragrant flowers, which are produced gradually over a long period of time (near 8 months), make this plant species economically important and thus a good choice for investigations on its drought resistance potential. There is little information on the ability of petunia to form symbiotic associations with AMF (Gaur *et al.,* 2004) however, Gerats *et al.* (2008) suggested that the high natural mutation of petunia could be useful for the study of the mechanistic aspects of the AMF symbiosis and is petunia therefore a useful model plant system for studying symbiosis within the Solanaceae. Knowledge of the symbiotic association between AMF and petunia can therefore be expected to contribute considerably to the functional and developmental analysis of AM symbiosis in the future.

The objective of the present study was to evaluate (1) the extent of root colonization of petunia by AMF; and (2) the effect of AMF and P availability on growth of petunia plants under water stress.

MATERIALS AND METHODS

Experimental Site

A greenhouse experiment was conducted in 2008 at the Agricollege of Vali-e-Asr university of Rafsanjan (30° 23′ 06″ N, 55° 55′ 30″ E), at 1523 m a.s. l.

Preparation of Plants

Seeds of petunia *(Petunia hybrida var. mix*) were surface sterilized in 5% sodium hypochlorite solution for 10 minutes and washed carefully with distilled water before sowing in 2-liter plastic containers. The soil used was an autoclaved sandy loam with the following characteristics: sand,69%; silt, 16%; clay, 15%; pH, 7.9; electrical conductivity (EC), 1.05 dS m^{-1}; Olsen P, 10 mg kg^{-1}; ammonium acetate extractable K, 2.2 meq L^{-1}; Ca and Mg in soil saturation extract, 3.5 and 7.5 meq L^{-1} respectively; diethylenetriamene pentaacetate extractable Zn, Fe, Mn, and Cu, 1.44, 2.54, 5.94, and 1.39 mg kg^{-1}, respectively.

Plants were grown for 30 days in a greenhouse (16 hours of light, 8 hours of dark, temperature of 28±3°C, relative humidity of 50-70%) and irrigated regularly to field capacity (FC) with a 50% Hoagland solution. The nutrient solution was renewed two times a week and the nutrient concentration increased to 100% Hoagland solution after the plants were 15 days old. After 30 days of development, plants with uniform height and size (4-6 leaf stage) were selected and carefully transferred to 5-liter plastic containers (3 plants per container) containing the same soil mixture as described above. Half of the containers received P at final concentration of 100 mg kg^{-1} dry soil.

Preparation of AMF Inocula

Pot cultures of the AM fungi *Glomus mosseae* and *Glomus intraradices* were initiated on wheat (*Triticum spp.*) in a greenhouse between February and May 2008. At maturity, the tops of the wheat plants were removed and substrate was allowed to dry for a week at 30±5°C. The roots were finely chopped and the dried root/soil mixture was thoroughly mixed to obtain a homogenous inoculum. The percentage of root colonization by mycorrhizae was assessed by the method of Phillips and Hayman, 1970. AM spores were extracted by wet sieving method

(Gerdemann and Nicolson, 1963) using 10 g samples from each pot. The washed spores were placed on a gridded tissue culture dish (60×15 mm with 2 mm grids) for counting.

Mycorrhizal Inoculation

The inocula consisted of soil, chopped wheat root fragments, spores and hyphae colonized with one of each of the fungi. 20 g (fresh mass) of inoculum with an average of 40 spores g^{-1} and 75% of roots colonized by AMF were placed at the bottom of the transplanting hole, immediately before planting. For the mixed treatment, 10 g of each inoculum was placed at the bottom of the transplanting hole before planting. Control plants were transplanted to soil containing 20 g of an autoclaved mixture of inoculum with both AMF species. To account for effects of nonmycorrhizal organisms potentially found in the inocula, all plants, including control plants, also received 20ml of filtered washings of a mixture of the fungal inocula. Plants were watered every two days with deionized water.

Drought Treatments

Drought treatments were started 30 days after transplantation. The three water regimes were 100% FC (as control), 75% FC (moderate stress) and 50%FC (severe stress). The water content of the soil mixture at FC level was calculated based on the difference between soil dry and wet weight after watering and ceasing the runoff. Thereafter, for 50 days (from 1 July to 20 August 2008), soil water contents were determined by weighing each container daily and adding water after weighing to maintain the predetermined water content in each pot. The amount of water added to each pot was used to determine water evapotranspired. During the experiment, the maximum temperature was 38±4°C, the minimum temperature was 23±6°C and the relative humidity was 55±5% with natural light (without additional artificial lightening).

Observation, Harvest and Analysis

Chlorophyll and Carotenoid Contents

Chlorophyll and carotenoid contents were determined by the method of Lichtenthaler *et al.* (1987). Fresh leaves (1 g) were triturated in 80% acetone. The absorbance of the extracts was measured at 645, 652, and 663 nm using a spectrophotometer U-2000 (Hitachi Instruments, Tokyo, Japan).

Growth Parameters

The number of flowers on each plant was counted every 10 days and the cumulative number of flowers was calculated at the end of experiment. Plant height and number of leaves on each plant was also recorded at the end of the experiment. The experiment was terminated by separating shoots from roots 50 days after treatments commencement. Shoots and roots were weighed immediately and then shoots were dried at 80°C for 24 h (Foliar dry weight, FDW). Roots were rinsed free from soil, cut into 1-cm fragments and thoroughly mixed. Sub samples (1 g) were saved for determination of root colonization by AMF, the remainder of the roots was dried and weighed (Root dry weight, RDW).

