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FULL LENGTH ARTICLE

Effects of different mycorrhiza species on grain yield, nutrient uptake and oil content of sunflower under water stress

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Abstract The role of arbuscular mycorrhizal fungi in alleviating water stress is well documented. In order to study the effects of water stress and two different mycorrhiza species on grain yield, nutrient uptake and oil content of sunflower, a field experiment as split plot design with three replications was conducted in the Research Field Station, Zabol University, Zabol, Iran in 2011. Water stress treatments included control as 90% of field capacity (W_1), 70% field capacity (W_2) and 50% field capacity (W_3) assigned to the main plots and two different mycorrhiza species, consisting of M_1 = control (without any inoculation), M_2 = *Glumus mossea* and M_3 = *Glumus etanicatum* as sub plots. Results showed that by increasing water stress from control (W_1) to W_3 treatment, grain yield was significantly decreased. The reduction in the level of W_3 was 15.05%. The content of potassium in seeds significantly decreased due to water stress but water stress upto W_2 treatment increased the content of phosphorus, nitrogen and oil content of seeds. In between two species of mycorrhiza in sunflower plants, *Glumus etanicatum* had the highest effect on grain yield and these elements in seeds and increased both.

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1. Introduction

Plants are exposed to a variety of biotic or abiotic stresses, such as drought, salt loading and freezing stress that influence

their development, growth and productivity. One of the major abiotic stresses that affect plant productivity is water stress, resulting from drought and salinity (Gueta-Dahan et al., 1997). Water stress is one of the major causes for crop loss worldwide, reducing average yields by 50% and over (Wang et al., 2003). Under such stress, water deficit in plant tissue develops, thus leading to a significant inhibition of photosynthesis. The ability to maintain the photosynthetic machinery functionality under water stress, therefore, is of major importance for drought tolerance. Plants react to water deficit with a rapid closure of stomata to avoid further water loss via transpiration (Cornic, 1994). Water stress adversely affects plant establishment and thereafter growth and development. Water stress reduces plant growth by affecting various physiological

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and biochemical processes, such as photosynthesis, respiration, translocation, ion uptake, carbohydrates, nutrient metabolism and growth promoters (Jaleel et al., 2008).

Water stress may affect the mineral-nutrient relations in plants. Generally, drought reduces both nutrient uptake by the roots and transport from the roots to the shoots, because of restricted transpiration rates and impaired active transport and membrane permeability (Alam, 1999).

The symbiosis of plant roots with AM fungi is known to be one of the most ancient and widespread plant strategies to enhance nutrient acquisition and to cope with environmental stress (Brachmann and Parniske, 2006). The intra-radical mycelium of these soil fungi proliferates in the root cortex of the host plant. Extra radical AM hyphae spread in the soil around the root and provide the surface area by which the AM fungus absorbs nutritional elements such as phosphorus (P), nitrogen (N), zinc (Zn) or copper (Cu) for transport and transfer to the host plant (Smith and Read, 2008).

AM fungus is the most common type of mycorrhizal association, occurring in 2/3 of land plants (Hodge, 2000). Mycorrhizal fungi have been reported in the roots of chickpea plants, improving the growth and yield of these plants, especially in phosphorus deficient soils (Zaidi et al., 2003). Many workers have reported the enhancement of phosphate uptake and growth of leguminous plants by vesicular arbuscular mycorrhizal fungi (AMF) (Atimanav and Adholeya, 2002).

Therefore, the main aim of this study was to investigate the effects of different mycorrhizal species on grain yield, nutrient uptake and oil content of sunflower under water stress conditions.

2. Materials and methods

A field experiment was conducted at the research farm of the Zabol university in Iran (latitude of 30° 54' N and longitude of 61° 41' E with an elevation of 481 m) in the 2011. The field soil was sandy loam in texture, having pH, 7.6; EC, 1.4 ds.m⁻¹; 0.04% N, 4.6 and 125 ppm of available P and K, respectively. Experiment laid out as split plot based on randomized complete block design with three replications. Three levels of water stress $W_1 = 90$ (control), $W_2 = 70$ and $W_3 = 50\%$ of the field capacity (FC), determined at the 0–15 cm soil depth by TDR, as main plots and two different Arbuscular mycorrhizal fungi consisting of $M_1 =$ control (without any inoculation), $M_2 =$ *Glomus mossea* and $M_3 =$ *Glomus etanicatum* as sub plots. Seeds of sunflower (Alester cultivar) were washed with distilled water then inoculation was performed by a suspension of any bacteria (108 cfu ml⁻¹) with perlite mixture. Mycorrhiza spores were added to each respective mycorrhizal treatment, non-mycorrhizal plants received mycorrhiza spore-free medium.

