

Effects of arbuscular mycorrhizal inoculation on growth, yield, nutrient uptake and irrigation water productivity of sunflowers grown under drought stress

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ABSTRACT

Drought stress is one of the most important limiting factors for field crops in arid and semi-arid regions. Yield assessments under drought stress conditions, using soil microorganisms to reduce the damage from drought stress, demonstrate that soil microorganisms are sustainable solutions for crop production in such climates. Therefore, we undertook a two year experiment to understand the effects of root colonization by two species of mycorrhizal fungi (*Glomus mosseae* and *Glomus hoi*), under different drought stress conditions, quantifying sunflower growth, nutrient uptake, yield, yield components, oil percentage and irrigation water productivity. The experiment was conducted at Tarbiat Modares University, located in the semi-arid region of Iran, during the 2006 and 2007 growing seasons. Drought stress was induced at two stress levels by irrigating after 60 and 80% water depletion, which were defined as mild and severe drought stress, respectively. Irrigating after 40% water depletion was considered to be normal irrigation (no stress). The results indicated that irrespective of the mycorrhizal species and the drought stress intensity, inoculated plants produced more dry matter, heavier seeds and greater seed and oil yields than did non-inoculated plants. Interestingly, the seed yields of plants inoculated with *G. mosseae* under each irrigation regime were higher than those of plants inoculated with *G. hoi*, or of the non-inoculated plants. Although drought stress reduced the N percentages in the leaves and seeds, mycorrhizal fungi enhanced the N percentages, particularly when the plants were inoculated with *G. mosseae*. In addition, seed P percentages were not affected by mycorrhizae in 2006. In contrast, the highest P percentages in both leaves and seeds were obtained from plants inoculated with *G. mosseae* in 2007. Overall, our results show that *G. mosseae* is more efficient under drought stress, and better supports sunflower plants. The application of these microorganisms could be critical in the cultivation of sunflowers under arid and semi-arid conditions, where water is the most important factor in determining plant growth and yield.

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1. Introduction

Water is one of the major limiting factors affecting plant growth, development and yield, especially in arid and semi-arid regions, where plants are often exposed to periods of water shortage (drought stress). Drought is a major cause of crop losses worldwide, reducing average yields by more than 50% (Wang et al., 2003). Among the diverse impacts of drought on plant development in these arid ecosystems, restricted water and nutrient availability are commonly discussed (Agnew and Warren, 1996). Fortunately, arbuscular mycorrhizal symbiosis can protect crops against the detrimental effects of drought stress. The symbiotic interactions of plants with mycorrhizal fungi are agriculturally and ecologically important (Indrasumunar, 2007). Over 90% of plants will engage in arbuscular mycorrhizal symbiosis, which mainly

improves the nutrient uptake of phosphorus, and several other (still disputed) nutrients (Bonfante, 2003; Brundrett, 2002). During the formation of arbuscular mycorrhizae, fungal hyphae enter the epidermal, exodermal and cortical cell layers of the roots, reaching the inner cortex, where the functional units, the arbuscules, develop. The fungi also form hyphae outside of the plant, extending the root–soil interface to facilitate the uptake of nutrients such as phosphates and water (Kistner and Parniske, 2002). Arbuscular mycorrhizal fungi enable the host plant to establish itself and grow more efficiently, even under conditions of biotic and abiotic stress, including drought (Porcel and Ruiz-Lozano, 2004), through a series of complex communications between the host and the fungus (Harrier, 2001). It has been shown that associations between roots and arbuscular mycorrhizal fungi enhance the amount of P taken up by plant roots under drought conditions (Ruiz-Lozano et al., 1995). As a result of the increased uptake of P, plants inoculated with mycorrhizae frequently produce higher yields than do those without arbuscular mycorrhizal fungi (Smith and Read, 1997). A significant osmotic adjustment based on the organic solutes,

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including soluble sugar, proline, etc., and inorganic ions, including potassium, calcium and magnesium, in the leaves of mycorrhizal plants have been reported (Wu and Xia, 2006). Also, improved P uptake due to the presence of arbuscular mycorrhizal fungi during periods of water shortage has been postulated as a primary mechanism for the enhanced drought tolerance of the host plants (Bethlenfalvay et al., 1988). However, other researchers consider that the drought tolerance of the host plants is independent of the P uptake stimulated by the arbuscular mycorrhizal fungi (Augé et al., 1994). Mycorrhizal colonization of the roots has been shown to increase drought tolerance in corn (Subramanian et al., 2006), wheat (Bryla and Duniway, 1997), soybean (Bethlenfalvay et al., 1988), onion (Azcón et al., 1996) and lettuce (Tobar et al., 1994; Azcón et al., 1996). There are also numerous reports of fungal symbionts (mycobionts) conferring host plant tolerance to various stresses, including drought, heat, salt, metals, and even diseases (Waller et al., 2005; Márquez et al., 2007; Rodríguez et al., 2008). However, most of the relevant experiments were conducted under controlled growth chamber or greenhouse conditions.

Sunflower (*Helianthus annuus* L.) is known as one of the most useful sources of edible oil in the world. The plant has a short vegetation period, is relatively drought tolerant and has shown potential for reducing the existing gap between the production and the consumption of edible oil because it contains 40–50% oil and 17–20% protein (Wang et al., 1997). Like other cultivated crops in arid and semi-arid climates, sunflower is subjected to drought stress. Yet, there is little information on the use of different species of mycorrhizal fungi under field conditions for improving sunflower yield and quality in semi-arid regions. This study investigates two different species of mycorrhizal fungi, investigating their efficiency under two different conditions of drought stress, by quantifying sunflower growth, nutrient uptake, yield, yield components, oil percentages and irrigation water productivity.

2. Materials and methods

2.1. Study site and climatic characteristics

Field experiments were conducted at the Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran (35°44' N, 51°10' E, altitude 1352 m), during the summers of 2006 and 2007. The average yearly precipitation (over a 30 year period), which occurs mostly during the autumn and winter months, is 298 mm for the site. The annual mean temperature is 19 °C. The average temperature in 2006 was similar to the long-term meteorological data trend, while in 2007, the average temperature (17 °C) was lower.