Nutrient Analysis

Dried samples were weighed 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 the volume was increased 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 phosphorous was measured using

spectrophotometry method (Chapman *et al.,* 1982).

Total Soluble Sugars and Proline

Total soluble sugars were determined according to Fales (1951). 0.1 g of well ground dry samples were homogenised in hot 80% ethanol and centrifuged at 2,900 rpm. The supernatant was taken and the residue was repeatedly washed with 80% ethanol and filtered to remove all the traces of soluble sugars and then the filtrate was added to the obtained supernatant. This was reacted with anthrone reagent and absorbance was read at 625 nm.

Free proline content was estimated following the method of Bates *et al.* (1973). Fresh leaves (0.5 g) were extracted in 3% sulphosalicylic acid and the homogenates were centrifuged at 10,000 rpm for 10 minutes. A 2 ml sample of the supernatant was reacted with 2 ml of acid ninhydrin reagent and 2 ml of glacial acetic acid in a test tube for 1 h at 100°C and the reaction terminated in an ice bath. The reaction mixture was extracted with 4 ml of toluene and mixed vigorously with a vortex for 15–20 seconds. The chromophore containing toluene was aspirated from the aqueous phase, warmed to room temperature and the absorbance was measured at 510 nm using toluene as blank. Proline concentration was calculated from a standard curve using 0–100 µg L^{-1} proline (Sigma).

AMF Colonization

Root samples for determination of root colonization with AMF were cleared with 10% (w/v) KOH and stained with 0.05% (v/v) trypan blue in lactophenol as described by Phillips and Hayman (1970), and microscopically examined for colonization by determining percentage root segments containing arbuscules and vesicles using a gridline intercept method (Bierman and Linderman 1981). Roots used to determine AMF colonization were dried, weighed, and added to the total.

Experimental Design

The experiment was a completely randomized design with three replicates and a factorial combination of two phosphorous rates, four types of mycorrhiza treatments (C: Control no mycorrhiza, M_1: *Glomus mosseae*, M_2: *Glomus intraradices*, M_3: M_1+M_2) and three watering regimes (D_1: 100% FC, D_2: 75% FC, D_3: 50% FC). Data were statistically analyzed by analysis of variance with the MSTATC PROGRAM (Michigan State University, East Lansing, Mich., USA). Probabilities of significance were used to test for significance among treatments and interactions and Duncan's multiple range test at 5% significant level was used to compare means.

RESULTS

Root Colonization

Root colonization by AMF ranged from 34% in plants inoculated with *G. mosseae* to 72 % in plants inoculated with *G. intraradices* (Table 1). Mycorrhizal colonization was slightly increased under water limitation in the medium while the degree of AMF colonization decreased in a more pronounced manner with phosphorous application. However, *G. intraradices* was found to be more infectious than *G. mosseae*. Sporulation by each mycobiont was much greater under well watered conditions. After 50 days of exposure to varying intensities of drought stress, severe drought treatment caused 22.4, 12.9 and 50.4% reductions in spore production in M_1, M_2 and M_3, respectively compared to well watered plants. Based on our results, spores population was increased with P application in M_2 and M_3 and decreased in M_1 although the differences were not significant in most cases.

Table 1. Mean root colonization and mean number of spores in mycorrhizal petunia plants under different irrigation regimes and P levels.

Mycorrhiza	Root colonization (%)		Number of spores 10 g^{-1} dry soil	
	-P[d]	+P[e]	-P	+P
D_1 (100% FC)				
M_1[a]	47_{f-g*}	34_{i}	89_{bc}	58_{de}
M_2[b]	64_{bc}	44_{h}	65_{c-e}	70_{c-e}
M_3[c]	59_{c-e}	52_{e-g}	112_{ab}	125_{a}
D_2 (75% FC)				
M_1	47_{f-g}	54_{d-f}	65_{c-e}	52_{ef}
M_2	72_{a}	51_{f-h}	60_{c-e}	68_{c-e}
M_3	45_{gh}	35_{i}	62_{c-e}	86_{b-d}
D_3 (50% FC)				
M_1	52_{e-g}	48_{f-h}	30_{fg}	45_{e-g}
M_2	67_{ab}	60_{b-d}	60_{c-e}	61_{c-e}
M_3	64_{bc}	64_{bc}	20_{gh}	62_{c-e}

[a] *G. mosseae;* [b] *G. intraradices*; [c] M1+M2; [d] Without phosphorous, [e] 100 mg P kg^{-1}dry soil.
* Different letters within a column indicate significant differences using the Duncan's multiple range test at 5% significant level.