There were six rows in each plot. The width and length of each row were 0.3 and 2 m, respectively. Before sowing, the soil was fertilized with N, P and K at a rate of 100, 50 and 50 kg ha⁻¹ as urea, single super phosphate and potassium sulfate, respectively. Half of the nitrogen was applied at sowing time and residue at the start of four leaves. Seeds were placed at 1–2 cm depth. At the harvesting stage, the two middle rows were used and seed yield, oil percentage and oil yield were assessed. Grain yield in each plot was measured with 10% humidity. To determine the oil content (% of d.m.) by a Soxhlet

apparatus petroleum ether at 40–60 °C was used as a solvent. To estimate the potassium concentration in seeds, samples of seeds were dry ashed at 500 °C and then determined by Jenway PFP7 Flame photometer (Keison Products, UK).

For nitrogen content, samples were digested according to the method of Chapaman and Pratt (1961), and total nitrogen content was determined using the Kjeldhal method. Phosphorus was estimated by the method given by Chapaman and Pratt (1961). Vanadate solution was added to the molybdate solution and cooled to room temperature; 250 mL of concentrated HNO₃ was then added and diluted to 1 L. Next, 0.5 g of plant material (seeds) was taken in 50 mL volumetric flasks and 10 mL of vanadomolybdate reagent was added to each flask. The volume was achieved with deionized water. The solution was kept for 30 min, and then the absorbance was taken at 420 nm with a spectrophotometer. Appropriate standards were run simultaneously.

2.1. Statistical analyses

All data were analyzed with SAS Institute Inc. 6.12 software. All data were first analyzed by ANOVA to determine significant ($P = 0.05$) treatment effects. Significant differences between individual means were determined using the Duncan's Multiple Range Test (DMRT) at 5% level of probability. Data points in the figures represent the means \pm SE of three independent experiments at least three replications per treatment combination each.

3. Results and discussion

3.1. Grain yield

Statistical analysis of data (ANOVA) showed that the grain yield was significantly affected by water stress (Table 1). By increasing water stress from control (W_1) to W_3 treatment, grain yield was reduced. This reduction in the level of W_3 was 15.05% (Table 2). Ashraf and Mehmood, (1990), reported that, even a short term water deficit stress can cause substantial losses in crop yield, which is in agreement with our results. Stone et al. (2001), indicated that water stress deficit causes considerable decrease in the yield and oil content of sunflower.

In this study although mycorrhiza treatment had no significant effect on grain yield (Table 1), but in between the two species of mycorrhiza, *G. etanicatum* had the highest effect on grain yield in sunflower (Table 2). It is known that different species of AM fungi differ in the type of benefits they confer on the growth and development of plants (Howeler et al., 1987).

Avis et al. (2008), studied the effect of *G. mossea* on the growth and productivity of legumes. They observed that arbuscular mycorrhiza was significantly affected when compared with nonmycorrhizal plants. Mycorrhizal plants performed better than non-mycorrhizal plants. Maximum flowers were produced at mycorrhizal treatments. The process of flowering and fruiting first appeared in the mycorrhizal plants.

Data in this study indicated that interaction between water stress and mycorrhiza had no significantly effect on the grain yield in sunflower (Table 1).

Table 1 Results of two-way analysis of variance (ANOVA) of water stress (W) and mycorrhiza species (M) effects and their interaction (W*M) for the variables listed.