2.2. Soil sampling and analysis

The field was kept fallow during the previous year to reduce the endogenous mycorrhizal fungi and eliminate their propagules, and to allow for the decomposition of the root debris from the previous crop. Prior to the beginning of the experiment, a composite soil sample was collected at depths of 0–30 and 30–60 cm, air-dried, crushed and tested for various physical and chemical properties. The research field had a sandy loam soil. Details of the soil properties are shown in Table 1. In addition, the soil was evaluated biologically. A wet-sieving technique was used to extract spores, and the most probable number (MPN) test was used to determine the number of propagules (kg^{-1}) in the soil. Because the number of extracted propagules from the soil was extremely low ($2\text{--}3 \text{ kg}^{-1}$), based on the wet-sieving technique and the MPN test no attempt was made to fumigate the soil before applying the treatments.

Table 1
Soil physico-chemical properties.

Properties	Depth (cm)			
	0–30		30–60	
	2006	2007	2006	2007
Organic matter (%)	1.05	1.06	1.2	0.9
pH (water 1:2 ratio)	7.8	7.2	6.5	7.00
Sand (%)	68	62	59	52
Silt (%)	18	22	29	30
Clay (%)	14	16	12	18
EC (dS m^{-1})	1.7	1.0	1.2	1.1
N (%)	0.7	0.55	0.8	0.8
P (mg kg^{-1})	7.5	8.9	n.d. ^a	n.d.
K (mg kg^{-1})	310	345	n.d.	n.d.
Fe (mg kg^{-1})	6.9	7.5	n.d.	n.d.
Zn (mg kg^{-1})	1.2	0.7	n.d.	n.d.
CaCO ₃ (%)	4.5	7.7	n.d.	n.d.
CEC ^b ($\text{cmol}_c \text{ kg}^{-1}$)	5.8	6.1	n.d.	n.d.
Field capacity (%)	22	21	n.d.	n.d.
Crop extractable water (%)	10	10	n.d.	n.d.
Available water (%)	12	11	n.d.	n.d.

^a Not determined.

^b Cation exchange capacity.

2.3. Field preparation and treatment application

Sunflowers were planted in different sections of the field each year. Plots were prepared after plowing and disk-harrowing. The plots were 5 m long and consisted of eight rows, 50 cm apart. There was a 2 m path between all of the plots, to eliminate the influence of lateral water movement. The mycorrhizal fungal inoculants consisted of spores and hyphal root fragments from stock cultures of *Glomus mosseae* and *Glomus hoi*. The dose of inocula ($125 \text{ spores per ml of inoculum}$) was 50 g m^{-2} . The *G. mosseae* and *G. hoi* inocula were purchased as pure isolates from the Agricultural and Biotechnology Research Institute, Karaj, Iran. These isolates were selected because of their commercial availability in Iran. Early-maturing sunflower seeds (cv. Alestar) were inoculated with the inoculants and sown during the first week of June. The distance between the plants in the rows was 20 cm; thus, the plant density was approximately $100,000 \text{ plants ha}^{-1}$. According to the recommended N requirements and soil N content for sunflowers, 130 kg ha^{-1} N was supplied from fertilizer, using urea as its N source, at two time points, before seed sowing and at the flowering stage (R₂ stage, as described by Schaefer and Miller, 1981). According to the results of the soil analysis, no P or K fertilization was required. The experimental design was arranged as completely randomized blocks, with a split-plot arrangement for the treatments, and four replications. To monitor the soil water content (θ_v), the Time Domain Reflectometry (TDR) method was used. TDR probe tubes were inserted into the soil in each experimental plot at a depth of 0–80 cm (at 20 cm intervals). Data for the soil volumetric water content were collected daily during the growing season using a TDR device (TRIME-FM, England). Irrigation was performed whenever 40% of the available water was consumed, until the seedlings were established. The various experimental irrigation regimes were initiated after the third leaves appeared. The three experimental irrigation regimes, irrigation after 40% of the water was depleted (normal irrigation), irrigation after 60% of the water was depleted (mild stress) and irrigation after 80% of the water was depleted (severe stress), were randomized for the main plots, while the three mycorrhizal treatments, *G. mosseae*, *G. hoi* and no inoculation (control), were randomized for the subplots. A polyethylene piping network with a volume counter was used for accurate and uniform irrigation, and the total amount of water consumed during the growing season, under normal irrigation, and the mild and severe stress conditions, was recorded as 6700,

4900 and 3350 m³ ha⁻¹ during the first year, and 6450, 4275 and 3150 m³ ha⁻¹ during the second year, respectively. During plant growth, weeds were manually controlled three times.

2.4. Data collecting

Eight weeks after the initiation of drought stress, the relative water content of leaves (RWC) was calculated. For this purpose, eight leaves from each plot were detached and immediately weighed to determine fresh weight (FW). The same tissues were immersed in distilled water and placed in darkness for 2 h at 25 °C; afterwards, their turgid weights (TW) were measured. The samples were then dried in an oven at 110 °C for 24 h to obtain their dry weights (DW). The relative water content for each sample was calculated using

$$\frac{FW - DW}{TW - DW} \times 100 \quad (1)$$

At the flowering stage, the leaf area index (LAI) was measured by a leaf area meter (Delta-T area meter; Delta-T Devices Ltd., Cambridge, UK), and then, similar leaves were selected to determine the N, P and K content. The leaf samples were oven-dried at 70 °C for 72 h and then powdered by an electric mill. Nitrogen was measured using the Kjeldahl method (Nelson and Sommers, 1973). The P content was measured spectrophotometrically after acetic acid extraction, and finally, potassium was measured using the flame photometry method. After the 80th day of drought stress induction, root samples were collected to evaluate mycorrhizal colonization. The samples were washed with distilled water and stained with Trypan blue, and the mycorrhizal colonization levels were determined using the gridline intersect method of Giovanetti and Mosse (1980).