Plant Growth Parameters

Phosphorous effects on plant growth in control and mycorrhizal treatments under different irrigation regimes are presented in Table 2. FFW and FDW were influenced positively by mycorrhizal infection irrespective of mycorrhizal type and water stress level. P application had no significant effect on FFW and FDW although a slight decrease in FFW and increase in FDW can be observed from the data (Table 2). FFW was lower in water stressed than in well-watered plants. Reductions in FFW due to water stress were more pronounced in -M than +M plants and the same results were obtained with FDW. However, the best results were recorded with M_1 under well watered condition (Table 2). RFW and RDW were also increased as a result of mycorrhizal inoculation and +M plant responses were more obvious under –P conditions. These parameters were decreased with drought stress intensity regardless of mycorrhizal treatments or P levels. Drought-stressed +M plants generally accumulated more fresh and dry mass in roots than drought-stressed or irrigated -M plants (Table 2).

In terms of plant height, all treatments showed increased height with P application including control and decreased height with increasing drought stress intensity. AM treatments could not improve plant height significantly. Although leaf number was not significantly influenced by AM treatments, an increase in this parameter could be observed especially with –P plants. Mycorrhizal colonization increased the petunia flower production regardless of mycorrhizal type, drought stress and P level, however, this effect was more prominent in –P plants under moderate drought stress. As expected, flower production was decreased severely by increasing drought stress but mycorrhizal treatments improved petunia flowering under water stress condition.

Plant Nutrient Status

Both +M and -M well-watered plants had higher P content than -M water stressed plants. Mycorrhizal symbiosis improved P uptake especially under severe drought stress without P application where the P contents of treated plants in M_1 and M_3 were 2.64 and 2.5 fold, respectively. Although not

Table 2. Means for growth parameters and water use efficiency of mycorrhizal petunia plants under different irrigation regimes with (+P) or without (-P) phosphorous application.

Treatments	Foliar fw (g)	Foliar dw (g)	Root fw (g)	Root dw (g)	Plant height (cm)	Leaf no	Flower no	WUE (mg ml^{-1})
Without phosphorous								
D_1 (100% FC)								
Cont.	11.80 $_{c-g}$	1.66 $_{e-g}$	1.08 $_{f-i}$	0.20 $_{f-i}$	39.55 $_{a-g}$	13.80 $_{b}$	14.00 $_{c-f}$	0.285 $_{b}$
M_1[a]	16.30 $_{b-e}$	2.74 $_{b-f}$	2.29 $_{bc}$	0.52 $_{ab}$	32.88 $_{c-i}$	25.53 $_{ab}$	27.00 $_{b-e}$	0.416 $_{ab}$
M_2[b]	20.84 $_{a-c}$	3.98 $_{ab}$	2.31 $_{bc}$	0.44 $_{bc}$	34.11 $_{c-i}$	29.10 $_{ab}$	35.00 $_{ab}$	0.506 $_{ab}$
M_3[c]	24.55 $_{ab}$	3.36 $_{a-e}$	2.94 $_{a}$	0.54 $_{a}$	38.58 $_{b-g}$	23.73 $_{ab}$	30.33 $_{b-d}$	0.484 $_{ab}$
D2 (75%FC)								
Cont.	9.33 $_{d-g}$	1.83 $_{e-g}$	0.87 $_{f-j}$	0.29 $_{d-i}$	36.00 $_{c-i}$	15.63 $_{b}$	6.67 $_{ef}$	0.321 $_{b}$
M_1	14.84 $_{b-e}$	2.45 $_{b-g}$	1.35 $_{d-g}$	0.33 $_{c-g}$	42.00 $_{a-g}$	18.00 $_{b}$	19.00 $_{b-f}$	0.409 $_{ab}$
M_2	16.24 $_{b-e}$	3.36 $_{a-e}$	1.65 $_{d-g}$	0.38 $_{c-e}$	47.22 $_{a-d}$	20.23 $_{b}$	23.00 $_{b-f}$	0.556 $_{ab}$
M_3	17.51 $_{b-e}$	3.57 $_{a-d}$	1.77 $_{c-e}$	0.36 $_{c-e}$	38.78 $_{a-g}$	26.20 $_{ab}$	30.33 $_{b-d}$	0.694 $_{a}$
D_3 (50%FC)								
Cont.	4.31 $_{fg}$	0.81 $_{g}$	0.52 $_{ij}$	0.14 $_{i}$	22.00 $_{i}$	13.67 $_{b}$	3.00 $_{f}$	0.428 $_{ab}$
M_1	7.95 $_{e-g}$	1.56 $_{fg}$	0.76 $_{g-j}$	0.18 $_{bc}$	29.77 $_{f-i}$	16.00 $_{b}$	7.00 $_{ef}$	0.395 $_{ab}$
M_2	16.24 $_{b-e}$	1.92 $_{d-g}$	0.93 $_{f-j}$	0.23 $_{f-i}$	23.33 $_{hi}$	12.77 $_{b}$	9.67 $_{d-f}$	0.436 $_{ab}$
M_3	17.21 $_{b-e}$	3.57 $_{a-d}$	1.57 $_{d-g}$	0.38 $_{c-e}$	36.00 $_{c-i}$	50.90 $_{a}$	12.33 $_{d-f}$	0.479 $_{ab}$
With phosphorous (100 mg kg^{-1})								
D_1 (100% FC)								
Cont.	18.35 $_{a-d}$	3.87 $_{a-c}$	1.75 $_{c-e}$	0.41 $_{b-d}$	42.80 $_{a-g}$	27.70 $_{ab}$	27.00 $_{b-e}$	0.677 $_{a}$
M_1	27.48 $_{a}$	4.12 $_{a}$	2.65 $_{ab}$	0.45 $_{bc}$	52.66 $_{ab}$	27.20 $_{ab}$	20.33 $_{b-f}$	0.455 $_{ab}$
M_2	21.00 $_{a-c}$	3.07 $_{b-f}$	1.97 $_{cd}$	0.34 $_{c-f}$	45.55 $_{a-e}$	20.33 $_{b}$	39.67 $_{a}$	0.384 $_{ab}$
M_3	15.34 $_{b-e}$	3.71 $_{a-c}$	1.48 $_{d-f}$	0.37 $_{c-e}$	42.05 $_{a-g}$	18.77 $_{b}$	29.67 $_{b-d}$	0.484 $_{ab}$
D_2 (75%FC)								
Cont.	9.68 $_{d-g}$	2.74 $_{b-f}$	1.15 $_{e-i}$	0.41 $_{b-d}$	48.00 $_{a-c}$	20.90 $_{b}$	13.00 $_{d-f}$	0.503 $_{ab}$
M_1	14.10 $_{c-f}$	2.39 $_{b-g}$	1.45 $_{d-g}$	0.32 $_{c-g}$	43.11 $_{a-f}$	12.00 $_{b}$	15.67 $_{c-f}$	0.453 $_{ab}$
M_2	13.97 $_{c-f}$	3.03 $_{b-f}$	1.72 $_{c-e}$	0.30 $_{d-h}$	53.88 $_{a}$	19.70 $_{b}$	26.33 $_{b-e}$	0.464 $_{ab}$
M_3	10.52 $_{d-g}$	2.55 $_{b-f}$	1.00 $_{f-i}$	0.23 $_{f-i}$	27.55 $_{g-i}$	14.10 $_{b}$	20.67 $_{b-f}$	0.525 $_{ab}$
D_3 (50%FC)								
Cont.	3.75 $_{g}$	1.73 $_{e-g}$	0.59 $_{ij}$	0.16 $_{i}$	32.11 $_{d-i}$	12.08 $_{b}$	3.67 $_{f}$	0.614 $_{ab}$
M_1	9.15 $_{d-g}$	2.59 $_{b-f}$	1.21 $_{d-i}$	0.45 $_{bc}$	31.11 $_{e-i}$	12.57 $_{b}$	7.67 $_{ef}$	0.632 $_{ab}$
M_2	9.71 $_{d-g}$	2.41 $_{b-g}$	1.48 $_{df}$	0.46 $_{bc}$	37.52 $_{b-h}$	17.87 $_{b}$	17.67 $_{c-f}$	0.605 $_{ab}$
M_3	7.95 $_{e-g}$	2.19 $_{c-g}$	0.87 $_{fg}$	0.21 $_{f-i}$	35.78 $_{c-i}$	14.67 $_{b}$	10.00 $_{d-f}$	0.423 $_{ab}$
LSD(P= 0.05)	8.42	1.12	0.544	0.116	12.77	24.74	18.18	0.284
ANOVA	P (Phosphorous); M (Mycorrhiza), D (Drought treatment)							
P	***	*	***	**	**	NS	NS	NS
M	**	*	*	**	**	NS	*	*
D	***	***	*	***	***	NS	***	*
P×M	**	*	***	***	NS	NS	*	*
P×D	NS	NS	NS	**	NS	NS	NS	*
M×D	***	***	***	***	*	NS	**	*
P×M×D	NS	NS	***	***	NS	NS	*	NS