Dependent variable	Df	Independent variable (mean square)				
		Grain yield	Oil percentage	Nitrogen	Phosphorus	Potassium
Replication	2	26557.2 ^a	47.08 ^b	1.12 ^b	57.62 ^c	42.01 ^c
Water stress (W)	2	290830.8 ^c	31.4 ^b	1.05 ^b	8.35 ^b	23.34 ^b
Error a	4	10535.1	12.44	0.117	3.99	11.85
Mycorrhiza species (M)	2	17929.4 ^a	12.28 ^b	1.56 ^b	7.91 ^b	0.07 ^a
W*M	4	48568.8 ^a	12.11 ^b	0.22 ^a	8.43 ^b	0.24 ^a
Error b	12	93185.5	4.36	0.23	2.45	1.51

Number represents *F*-values at 5% level.

^a ns, Non-significant.

^b Significant at $P < 0.05$.

^c Significant at $P < 0.01$.

3.2. Nutrient elements (N, P and K)

The current study revealed that water stress had a significant effect on the three nutrient elements including potassium, phosphorus and nitrogen in the seeds of sunflower (Table 1). The results showed that water stress increased the concentration of P and N in seeds, but this increase was only up to W₂ treatment. With the continued rise in water stress level, the concentration of these elements was decreased (Table 2). Unlike these two elements with increasing water stress levels from control (W₁) to W₃, the concentration of potassium in seeds was reduced (Table 2).

Nitrogen is the mineral element that plants require in the largest amounts and is a constituent of many plant cell components, including amino and nucleic acids. Drought conditions may reduce soil-N mineralization, thus lowering the N availability, a reduced crop N uptake may also be attributed to a decreased transpiration rate to transport N from roots to shoots (Tanguilig et al., 1987).

Drought and salinity affect plant growth similarly through water deficit, K⁺ is equally important for maintaining the turgor pressure in plants under either stress. The availability of K⁺ to the plant decreases with decreasing soil water content, due to the decreasing mobility of K⁺ under these conditions. Kuchenbuch et al. (1986), showed that low levels of soil moisture reduced root growth and the rate of potassium inflow in

onion plants in terms of both per unit of root growth and per unit of root length.

In this experiment, with the exception of potassium, mycorrhiza treatment had only significantly effect on nitrogen and phosphorus content in seeds (Table 1). Between the two species of mycorrhiza used in sunflower plants, *G. etanicatum* had more effect on these elements in seeds (Table 2).

Phosphorus is a constituent of nucleic acids, phospholipids, phosphoproteins, dinucleotides, and adenosine triphosphate. Hence, P is required for processes including the storage and transfer of energy, photosynthesis, the regulation of some enzymes, and the transport of carbohydrates. It is generally accepted that the uptake of P by crop plants is reduced in dry-soil conditions (Pinkerton and Simpson, 1986). For example, the translocation of P to the shoots is severely restricted even under relatively mild drought stress (Rasnick, 1970). However, Liebersbach et al. (2004), reported that the large amount of molecular exudates (i.e., mainly mucilage) from plants in dry soil counteract the reduced mobility of P under such conditions.

Khalil et al. (1994), studied the mycorrhizal dependency of soybean and corn. They found that, soybean had a higher mycorrhizal dependency than maize. When mycorrhizal and non-mycorrhizal plants were compared, the N, P, K, Ca and Mg uptake were significantly increased in mycorrhizal plants. Mycorrhizae of the host plant increases the absorption of

Table 2 Mean comparisons of some traits for sunflower at various levels of water stress and mycorrhizal inoculation.

Treatments	Grain yield	Oil percentage	Nitrogen	Phosphorus	Potassium
	(g.m ⁻²)		%	(mg/gDW)	
<i>Water stress</i>					
W ₁	2300.17a	37.7b	2.06b	9.03b	14.50a
W ₂	2100.3b	41.34a	2.86a	10.81a	11.82b
W ₃	1975.9b	40.23a	2.67a	10.56a	11.61b
<i>Mycorrhiza</i>					
M ₁	2121.6a	38.52b	2.41a	9.78b	12.74a
M ₂	2082.9a	40.84a	2.71a	10.21a	12.57a
M ₃	2171.9a	39.91ab	2.91a	10.41a	12.61a

Means in each column and for each treatment, followed by the same letter are not significantly different at 5% of probability level-using Duncan's Multiple Range Test.