To determine the dry matter weight and seed yield for the sunflowers, 8 m² of each plot was hand-harvested at the physiological maturity stage. The oil percentages of the seeds were determined using an Inframatic 8620 (Percor, Germany). The oil yield was calculated by multiplying the seed yield quantity by the oil percentage. Phosphorus and potassium percentages in the seeds were measured using calorimetric (using a 6505 JenWay spectrophotometer) and flame (using a JenWay PFP7 flame-photometer) photometry methods, respectively. Irrigation water productivity (IWP) was calculated by dividing the dry matter yield (kg ha⁻¹) by the total seasonal irrigation water applied (m³). Mycorrhizal dependency was calculated for the sunflower yields in 2006 and 2007 using the following formula (Audet and Charest, 2006):

$$MD (\%) = \frac{\text{seed yield (M+)} - \text{seed yield (M-)}}{\text{seed yield (M+)}} \times 100 \quad (2)$$

where MD represents mycorrhizal dependency, for M+ (inoculated) and M- (not inoculated) plants.

2.5. Statistical analysis

The obtained data were subjected to an analysis of variance (ANOVA) with SAS 8.1 software. Probabilities of significance ($P \leq 0.01$ or 0.05) were used to test the significance among the main treatment effects, treatment combinations and interactions. In addition, the LSD test at a 0.05 probability level was used to compare the means.

3. Results

3.1. Mycorrhizal colonization rate

Irrespective of the drought stress intensity, the rate of root colonization in the non-inoculated plants was significantly lower than

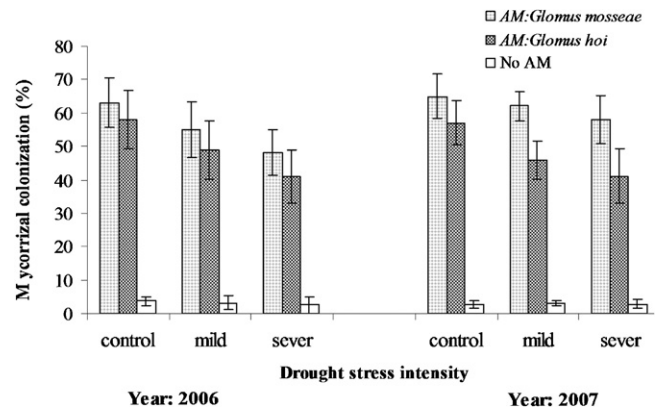


Fig. 1. Mycorrhizal colonization by *Glomus mosseae*, *Glomus hoi* and non-inoculated field grown sunflower plants under severe, mild and no drought stress (control) conditions ($n = 4$). Bars show standard error.

in the inoculated plants, during both years (Fig. 1). This demonstrates the lack of any mycorrhizal activity in the soil. After the 80th day of drought stress induction, the severe, mild, and no drought stress (control) treatments demonstrated 48, 55 and 63% colonization with *G. mosseae* in 2006, and 58, 62 and 65% colonization in 2007, as well as 41, 49 and 58% colonization with *G. hoi* in 2006, and 41, 46 and 57% colonization in 2007. Mycorrhizal colonization in the control plants remained at less than 4%, regardless of the irrigation treatment (Fig. 1). Generally, the highest colonization was observed in the roots inoculated with *G. mosseae* at each level of drought stress. However, increases in drought stress intensity decreased the colonization percentage (Fig. 1).

3.2. Leaf area index, relative water content and yield components

The LAI increased by 4% in 2007, in comparison with 2006, primarily because of more favorable climatic conditions (Table 2). During both years, drought stress significantly decreased the LAI, the RWC, the filled seed number and the 1000-seed weight (Table 2). When compared with normal irrigation, mild and severe drought stress decreased the LAI by 30 and 47% in 2006, and by 19 and 37% in 2007. Furthermore, during both experimental years, the RWC decreased linearly with increasing drought stress intensity (Table 2).

The main effects of mycorrhizal inoculation showed that, firstly, mycorrhizal inoculation (with either species) increased the LAI, the RWC, the filled seed number and the 1000-seed weight, when compared with the control treatment. Secondly, *G. mosseae* performed more favorably at improving these traits than did *G. hoi* (Table 2). Although, the interaction between mycorrhizal inoculation and drought stress was not significant, a comparison between the combined treatments indicated that under normal irrigation and under drought stress conditions, *G. mosseae* inoculation significantly increased the filled seed number and the 1000-seed weight when compared with *G. hoi* inoculation or with the control treatment (Table 2). Although there was no significant difference between the inoculated and the non-inoculated plants as far as their RWC in 2007, in 2006 the mycorrhizal inoculated plants showed a higher RWC than did the non-inoculated ones. Mycorrhizal inoculation had no significant effect on the RWC of either non-stressed or severely stressed plants during both years. But, under mild stress conditions, plants inoculated with *G. mosseae* showed a higher RWC than did plants inoculated with *G. hoi* (Table 2).

Table 2

Mean comparisons of treatment combinations, main effects and interactions of drought stress and mycorrhizal inoculation on LAI, RWC and yield components for sunflowers.