*P≤ 0.05; ** P≤ 0.01; *** P≤ 0.001, NS: Not significant.
[a] *G. mosseae;* [b] *G. intraradices*, [c] M1+M2.
Different letters within a column indicate significant differences using the Duncan's multiple range test at 5% significant level.

significant in most cases, drought stress affected P uptake. Addition of P did not change the uptake capacity of +M or -M plants although a marginal increase was observed in -M plants irrespective of drought stress levels (Table 3). Drought stressed -M plants appeared to accumulate more K in comparison with +M plants under +P condition and less K under -P status. However, K uptake was not significantly affected by any treatments under our experimental conditions (Table 3). The results of Fe and Cu uptake showed a fluctuating pattern in response to different treatments but in general, the observed differences were not statistically significant. In the case of Mn, +M plants accumulated less Mn than -M plants except with +M+P under severe drought stress. The ANOVA showed significant effect of P and drought stress level as well as interaction between the mycorrhiza and phosphorous factors, mycorrhiza and drought and phosphorous and drought on Zn uptake.

Chlorophyll and Carotenoids Content

P application generally reduced chlorophyll (a, b and total) and carotenoid contents (Table 4). Mycorrhizal treatments could not improve these characteristics where most of +M plants had lower chlorophyll and carotenoid contents or remained unaffected. Chlorophyll and carotenoid contents decreased significantly under drought stress in -M plants. It would appear that AM treatments could moderate the adverse effects of drought on these parameters (Table 4).

Total Soluble Sugars and Proline

In-M-P plants, increases in sugar content of leaves were observed in response to drought condition where the values of 138% and 150.5% were recorded with moderate and severe stresses, respectively. In contrast, -M+P plants showed decreased TSS with increasing drought stress intensity. In +M drought-stressed plants, the total soluble sugar content increased irrespective of drought intensity and P level.