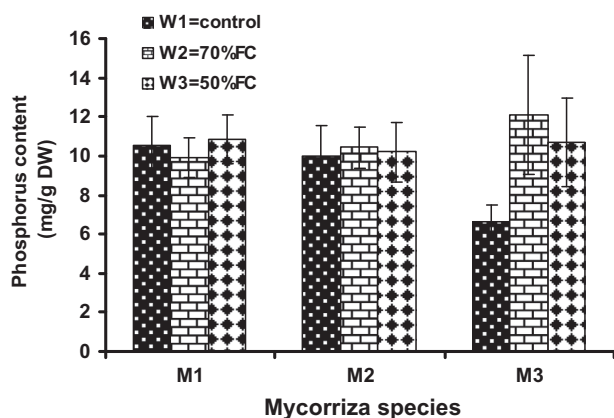


Figure 1 Effect of the water stress and mycorrhiza species on phosphorus content in seed.

food elements in soil. Unlike phosphorus, nitrogen is an important element. Studies show that a mycorrhizae hosted plant increases the absorption of nitrogen (Bolan, 1991). Mycorrhiza hyphae have the ability of absorbing the soil nitrogen and transferring it to the plant's root (Bago et al., 1996). In the case of potassium absorption by mycorrhizae some researchers say that it has no effect and some are saying it is useful (Barea, 1992). Among the three elements studied in these experiments, interaction between water stress and mycorrhizal treatment had only significant effect on P content in the seeds of sunflower (Table 1). The highest content of P was obtained at W₂M₃ treatment (Fig. 1).

Mycorrhizal fungi are effective in attracting food elements like phosphorus and nitrogen and the absorption of water in tension conditions, and in producing botanic hormones and harmonizing the environmental stress effect, increasing the plant's resistance to abiotic stress (Portas and Taylor, 1975).

3.3. Oil content

Statistical analysis of data showed water stress had significant effect on oil content in sunflower (Table 1). The result of this study indicated that water stress treatments up to W₂ increased the oil content but by increasing water stress from W₂ to W₃ the oil content decreased (Table 2). The oil content in sunflower seed depends on weather conditions, disease attacks

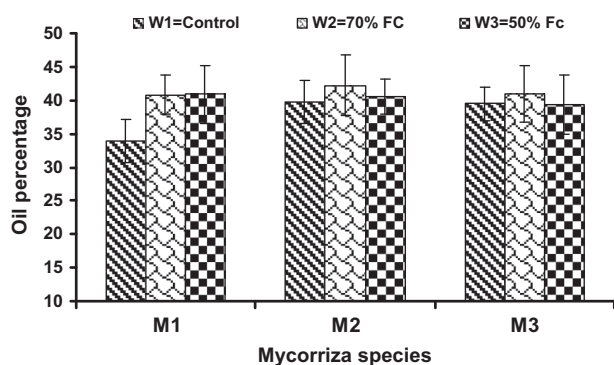


Figure 2 Effect of the water stress and mycorrhiza species on oil content in seed.

during the season, and the hybrid's characteristics. According to Škorić et al. (1994), oil synthesis ceases in sunflower seeds whenever the plant is exposed to any kind of stress. Škorić (1992), indicated that oil content in sunflower seed depends on mean daily air temperature, water supply at seed fill and the duration of the seed filling stage. Harris et al. (1978), noted that the oil content of sunflower seeds decreased with increasing air temperature.

Our results showed that the oil content of seeds in sunflower was significantly affected by mycorrhizal inoculation (Table 1). Results showed that the use of mycorrhiza increases the amount of oil content compared to the control treatment. In between two species of mycorrhiza, *G. mossea* had the highest effect of increasing oil content in seeds (Table 2).

Data in this study indicated that interaction between water stress and mycorrhiza inoculation had significant effect on oil content (Table 1). The highest oil content of seeds was obtained at W₂M₂ treatment (Fig. 2).

4. Conclusion

In the present study, water stress significantly decreased the grain yield of sunflower (Alester cultivar) but the application of mycorrhiza, increased the grain yield and oil content significantly compared to the control treatment. Water stress decreased the potassium content of seeds but by increasing water stress from control up to W₂ treatment, the content of nitrogen and phosphorus increased. In between two species of mycorrhiza, *G. etanicatum* had the highest effect in increasing grain yield and nutrient content of sunflower.

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