Treatments		Traits							
Treatment combinations		LAI		RWC (%)		Number of filled seed		1000 seed weight (g)	
Drought stress (S)	Mycorrhiza (M)	2006	2007	2006	2007	2006	2007	2006	2007
Ns	M ₀	2.36 a	2.50 a	77 a	78 a	5717 bc	5824 ab	59.3 a	60.9 a
Ns	M ₁	2.28 a	2.11 b	74 ab	77 a	6187 a	6175 a	64.8 a	66.5 a
Ns	M ₂	2.06 a	1.77 bc	71 ab	76 a	5851 b	5882 ab	61.3 a	65 a
Ms	M ₀	1.62 b	1.75 bc	76 a	67 b	5200 f	4100 d	30.7 cd	32 de
Ms	M ₁	1.58 bc	1.76 bc	69 b	66 bc	5663 cd	4566 c	44.3 b	45.7 b
Ms	M ₂	1.43 bc	1.62 cd	60 c	63 bcd	5473 e	4492 cd	36.9 c	40.3 bc
Ss	M ₀	1.32 bcd	1.54 cde	61 c	61 d	5045 f	4943 c	25.4 d	26.8 e
Ss	M ₁	1.22 cd	1.29 de	62 c	62 bcd	5519 de	5526 b	33.4 c	34.7 cd
Ss	M ₂	1.04 d	1.16 e	58 c	59 d	5129 f	3562 e	26 d	29.3 de
Mean		1.66	1.72	68	68	5532	5007	42	45
P-Value		**	**	**	**	**	**	**	*
SE		0.09	0.08	1.3	1.3	63	199	2.6	2.6
<i>SME</i>									
Ns		2.23 a	2.12 a	74 a	77 a	5918 a	5960 a	61.8 a	64.1 a
Ms		1.54 b	1.71 b	68 b	65 b	5445 b	4386 b	37.34 b	39.3 b
Ss		1.19 b	1.33 c	59 c	61 c	5231 c	4677 b	28.30 c	30.3 c
P-Value		**	**	**	**	**	**	**	**
<i>MME</i>									
M ₀		1.51 b	1.52 c	63 b	66 a	5321 c	4955 ab	38.4 b	39.9 b
M ₁		1.77 a	1.93 a	71 a	68 a	5790 a	5422 a	47.5 a	49 a
M ₂		1.69 a	1.72 b	68 a	68 a	5485 b	4645 b	41.4 b	44.8 a
P-Value		*	*	**	ns	**	*	*	*
<i>S × M interaction</i>									
P-Value		ns	ns	ns	ns	ns	ns	ns	ns

M₀, non mycorrhizal inoculation; M₁, inoculation with *G. mosseae*; M₂, inoculation with *G. hoi*; Ns, non-stress; Ms, mild drought stress; Ss, severe drought stress. SME, stress main effect; MME, mycorrhiza main effect; ns, not significant; SE, standard error.

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

Means within each column of each section followed by the same letter are not significantly different ($P \leq 0.05$).

3.3. Dry matter yield, seed yield and irrigation water productivity

An analysis of variance showed that both drought stress and mycorrhizal treatments significantly affected the dry matter yield, seed yield and irrigation water productivity (Table 3). The results demonstrated that both mild and severe drought stress significantly decreased the dry matter yield. There were decreases of 28 and 48% in 2006, and of 26 and 45% in 2007, when compared with plants that had the normal irrigation treatment. During both years, the induction of mild or severe drought stress consistently resulted in lower seed yield than that found in non-stressed plants (Table 3). Seed yield decreased by 38% in 2006 and 37% in 2007 due to mild stress, and 50% in 2006 and 2007 due to severe stress, relative to non-stressed plants. Low sunflower seed yield under limited irrigation conditions could result from reduced yield components, such as capitule diameter, seed number per capitule and 1000-seed weight. Furthermore, dry matter yield and seed yield in mycorrhizally inoculated plants were higher than in non-inoculated ones, regardless of the drought stress intensity (Table 3). Remarkably, plants inoculated with *G. mosseae* produced higher dry matter and seed yield in comparison with non-inoculated plants and plants inoculated with *G. hoi* that were grown under drought stress conditions (Table 3). Generally, mycorrhizally inoculated plants grown under normal irrigation conditions displayed significantly increased yields when compared with non-inoculated plants. However, the two fungal species varied in their potential with regard to various parameters. Of the two mycorrhizal species, *G. mosseae* responded more effectively, as it resulted in a significant increase in sunflower yield compared with non-mycorrhizal plants, while *G. hoi* responded less effectively.

Irrigation water productivity describes the quantitative relationship between plant growth and water consumption (Sezen

et al., 2011). Table 3 shows that during both years, the severe drought stress treatment had greater irrigation water productivity than the other irrigation treatments. In other words, irrigation water productivity increased during conditions of limited water. For the no-stress and mild-stress treatment conditions, irrigation water productivity decreased due to increased deep water percolation and evaporation. Plants inoculated with *G. mosseae* showed the maximum irrigation water productivity during both years; however, there was no significant difference between *G. mosseae* and *G. hoi* (Table 3). Under both normal irrigation and mild stress conditions, non-inoculated plants showed the highest irrigation water productivity values (Table 3). In contrast, under severe drought stress conditions, irrigation water productivity was found to be higher in plants inoculated with *G. mosseae* than in non-inoculated plants, or in plants inoculated with *G. hoi* (Table 3).

3.4. Seed oil content and oil yield

Drought stress, and inoculation with mycorrhizae, significantly affected the seed oil percentage and oil yield during both experimental years (Table 3). Nevertheless, no significant interaction between the irrigation regimes and the mycorrhizal treatments was detected during either year. A comparison of means revealed that severe drought stress significantly reduced seed oil percentages, so that the difference between the severe stress and the no-stress treatments was 12% in 2006 and 10% in 2007. Mycorrhizal inoculation significantly increased seed oil percentages and oil yields during both years (Table 3). The efficiency of the *Glomus* species demonstrated that, under normal irrigation and water stress conditions, *G. mosseae* application led to an increase in seed oil percentages and oil yields when compared with the *G. hoi*-inoculated or non-inoculated plants (Table 3).

Table 3
Mean comparisons of treatment combinations, main effects and interactions of drought stress and mycorrhizal inoculation on yield, Irrigation water productivity, seed oil content and oil yield of sunflower.