Leaf proline accumulation was increased in response to drought condition regardless of mycorrhizal treatment (Table 4). Overall mycorrhizal treatments stimulated proline accumulation in leaves although an irregularity was observed with M_2 where the proline content was decreased in moderate drought compare with well watered plants.

Effect of P application on proline accumulation did not show a clear pattern and a fluctuation in the results was observed (Table 4).

Water Use Efficiency (WUE)

The +M-P plants used less water to produce one unit of FDW than -M plants and the best result was obtained with M_3 where an increase of about 60% in WUE was recorded. P application was able to improve WUE in -M plants. Both +M and -M plants had the same WUE when grown either under water-stressed or well-watered conditions (Table 2).

DISCUSSION

The results of the present study demonstrated that applied AMF established well in the petunia plants. The extent of AM infection in petunia was related to a low P concentration which agrees with other studies indicating that plant P status influences mycorrhizal infection (Charron *et al.,* 2001; Arines and Vilarifm, 1989; Antunes and Cardoso, 1991). The role of P in root colonization by AMF has been linked to the concentration of soluble carbohydrates either in the root or in root exudates of AM infected host (Valentine *et al.,* 2001)

In our work, AM inoculation significantly stimulated growth parameters (e.g., FFW, FDW, RFW, RDW and flower number) of petunia plants especially without P

Table 3. Means for some nutrient contents of mycorrhizal petunia plants under different irrigation regimes with (+P) or without (-P) phosphorous application.

Treatments	P (%)	K (%)	Fe (mg kg^{-1})	Mn (mg kg^{-1})	Cu (mg kg^{-1})	Zn (mg kg^{-1})
	Without phosphorous					
D_1 (100% FC)						
Cont.	0.058_{d-f}	3.93_{ab}	437.3_{ab}	117.1_{a}	27.53_{ab}	112.4_{b-g}
M_1[a]	0.107_{a-e}	2.39_{b}	488.6_{ab}	92.80_{ab}	15.20_{b}	119.2_{a-f}
M_2[b]	0.139_{a}	3.80_{ab}	504.8_{ab}	91.17_{ab}	22.80_{ab}	143.8_{ab}
M_3[c]	0.114_{a-d}	3.53_{ab}	467.3_{ab}	86.47_{ab}	18.37_{b}	150.8_{a}
D_2 (75% FC)						
Cont.	0.061_{c-f}	2.70_{b}	404.9_{ab}	96.57_{ab}	17.83_{b}	117.6_{a-f}
M_1	0.124_{a-c}	3.00_{b}	442.2_{ab}	88.67_{ab}	21.93_{ab}	136.2_{a-d}
M_2	0.116_{a-d}	3.50_{ab}	522.9_{ab}	81.33_{ab}	22.30_{ab}	128.2_{a-e}
M_3	0.091_{a-f}	3.53_{ab}	510.2_{ab}	87.47_{ab}	31.10_{ab}	146.3_{ab}
D_3 (50% FC)						
Cont.	0.048_{ef}	2.57_{b}	537.5_{ab}	93.30_{ab}	15.87_{b}	98.00_{e-h}
M_1	0.127_{ab}	3.53_{ab}	224.4_{b}	93.33_{ab}	38.13_{ab}	121.7_{a-f}
M_2	0.093_{a-f}	3.50_{ab}	467.5_{ab}	83.93_{ab}	20.10_{ab}	138.0_{a-c}
M_3	0.120_{a-d}	3.70_{ab}	389.0_{ab}	81.53_{ab}	17.30_{b}	130.8_{a-e}
	With phosphorous(100mg.kg^{-1})					
D_1 (100% FC)						
Cont.	0.069_{b-f}	3.87_{ab}	689.9_{a}	119.8_{a}	15.10_{b}	136.2_{a-d}
M_1	0.122_{a-c}	2.23_{b}	569.4_{ab}	92.83_{ab}	51.43_{ab}	96.20_{e-h}
M_2	0.117_{a-d}	2.83_{b}	651.8_{ab}	82.87_{ab}	21.57_{ab}	96.57_{e-h}
M_3	0.073_{b-f}	2.63_{b}	675.8_{ab}	88.50_{ab}	21.17_{ab}	99.73_{d-h}
D_2 (75% FC)						
Cont.	0.063_{c-f}	3.37_{b}	653.1_{ab}	107.5_{ab}	20.57_{ab}	119.9_{a-f}
M_1	0.121_{a-c}	2.83_{b}	543.7_{ab}	104.6_{ab}	23.70_{ab}	89.97_{f-h}
M_2	0.112_{a-d}	2.77_{b}	452.0_{ab}	78.20_{ab}	22.30_{ab}	86.33_{f-h}
M_3	0.073_{b-f}	2.41_{b}	502.8_{ab}	82.10_{ab}	20.40_{ab}	102.5_{c-h}
D_3 (50% FC)						
Cont.	0.044_{f}	5.69_{a}	332.3_{ab}	73.00_{b}	62.07_{a}	43.00_{i}
M_1	0.129_{ab}	3.63_{ab}	664.2_{ab}	107.1_{ab}	26.00_{ab}	78.63_{gh}
M_2	0.095_{a-f}	3.90_{ab}	430.6_{ab}	81.87_{ab}	20.50_{ab}	72.87_{hi}
M_3	0.099_{a-f}	3.70_{ab}	408.6_{ab}	86.57_{ab}	22.30_{ab}	79.73_{gh}
LSD (P= 0.05)	0.052	1.85	372	34.52	34.60	30.90
ANOVA	P (Phosphorous); M (Mycorrhiza), D (Drought treatment)					
P	NS	***	NS	***	**	***
M	***	NS	***	*	**	NS
D	***	NS	NS	NS	**	***
P×M	NS	NS	***	***	**	**
P×D	***	NS	***	***	**	*
M×D	*	***	***	NS	**	*
P×M×D	***	***	NS	***	NS	NS