Treatments		Traits									
Treatment combinations		Dry matter yield (kg ha ⁻¹)		Seed yield (kg ha ⁻¹)		Irrigation water productivity (kg m ⁻³ ha ⁻¹)		Seed oil content (%)		Oil yield (kg ha ⁻¹)	
Drought stress (S)	Mycorrhiza (M)	2006	2007	2006	2007	2006	2007	2006	2007	2006	2007
Ns	M ₀	6637 b	7069 b	2341 b	2453 b	1.07 bc	1.18 c	45 bc	46.2 bc	1053 b	1133 b
Ns	M ₁	7230 a	7660 a	2619 a	2792 a	1.05 cd	1.16 c	49.2 a	52 a	1289 a	1453 a
Ns	M ₂	7039 a	7507 a	2344 b	2576 b	0.99 def	1.09 c	45.7 b	47.5 b	1073 b	1224 b
Ms	M ₀	4598 e	5031 e	1266 ef	1328 f	1.11 ab	1.38 a	41.2 e	44.2 bcd	521 e	585 ef
Ms	M ₁	5462 c	5892 c	1741 c	1914 c	1.02 cde	1.28 b	43.7 cd	46.2 bc	761 c	885 c
Ms	M ₂	5029 d	5497 d	1513 d	1696 d	0.94 f	1.17 c	42.5 de	45 bc	643 d	762 d
Ss	M ₀	3281 g	3713 g	1091 g	1096 g	1.12 ab	1.35 ab	39.5 f	41 d	432 f	447 g
Ss	M ₁	3780 f	4248 f	1402 de	1517 e	1.16 a	1.37 a	42.7 de	45.5 bc	598 d	689 de
Ss	M ₂	3901 f	4333 f	1135 fg	1280 f	0.97 ef	1.17 c	41.2 e	43.7 cd	469 ef	559 f
Mean		5218	5661	1717	1851	1.05	1.24	43	46	760	860
P-Value		**	**	**	**	**	**	*	*	**	**
SE		241	240	93	100	0.01	0.02	0.5	0.8	49	56
SME											
Ns		6969 a	7412 a	2434 a	2607 a	1.03 b	1.14 b	46.6 a	48.5 a	1138 a	1270 a
Ms		5030 b	5473 b	1507 b	1646 b	1.02 b	1.28 a	42.5 b	45.1 b	642 b	744 b
Ss		3654 c	4098 c	1210 c	1298 c	1.08 a	1.30 a	41.1 c	43.4 b	500 c	565 c
P-Value		**	**	**	**	*	*	**	*	**	**
MME											
M ₀		4838 c	5271 c	1566 c	1626 c	0.96 b	1.14 b	41.9 c	43.8 b	669 c	722 c
M ₁		5491 a	5933 a	1921 a	2074 a	1.10 a	1.30 a	45.2 a	47.9 a	883 a	1009 a
M ₂		5323 b	5779 b	1664 b	1851 b	1.08 a	1.27 a	43.1 b	45.4 b	728 b	848 b
P-Value		**	**	**	**	**	*	**	*	**	**
S × M interaction											
P-Value		ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

M₀, non mycorrhizal inoculation; M₁, inoculation with *G. mosseae*; M₂, inoculation with *G. hoi*; Ns, non-stress; Ms, mild drought stress; Ss, severe drought stress; SME, stress main effect; MME, mycorrhiza main effect; ns, not significant; SE, standard error.

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

Means within each column of each section followed by the same letter are not significantly different ($P \leq 0.05$).

Among all of the treatment combinations, the highest and lowest oil yields were obtained from non-stressed plants inoculated with *G. mosseae* (1289 and 1453 kg ha⁻¹ in 2006 and 2007, respectively) and from non-inoculated plants grown under severe drought stress conditions (432 and 447 kg ha⁻¹ in 2006 and 2007, respectively). Although, mycorrhizal inoculation increased oil yields in both the normally irrigated and stressed plants, its impact was more pronounced under mild stress conditions (Table 3). However, regardless of the drought stress intensity, the seed oil percentages obtained, and the oil yields from inoculated plants, were higher than those from the non-inoculated plants.

3.5. Nutrient content in the leaves and seeds

Drought stress significantly affected N percentages in both the leaves and seeds, while not having a significant effect on the leaf P and K percentages (Table 4). In addition, the results showed that the effect of mycorrhizal treatment was significant on all parameters related to nutritional status, except for leaf and seed P percentages in 2006, seed K percentages in 2007 and leaf K percentages during both years of the experiment (Table 4). The interaction between drought stress and mycorrhizae was not significant for the nutrient content of the leaves and seeds (Table 4). Although drought stress reduced the N percentages in the leaves and seeds, mycorrhizal fungi enhanced the N percentages, particularly when the plants were inoculated with *G. mosseae*. During both years, the leaf and seed N percentages in the stressed plants decreased in comparison

with the normally irrigated plants. The percentages of reduction were 31% (for leaves) and 9% (for seeds) for the non-inoculated plants, 14% (for leaves) and 6% (for seeds) for the plants inoculated with *G. mosseae* and 25% (for leaves) and 8% (for seeds) for the plants inoculated with *G. hoi*. A comparison of the means demonstrated that the inoculated plants had higher N percentages in both the leaves and the seeds under the different drought stress intensities (Table 4).

Although the irrigation regimes did not have a significant effect on the leaf P percentages, the P percentages decreased with increasing drought stress intensity (Table 4). In addition, the highest seed P percentages were observed in the normally irrigated plots. Seed P percentages were not affected by mycorrhizae in 2006. In contrast, the highest P percentages in both leaves and seeds were obtained from plants inoculated with *G. mosseae* in 2007 (Table 4). When averaged over the two years, plants inoculated with *G. mosseae* had increased their leaf P percentages by 14 and 10% when compared with both the control treatment (non-inoculated plants), and with those plants that were inoculated with *G. hoi*. Under severe drought stress conditions, mycorrhizal inoculation significantly increased the P percentage in the seeds (Table 4).

Leaf K percentages were affected by neither irrigation regimes nor mycorrhizal treatments (Table 4). Nonetheless, the main effect of drought stress (in 2007) and mycorrhizal inoculation (in 2006) was a significant change in the seed K percentages. Moreover, the highest leaf K percentages were obtained when the plants were inoculated with *G. mosseae* and irrigated normally, during both years (Table 4).

Table 4

Mean comparisons of treatment combinations, main effects and interactions of drought stress and mycorrhizal inoculation on N, P and K concentration of sunflower leaf and seed.