*P≤ 0.05; ** P≤ 0.01; *** P≤ 0.001, NS: Not significant.

[a] *G. mosseae;* [b] *G. intraradices*, [c] M1+M2.

Different letters within a column indicate significant differences using the Duncan's multiple range test at 5% significant level.

Table 4. Means for chlorophyll a, b, total chlorophyll, carotenoids, total soluble sugars and praline contents of mycorrhizal petunia plants under different irrigation regimes with (+P) or without (-P) phosphorous application.

Treatments	Chl. A (mg g^{-1} fw)	Chl. B (mg g^{-1} fw)	TChl. (mg g^{-1} fw)	Cart. (mg g^{-1} fw)	TSS (mg g^{-1} fw)	Proline (mg g^{-1} fw)
Without phosphorous						
D_1 (100% FC)						
Cont.	0.093 $_{a}$	0.056 $_{b}$	0.176 $_{ab}$	0.057 $_{c-g}$	129.0 $_{g}$	48.80 $_{b-f}$
M_1 [a]	0.091 $_{ab}$	0.047 $_{b}$	0.170 $_{ab}$	0.066 $_{b-d}$	425.5 $_{d-g}$	58.13 $_{b-f}$
M_2 [b]	0.063 $_{d-f}$	0.030 $_{b}$	0.098 $_{d-g}$	0.043 $_{f-h}$	536.8 $_{b-g}$	68.74 $_{a-f}$
M_3 [c]	0.067 $^{d-f}$	0.030 $_{b}$	0.102 $_{c-g}$	0.053 $_{d-g}$	471.6 $_{c-g}$	31.93 $_{d-f}$
D2 (75%FC)						
Cont.	0.079 $_{cd}$	0.039 $_{b}$	0.126 $_{b-e}$	0.058 $_{c-g}$	307.4 $_{fg}$	82.74 $_{a-e}$
M_1	0.080 $_{cd}$	0.041 $_{b}$	0.131 $_{b-e}$	0.066 $_{b-d}$	933.6 $_{a-c}$	78.73 $_{a-f}$
M_2	0.053 $_{f-h}$	0.027 $_{b}$	0.085 $_{e-h}$	0.049 $_{d-g}$	549.0 $_{b-g}$	26.77 $_{f}$
M_3	0.077 $_{de}$	0.036 $_{b}$	0.109 $_{c-f}$	0.061 $_{c-f}$	715.8 $_{a-f}$	36.79 $_{c-f}$
D_3 (50%FC)						
Cont.	0.053 $_{f-h}$	0.029 $_{b}$	0.088 $_{e-g}$	0.051 $_{d-g}$	462.5 $_{d-g}$	91.00 $_{a-c}$
M_1	0.063 $_{d-f}$	0.224 $_{a}$	0.190 $_{a}$	0.082 $_{ab}$	432.2 $_{d-g}$	86.54 $_{a-d}$
M_2	0.077 $_{de}$	0.042 $_{b}$	0.128 $_{b-e}$	0.090 $_{a}$	518.5 $_{c-g}$	31.47 $_{d-f}$
M_3	0.049 $_{f-h}$	0.027 $_{b}$	0.082 $_{e-h}$	0.058 $_{c-g}$	556.1 $_{b-g}$	54.31 $_{b-f}$
With phosphorous (100 mg kg^{-1})						
D_1 (100% FC)						
Cont.	0.050 $_{f-h}$	0.029 $_{b}$	0.086 $_{e-g}$	0.049 $_{d-g}$	531.0 $_{b-g}$	36.41 $_{c-f}$
M_1	0.034 $_{h-j}$	0.019 $_{b}$	0.060 $_{f-h}$	0.040 $_{gh}$	548.7 $_{b-g}$	64.32 $_{b-f}$
M_2	0.030 $_{i-k}$	0.019 $_{b}$	0.053 $_{f-h}$	0.041 $_{gh}$	522.3 $_{c-g}$	78.31 $_{a-f}$
M_3	0.048 $_{f-i}$	0.026 $_{b}$	0.081 $_{e-h}$	0.048 $_{d-g}$	833.4 $_{a-e}$	28.74 $_{ef}$
D_2 (75%FC)						
Cont.	0.042 $_{g-j}$	0.024 $_{b}$	0.070 $_{e-h}$	0.050 $_{d-g}$	323.4 $_{fg}$	34.60 $_{d-f}$
M_1	0.060 $_{e-g}$	0.028 $_{b}$	0.095 $_{d-g}$	0.044 $_{e-h}$	856.1 $_{a-d}$	37.75 $_{c-f}$
M_2	0.030 $_{i-k}$	0.044 $_{b}$	0.150 $_{a-d}$	0.064 $_{b-e}$	686.7 $_{a-f}$	44.60 $_{b-f}$
M_3	0.041 $_{h-j}$	0.025 $_{b}$	0.072 $_{e-h}$	0.054 $_{d-g}$	738.2 $_{a-f}$	51.67 $_{b-f}$
D_3 (50%FC)						
Cont.	0.013 $_{k}$	0.009 $_{b}$	0.024 $_{h}$	0.028 $_{h}$	336.4 $_{fg}$	38.31 $_{c-f}$
M_1	0.0042 $_{g-j}$	0.042 $_{b}$	0.160 $_{a-c}$	0.075 $_{a-c}$	989.4 $_{a}$	94.00 $_{ab}$
M_2	0.026 $_{jk}$	0.014 $_{b}$	0.044 $_{gh}$	0.029 $_{h}$	378.7 $_{f-g}$	117.9 $_{a}$
M_3	0.041 $_{h-j}$	0.024 $_{b}$	0.069 $_{e-h}$	0.045 $_{e-h}$	269.4 $_{fg}$	48.79 $_{b-f}$
LSD (P=0.05)	0.016	0.104	0.052	0.016	387.4	45.33
ANOVA	P (Phosphorous); M (Mycorrhiza), D (Drought treatment)					
P	***	NS	***	***	*	NS
M	***	NS	***	**	**	*
D	**	NS	NS	*	NS	*
P×M	NS	NS	*	*	*	**
P×D	**	NS	*	**	*	NS
M×D	***	NS	***	**	*	NS
P×M×D	*	NS	*	***	*	NS