Treatments		Traits											
Treatment combinations		Leaf N		Seed N		Leaf P		Seed P		Leaf K		Seed K	
Drought stress (S)	Mycorrhiza (M)	% on dry matter basis		% on dry matter basis		% on dry matter basis		% on dry matter basis		% on dry matter basis		% on dry matter basis	
		2006	2007	2006	2007	2006	2007	2006	2007	2006	2007	2006	2007
Ns	M ₀	3.12 bc	3.39 bc	2.84 b	3.28 bc	0.42 abc	0.63 bcd	0.69 ab	0.80 abc	2.25 a	2.93 a	0.42 ab	0.47 abc
Ns	M ₁	3.58 a	3.89 a	3.09 a	3.55 a	0.50 ab	0.72 a	0.78 a	0.87 a	2.31 a	2.96 a	0.46 a	0.50 a
Ns	M ₂	3.24 b	3.59 b	2.94 ab	3.38 b	0.49 abc	0.66 ab	0.69 ab	0.83 ab	2.14 a	2.95 a	0.42 ab	0.48 ab
Ms	M ₀	2.21 d	2.59 g	2.59 c	3.11 de	0.38 bc	0.60 cd	0.61 b	0.75 bc	1.96 a	2.88 a	0.37 bc	0.44 abcd
Ms	M ₁	2.86 c	3.16 cd	2.85 b	3.38 b	0.52 a	0.68 ab	0.69 ab	0.82 ab	2.11 a	2.92 a	0.45 a	0.48 ab
Ms	M ₂	2.47 d	2.94 de	2.85 b	3.20 cd	0.38 bc	0.63 bcd	0.73 ab	0.80 abc	2.16 a	2.90 a	0.42 ab	0.45 abc
Ss	M ₀	1.88 e	2.28 h	2.54 c	2.97 e	0.38 bc	0.58 d	0.63 b	0.73 c	1.97 a	2.82 a	0.35 bc	0.42 cd
Ss	M ₁	2.44 d	2.88 ef	2.95 ab	3.28 bc	0.40 abc	0.62 bcd	0.72 ab	0.80 abc	2.20 a	2.88 a	0.46 a	0.45 abcd
Ss	M ₂	2.23 d	2.64 fg	2.54 c	3.08 de	0.37 c	0.59 cd	0.60 b	0.75 bc	2.06 a	2.84 a	0.33 c	0.41 d
Mean		2.67	3.04	2.80	3.25	0.43	0.64	0.69	0.80	2.13	2.90	0.41	0.46
P-Value		**	**	*	**	*	*	*	*	ns	ns	*	*
SE		0.1	0.09	0.04	0.03	0.01	0.01	0.01	0.01	0.04	0.02	0.01	0.009
<i>SME</i>													
Ns		3.13 a	3.62 a	2.95 a	3.41 a	0.47 a	0.67 a	0.72 a	0.84 a	2.23 a	2.95 a	0.43 a	0.49 a
Ms		2.51 b	2.90 b	2.76 b	3.23 b	0.43 a	0.64 a	0.68 ab	0.79 b	2.07 a	2.90 a	0.42 a	0.46 ab
Ss		2.18 c	2.60 c	2.68 b	3.11 c	0.38 a	0.60 a	0.65 b	0.76 b	2.07 a	2.85 a	0.38 a	0.43 b
P-Value		**	**	*	**	ns	ns	*	*	ns	ns	ns	*
<i>MME</i>													
M ₀		2.40 c	2.75 c	2.65 c	3.12 b	0.39 a	0.61 b	0.64 a	0.76 b	2.06 a	2.88 a	0.38 b	0.45 a
M ₁		2.96 a	3.31 a	2.98 a	3.41 a	0.47 a	0.68 a	0.67 a	0.83 a	2.02 a	2.92 a	0.45 a	0.48 a
M ₂		2.65 b	3.06 b	2.78 b	3.22 b	0.41 a	0.63 b	0.73 a	0.80 ab	2.12 a	2.90 a	0.39 b	0.45 a
P-Value		**	**	**	**	ns	**	ns	*	ns	ns	*	ns
<i>S × M interaction</i>													
P-Value		ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

M₀, non mycorrhizal inoculation; M₁, inoculation with *G. mosseae*; M₂, inoculation with *G. hoi*; Ns, non-stress; Ms, mild drought stress; Ss, severe drought stress; SME, stress main effect; MME, mycorrhiza main effect; ns, not significant; SE, standard error.

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

Means within each column of each section followed by the same letter are not significantly different ($P \leq 0.05$).

The results demonstrated that plants inoculated with *G. mosseae* accumulated more K in their seeds when compared with the plants inoculated with *G. hoi* or with the non-inoculated plants grown under different drought stress intensity (Table 4).

3.6. Mycorrhizal dependency

Mycorrhizal dependency was more pronounced in plants grown under drought stress conditions than in normally irrigated ones (Fig. 2). Plants inoculated with *G. mosseae* and grown under either mild or severe drought stress conditions demonstrated increased

mycorrhizal dependency when compared with plants inoculated with *G. hoi* (Fig. 2).

4. Discussion

The results demonstrated that non-inoculated plants, grown either under normal or limited irrigation, had the lowest mycorrhizal colonization rates (less than 4%). This resulted from the low population level of mycorrhizae in the soil. Drought stress also had a negative effect on mycorrhizal colonization. In contrast, the highest colonization rate was obtained in inoculated plants that were not subjected to any drought stress. It has been reported that drought stress significantly decreases mycorrhizal colonization in watermelon (Wu and Xia, 2006). This is most likely because the contents of the mycorrhizal fungi are absorbed by the host plants under stressful conditions (Evelin et al., 2009). A higher colonization rate in plants inoculated with *G. mosseae* rather than in those inoculated with *G. hoi*, under both irrigation regimes (stress and no-stress conditions), might be due to a greater adaptation of this species to its native soil, in the sense of benefiting its hosts best, given these soil conditions. The different levels of mycorrhizal colonization and efficiency might be due to the different behaviors of each mycorrhizal fungal species, even under similar conditions. The high colonization rate of *G. mosseae*, regardless of soil water content, suggests that this species is the best adapted, or the most aggressive colonizer, under drought stress conditions.

In the present study, drought stress decreased the LAI and the RWC, which is supported by the results of other sunflower research (Connor and Sadras, 1992; Unger, 1992). The principal

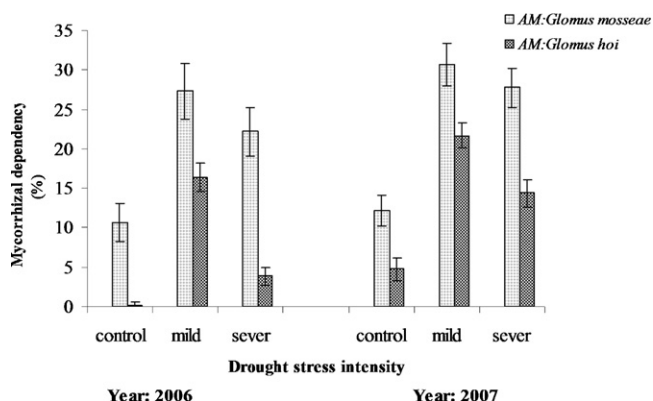


Fig. 2. Mycorrhizal dependency of *Glomus mosseae* and *Glomus hoi* in field grown sunflower plants under severe, mild and no drought stress (control) conditions ($n = 4$). Bars show standard error.

reason for reduced leaf expansion under drought stress conditions is the reduction in leaf cell hydraulic conductivity, which, in turn, decreases water transport and hinders both cell enlargement and cell division, and finally, leaf development. A significant and direct correlation between the RWC and the LAI ($r^2_{\text{year 2006}} = 0.70^{**}$, $r^2_{\text{year 2007}} = 0.65^{**}$) was found in support of this claim. Irrigation after 80% water depletion decreased the filled seed number and the 1000-seed weight to a greater degree than did irrigation after 60 or 40% water depletion. It has been reported that drought stress at the sunflower seed filling stage reduces the rate of photosynthesis and the rate of assimilative transport, leading to a loss of seed weight (Pandey et al., 2000). We also observed positive effects from arbuscular mycorrhizal fungi on the LAI and the sunflower yield components, under both normal and limited irrigation. Positive effects from mycorrhizal inoculation are attributed to improvements in the uptake of nutrients (Bethlenfalvay et al., 1988), greater water absorption by hyphae (Faber et al., 1991) and changes in plant stomatal sensitivity, perhaps by increasing abscisic acid production (Ruiz-Lozano et al., 1995). During both years, in the normally irrigated plots, the RWC was the same in both the non-inoculated and the inoculated plants. These results were also observed by Diallo et al. (2001) in cowpea plants inoculated with *Glomus intraradices*. Increases in the RWC under severe drought stress and inoculation with *G. mosseae* demonstrated that these plants had more hydraulic conductivity in their roots at a low water potential. The improved root conductance is associated with a longer root and an alteration in the root system induced by mycorrhizae (Kapoor et al., 2008). Strong and positive correlations between the root colonization rate and the LAI ($r^2_{\text{year 2006}} = 0.72^{**}$, $r^2_{\text{year 2007}} = 0.68^{**}$), and also between the LAI and the seed yield ($r^2_{\text{year 2006}} = 0.80^{**}$, $r^2_{\text{year 2007}} = 0.66^{**}$) indicate that mycorrhizal symbiosis enhances the photosynthetic source of plants through the increase in the LAI, so that crops with higher production capacities can produce a greater seed yield.

Our results confirm that drought stress significantly decreases both dry matter yield and sunflower seed yield. Drought stress reduces sunflower yield through three principal mechanisms. First, whole canopy absorption of incident photosynthetically active radiation may be reduced by a drought-induced limitation of the leaf expansion area (Xianshi et al., 1998). Second, drought stress reduces the efficiency with which absorbed PAR is used by the crop to produce new dry matter (Stone et al., 2001). Third, drought stress may limit the seed yield by reducing the harvest index (Earl and Davis, 2003).

Mycorrhizal colonization, especially by *G. mosseae*, improved growth, dry matter yield and seed yield in sunflowers exposed to various intensities of drought stress. Previous researchers have found that the efficiency of arbuscular mycorrhizal fungi would increase with increasing drought stress (Audet and Charest, 2006; Subramanian et al., 2006; Miransari et al., 2008). Other authors have suggested that increases in crop yield under drought stress conditions resulting from arbuscular mycorrhizal colonization may be explained by a greater root surface absorption area, densely proliferated root growth (Kothari et al., 1990) or hydraulic differences between root systems (Augé et al., 1994). Augé (2001) has explained that mycorrhizal symbiosis improves the hydraulic conductivity of the roots at low soil water potentials, which ultimately influences the water potential, transpiration rate, leaf resistance and crop yield. In the current experiment, different species of fungi differ in their potential to facilitate greater dry matter yield and seed yield. Between the two different mycorrhizal species used, *G. mosseae* was most efficient, so that an increase of 3 and 14% (averaged over the two years) in dry matter yield and seed yield was observed, when compared with plants inoculated with *G. hoi*. Because different fungi have different colonization rates (Ortas et al., 2011),

it is clear that various mycorrhizal species have different root colonization capacities and also different influences on plant growth and performance. Our results showed that *G. mosseae* was a more effective fungal symbiont for increasing the drought tolerance of sunflowers, both in terms of growth under drought conditions and in terms of water productivity.

Drought stress increased IWP slightly during both years. As mentioned earlier, for the normally irrigated treatment, IWP was lower due to increased deep water percolation and evaporation. Our results demonstrated that plants inoculated with *G. mosseae* were more efficient in producing dry matter in response to applied irrigation than were plants inoculated with *G. hoi* or non-inoculated plants under drought stress conditions. In addition, regardless of the mycorrhizal species, symbiosis with mycorrhizal fungi had a positive effect on IWP when compared with non-inoculated plants. Shahhosseini et al. (2012) have observed increases in stomatal conductance, root development, water absorption and nutrient uptake in mycorrhizally inoculated plants, resulting in increased yield and IWP. Furthermore, differences between mycorrhizal species with regard to IWP are due to differences in their capacity to produce external mycelia (Augé et al., 2004), which supply the plant with a higher degree of water availability in the soil.