*P≤ 0.05; ** P≤ 0.01; *** P≤ 0.001, NS: Not significant.

[a] *G. mosseae;* [b] *G. intraradices*, [c] M1+M2.

Different letters within a column indicate significant differences using the Duncan's multiple range test at 5% significant level.

application. Hayman (1987) mentioned that if P fertilization is sufficient to provide what the host plant needs without decreasing the colonization level, then AM fungi may induce C loss from the host plant to the symbiont without the reciprocal transfer of P. This may explain a possible decrease in growth parameters of petunia plants colonized by different mycorrhiza with P application. However, for commercial AMF inoculation programs, it is fundamental to test various endophytes over a broad range of P levels to avoid 'fertilizer induced pathogenicity of mycorrhizal fungi (Kiernan *et al.,* 1983).

The data also indicate the potential role of AM fungi in the growth and mineral nutrition of tested plants under water stress condition. The improved WUE in +M-P plants (not in severe drought stress level) as compared to -M plants may indicate that AMF increased the ability of roots to absorb soil moisture, thus maintaining more open stomata in leaves and enhancing dry mass production. The +M petunia plants in this study produced more RDW than -M plants without P application (Bethlenfalvay *et al.,* 1988). This might partially explain why +M plants had higher WUE than -M plants (Ghazi and Al-Karaki, 1998).

The effects of the two fungal species on fresh and dry biomass, root colonization, and nutrient uptake observed in this experiment differed. Daniels and Menge (1981) as well as Schubert and Hayman (1986) reported that different AM fungal species have different effects on the growth of host plants in terms of dry mass production. Ferguson and Woodhead (1982) explained this phenomenon by differences in colonization levels, development and density of external mycelium and efficiency in essential nutrient translocation. In our experiment, in spite of higher root colonization of *G. intraradices*, *G. mosseae* had a more positive effect on nutrient uptake by the host showing that the infection extent can not be considered as a determinant point in host nutrition and hyphal translocation capacity. Density may play a more important role, however, extraradical hyphal development was not evaluated in this experiment.

A variety of mechanisms have been proposed as to how mycorrhizae may ameliorate the effects of water stress in plants. For example, mycorrhizal colonization may increase root length density or alter root system morphology, enabling colonized plants to explore more soil volume and extract more water than uncolonized plants during drought (Kothari *et al.,* 1990; Davis *et al.,* 1996; Bryla and Duniway, 1997a). Mycorrhizal hyphae may also directly enhance root water uptake, increasing water supply which would help sustain physiological activity within plants (Allen, 1982). Improved drought tolerance and better drought recovery by mycorrhizal plants has also been linked to improved P uptake (Nelsen and Safir, 1982; Graham *et al.,* 1987; Fitter, 1988). Our findings support all of these views because mycorrhizae enhanced uptake of P and root growth of water-stressed petunia plants compared to those without mycorrhizal colonization.