During both experimental years, increases in each level of drought stress resulted in significant decreases in seed oil percentages. Previous studies have reported contradictory results, showing that drought stress does not affect sunflower seed oil percentages (Sing and Bhunia, 1997; Pejić et al., 2009). They reasoned that because seed oil percentage is a qualitative trait controlled by many genes, it is not probable that all of the genes controlling seed oil percentage would be inhibited by drought stress. In contrast, other investigators have concluded that sunflower seed oil percentage is diminished under water deficiency conditions (Unger and Paul, 1982; Connor and Sadras, 1992). Connor and Sadras (1992) suggested that abscisic acid produced in the leaves of stressed plants is translocated to the seeds, and thus contributes to the decline in the seed oil percentage. These findings are in agreement with our results.

Inoculation by *G. mosseae* improved both seed oil percentage and oil yield in plants exposed to various intensities of drought stress. Unfortunately, there are no comprehensive data demonstrating how mycorrhizae affect seed oil percentages. Increases in oil percentages due to the application of mycorrhizae might be due to increases in P availability in the plants because of the mycorrhizal association. There are many reports stating that P absorption and availability would be increased in mycorrhizally inoculated plants (Martin et al., 2012; Grace et al., 2009). Because P is one of the main elements in phospholipid structures, increases in P availability by mycorrhizae can improve the metabolic pathways leading to oil production.

In the present study, we found that drought stress significantly decreased leaf and seed N percentages, and the P and K percentages in the seeds. In contrast, drought stress had no significant effect on leaf P and K percentages. Decreases in the transpiration rate due to drought stress reduce the flow of the water mass through the soil, consequently decreasing the N absorbed by the plants. Of the three principal mechanisms (mass flow, diffusion and contact exchange) affecting plant absorption of elements, diffusion is the most influential for K (Schaff and Skogley, 1982). Because soil water potential would be decreased due to drought stress, diffusion coefficients, and consequently K absorption, would decrease.

Mycorrhizal fungi enhanced N percentages in sunflower leaves and seeds, particularly when the plants were exposed to drought stress conditions. In addition, plants inoculated with *G. mosseae* accumulated the highest N when compared with the plants inoculated with *G. hoi*, under varying intensities of drought stress. Mycorrhizal fungal associations increase nutrient uptake

by increasing the absorptive system surface area of plants and by exploring the soil with extra-radical hyphae beyond the root hairs. It has been reported that mycorrhizal fungi increase N uptake in drought-stressed plants (Busse and Ellis, 1985) due to increases in root hydraulic conductivity (Graham and Syversen, 1984). It has been suggested that mycorrhizal fungi acquire N from organic substances by enhancing decomposition (Hodge et al., 2001; Goussous and Mohammad, 2009). These nutritional changes assist in maintaining the water status of the host plant under conditions of water shortage. As mentioned earlier, the mycorrhizal hyphae of *G. mosseae* contributed more to the uptake of N when compared with the plants inoculated by *G. hoi*. It has been reported that *G. mosseae* is better able to penetrate the host plant roots compared with other species (Martin et al., 2012).

The results obtained from both experimental years indicated that leaf and seed P percentages in inoculated plants were higher than those in non-inoculated plants. Several studies have demonstrated that plants colonized by mycorrhizal fungi are much more efficient in taking up soil P than non-inoculated plants (Smith and Gianinazzi-Pearson, 1988; Asmah, 1995). There are various possible mechanisms by which P availability and absorption can be enhanced through the application of mycorrhizal fungi: (i) by enhancing the P uptake that is facilitated by the extensive hyphae of the fungus, allowing them to explore more soil volume than can the non-mycorrhizal plants (Evelin et al., 2009) and (ii) by increasing the microbial biomass in the mycorrhizal plots. Microbial biomass increases when mycorrhizal fungi are added to the soil, increasing the release of CO₂, which, in turn forms H₂CO₃ in the soil solution. The resulting weak acid can dissolve primary phosphorus-containing minerals, thereby increasing P availability. A close relationship between the P content and drought tolerance has been reported previously (Subramanian et al., 2006). It has also been reported that P concentrations in the leaves may affect the stomatal response to environmental stress, perhaps by affecting the energetics involved in guard cell osmotic parameters or by wall stiffening governing the stomatal movements (Augé, 2001). It is reasonable, then that plants inoculated with mycorrhizal fungi show higher performance when compared with non-inoculated plants, even under stressful conditions.

Mycorrhizal treatments increased the leaf and seed K percentages during both years, but the differences were only significant for the seed K percentages in 2007. Potassium plays a key role in plant metabolism, particularly under drought stress conditions. It activates a range of enzymes, and plays an important role in stomatal movements and protein synthesis (Evelin et al., 2009). As our findings have also revealed, it has been reported that mycorrhizal colonization can enhance K absorption by plants (Giri et al., 2007). Increased K concentration can be a consequence of the increased P availability from mycorrhizal fungal activity. In addition, a synergistic relationship between P and K has been reported by Cardoso and Kuyper (2006). They have stated that P availability in the root zone increased K absorption in plants. Ruiz-Lozano et al. (1995) believe that the mitigation of drought stress by different mycorrhizal fungal species can be described by specific physiological (CO₂ fixation, transpiration, water use) and nutritional (P and K) mechanisms, according to which species of fungus are involved in the symbiotic association. Our results support their statements and show that there are differences in the symbiotic physiology of different mycorrhizal species.

5. Conclusion

Our results clearly illustrate that *G. mosseae* is more efficient under drought stress, and better supports sunflower plants. The application of these microorganisms could be critical in the

cultivation of sunflowers under arid and semi-arid conditions, where water is the most important factor in determining plant growth and yield.

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