Our data indicated that the content of soluble sugar in +M leaves was higher than that in -M leaves in response to drought treatments, confirming earlier findings (Wu and Xia, 2006) and suggesting that natural physiological metabolisms of -M leaves were less than those of +M leaves during water stress . On the other hand, this result also suggested that AM inoculation helped soluble sugar accumulation under adverse conditions, potentially resulting in a decrease of osmotic potentials in host cells. Thus, +M plants could take up more water from the soil under water stress conditions. Another biochemical mechanism of drought tolerance in plants is the rapid accumulation of proline, commonly produced for osmoregulation of cells (Bray 1993, Maggio *et al.,* 2002). Our findings did not show any dramatic increases in proline contents in leaves of petunia plants which is not in agreement with the results suggesting that AM fungi can enhance the osmotic adjustment of plants through accumulation of proline (Schellembaum *et al.,* 1988; Poecel *et al.,* 2004). This may be attributed to either greater drought resistance or less injury of +M

petunia plants grown under drought stress conditions. The result agrees with previous reports obtained from *Citrus tangerine* (Wu and Xia, 2006), *Zea mays* (Ramakrishnan *et al.*, 1988), *Lactuca sativa* (Ruiz-Lozano and Azco´n 1997) and *Glycine max* (Porcel *et al.,* 2004).

Our results agree with several reports of decreasing contents of chlorophylls and carotenoids under drought or salt stress as reported for a number of plant species (Logini *et al.,* 1999; Agastian *et al.,* 2000). The decrease in Chl. a from drought stress can influence light harvesting complexes of thylakoid membranes leading to a reduction in dry mass production (Parida *et al.,* 2003) as apparent in this study (Table 2). The increased total chlorophyll concentration brought about by AM inoculations, however, was strictly due to an increase in Chl. a. The concentration of Chl. b is known to be related to the amount of the light harvesting complex of photosystem II and was not altered by AM treatments.

Plenchette and Duponnois (2005) reported that the formation of AM stimulates the absorption of phosphorus in host plants. Similar results were observed in this study that the formation of AM improved the phosphorus absorption in plants although the differences were not statistically significant but tissue P concentrations in -M plants were always less than those in +M plants. Based on the results (data not shown), the pH and calcium carbonate content of our experimental soil was high (more than 8% and 27% respectively). Since we used phosphoric acid as P source in the experiment, it could have resulted in increment of Ca^{2+} as well as $Ca_3(PO_4)_2$ concentrations. $Ca_3(PO_4)_2$ is an insoluble form of phosphorous so it may partially explain the low P content of plants. AMF treatments increased Zn uptake in the absence of P which corroborates research showing increased Zn uptake by mycorrhizae (Sharma *et al.,* 1991, Burkert *et al.,* 1994). Bell *et al.,* 1989) also reported decrease in Zn uptake in the presence of P with mycorrhizal peanut plants. Mn contents of plants were lower in +M than -M plants (Table 2) confirming earlier reports on maize, red clover and soybean (Arines *et al.,* 1992; Leidi *et al.,* 1987; Liu *et al.,* 2000). It has been proposed that AMF modifies root exudation, thereby decreasing Mn acquisition by depressing the number of Mn-reducing bacteria or by increasing the number of Mn-oxidizing bacteria in the rhizosphere.

In conclusion, water stress has been shown to adversely affect growth, nutrition and flowering of petunia plants. This study confirms that mycorrhizal colonization can mitigate the adverse effects of water stress on treated plants restoring most of the key growth parameters to levels similar or close to those in unstressed plants.

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Abbreviations:

+M: With mycorrhizae; -M: Without mycorrhizae; AMF: Arbuscular mycorrhizal fungi; +P: With phosphorous; -P: Without phosphorous; FFW: Foliar fresh weight; FDW: Foliar dry weight; RFW: Root fresh weight; RDW: Root dry weight; Chl: Chlorophyll; Cart: Cartenoids.

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پاسخ گیاهان اطلسی مایه کوبی شده با *Glomus mosseae* و *Glomus intraradices* به تنش خشکی و فسفر

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چکیده

به منظورارزیابی میزان مقاومت به خشکی گیاهان اطلسی (*Petunia hybrida cv.* Mix) آلوده به میکوریز، یک آزمایش گلخانه ای اجراگردید که در آن دو گونه مختلف میکوریز (*Glomus mosseae, Glomus intraradices*) به دو صورت کاربرد جداگانه و ترکیبی در دو سطح فسفر(صفرو۱۰۰ میلی گرم به ازا هرکیلوگرم خاک خشک) و سه رژیم آبیاری (۱۰۰٪ ظرفیت مزرعه به عنوان شاهد، ۷۵٪ ظرفیت مزرعه به عنوان تنش ملایم و ۵۰٪ ظرفیت مزرعه به عنوان تنش شدید) به‌کار برده شد. هر دو گونه میکوریز به‌خوبی بر روی ریشه گیاهان اطلسی مستقر گردیدند که میزان تجمع آنها با کاهش فسفر افزایش و با افزایش تنش خشکی کاهش یافت. تجمع میکوریز عموماً باعث افزایش رشد رویشی و زایشی در تمامی سطوح خشکی و فسفر گردید. در پاسخ به تنش خشکی، میزان قندهای محلول در برگ گیاهان میکوریز نسبت به گیاهان غیر میکوریز افزایش نشان داد اما پرولین درتیمارهای میکوریز و در شرائط مشابه افزایش چندانی نشان نداد. نتایج این تحقیق تائیدی است براین واقعیت که آلودگی میکوریزایی قادر به تعدیل اثرات مخرب تنش آبی بر گیاهان تیمار شده می-باشد و تاحدودی موجب حفظ شاخص های کلیدی رشد در سطحی شبیه یا نزدیک به گیاهانی می-گردد که درشرائط بدون تنش قراردارند.