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ORIGINAL ARTICLE

# Alleviation of drought stress of marigold (*Tagetes erecta*) plants by using arbuscular mycorrhizal fungi

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**Abstract** The effect of an arbuscular mycorrhizal fungus “AMF” (*Glomus constrictum* Trappe) on growth, pigments, and phosphorous content of marigold (*Tagetes erecta*) plant grown under different levels of drought stress was investigated. The applied drought stress levels reduced growth vigor (i.e. plant height, shoot dry weight, flower diameter as well as its fresh and dry weights) of mycorrhizal and non-mycorrhizal plant as compared to control plant (non-drought stressed plant). The presence of mycorrhizal fungus, however, stimulated all growth parameters of the treated plant comparing to non-mycorrhizal treated plant. The photosynthetic pigments (carotene in flowers and chlorophylls *a* and *b* in leaves) were also stimulated by the mycorrhizal fungi of well-watered as well as of water-stressed plants. The total pigments of mycorrhizal plants grown under well-watered conditions were higher than those of non-mycorrhizal ones by 60%. In most cases, drought-stressed mycorrhizal plants were significantly better than those of the non-mycorrhizal plants. So, the overall results suggest that mycorrhizal fungal colonization affects host plant positively on growth, pigments, and phosphorous content, flower quality and thereby alleviates the stress imposed by water with holding.

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## 1. Introduction

Marigold (*Tagetes erecta* L.) belongs to Asteraceae family and is a herbaceous plant with aromatic, pinnately divided leaves and is usually used as a bedding plant, cut flower, or as a coloring agent in poultry feed to obtain yellow egg yolks (Dole and Wilkins, 2005). *T. erecta* L. has smaller flowers and leaves than those of most other marigolds. The plants brighten up any sunny area in the landscape and attract attention. Moreover, marigold plants are considered a very valuable enter crop for controlling plant parasitic nematode as recorded by Basu and Roy (1975). The aerial parts of the plant contain high

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quality of essential oil that can be used for scenting soaps, perfumery, cosmetic, and pharmaceutical industries.

Arbuscular mycorrhizal (AM) fungal symbiosis is widely believed that it protects host plants from detrimental effects of drought (Augé, 2001; Abdel-Fattah et al., 2002; Ruiz-Lozano, 2003). Possible mechanisms for improving drought resistance of the mycorrhizal plants could be due to an increased in root hydraulic conductivity (Robert et al., 2008), stomatal regulation or transpiration rate (Allen and Boosalis, 1983), enhanced water uptake at low soil moisture levels as a result of extraradical hyphae (Fagbola et al., 2001), osmotic adjustment which promotes turgor maintenance even at low tissue water potential (Augé et al., 1986), increased photosynthetic activity, proline and carbohydrate accumulation, and increased nutritional status in mycorrhizal plants (Scheilenbaum et al., 1999). These mechanisms may be important in adaptation of the mycorrhizal plants to drought conditions. 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 which copes with the environmental stress (Brachmann and Parniske, 2006). The intra-radical mycelium of these soil fungi proliferates in root cortex of the host plant. Extraradical AM hyphae spread in the soil around the root and provide a surface area by which the AM fungus absorbs nutritional elements “such as phosphorus (P), nitrogen (N), zinc (Zn), or copper (Cu)” and transports and transfers them to the host plant (Smith and Read, 2008).

One of the increasing interest and economic importance is the variation of the mycorrhizal responsiveness of a specific fungus by the variations in cultivars of the same host plants. This concept has been reported for field-grown crops, including basil (Gupta et al., 2000), grapes (Karagiannidis et al., 1995), onions (Tawaraya et al., 2001), wheat (Zhu et al., 2001), marigold (Robert et al., 2003), *Brodiaea laxa* (Scagel, 2004), *Coriander* spp. (Aliabadi et al., 2008), *Viola calaminaria* (Fernández et al., 2008), *Calendula* spp. (Rahmani et al., 2008), and medicinal and aromatic plants (Hosseini et al., 2009).

Marigolds are annual plants that generally respond to mycorrhizal infection, but they do not always exhibit significant responsiveness under P-limiting conditions (Koide et al., 1999). However, plants of this nature may still benefit from the symbiosis, if not by enhancing growth, and hence increasing disease resistance (Linderman, 2000), and environmental stress tolerance (Cantrell and Linderman, 2001), or other physiological changes (Koide, 2000).

Therefore, the objective of this study was to investigate the effects of arbuscular mycorrhizal fungus (*Glomus constrictum*) on growth, phosphorus content, and flower quality of marigold plants subjected to various levels of drought stress conditions.

## 2. Materials and methods

### 2.1. Growth conditions

Seeds germination and transplanting of marigold (*T. erecta*) plants, cultivar ‘Jubilee’ were carried out in a greenhouse with a temperature of 27/18 °C day/night, and a supplemental light of 750  $\mu\text{mol m}^{-2} \text{s}^{-1}$  (at a canopy level) provided by high-vapor pressure sodium lamps for 14 h day<sup>-1</sup>, and misted twice daily until transplanted, Plant Production Departments, College of Food and Agricultural Sciences, King Saud University, Riyadh, Saudi Arabia. Seeds were germinated in 72-cell plug

flats containing a seedling germination mix of 50% fine peat moss and 50% vermiculite. Seeds were sown on 16 September, 2009 and transplanted 2 weeks later into plastic pots of 25 cm diameter with one seedling per pot containing a mixture of one coarse sand:one low P sandy loam soil (v/v) media with a textural analysis of 77% sand, 9% clay, and 14% silt. The sandy loamy soil was collected from the experimental and agricultural research station at Dirab, Riyadh region, Saudi Arabia. Prior to the study, the mix was steam-pasteurized with AM fungal inoculants or non-inoculated controls and then cooled and stored for at least a week prior to use. Soil characteristics were as follows: the water pH 7.8; 23 mg phosphorus kg<sup>-1</sup>; 17 mg nitrogen kg<sup>-1</sup>; 28 mg potassium kg<sup>-1</sup>, and 34 mg magnesium kg<sup>-1</sup> soil.

### 2.2. Mycorrhizal fungal inoculum preparation

The mycorrhizal fungus was originally isolated from the Dirab Experimental Station of College of Food and Agricultural Sciences, Riyadh region, Saudi Arabia. The fungus was propagated in pot culture on roots of bunching onion (*Allium cepa* L. ‘White Lisbon’) grown in loam:sand (1:1) medium for 5 months. The non-mycorrhizal control soil had a similar culture, but without AM fungi. Inocula consisted of a mixture of soil medium, extraradical hyphae, and spores, and colonized root segments ( $\leq 2$  mm in length). The inoculum (5 g of soil containing spores of mycorrhizal fungus) was placed 3 cm below the surface of the soil (before sowing) to produce mycorrhizal plants. The non-mycorrhizal treatment received an equal amount of sterilized soil inoculum to provide the same microflora without mycorrhizal fungi.

### 2.3. Experimental design

Pots were arranged on a greenhouse bench in a 2  $\times$  4 factorial randomized block design included two mycorrhizal treatments (with mycorrhizal fungi “M+” and non-mycorrhizal fungi “M-”), and four levels of drought stress (no drought, mild drought, moderate drought, and severe drought). Plants were carefully watered as needed with tap water to maintain soil moisture near field capacity for 3 weeks, and then plants were subjected to eight treatments (five pots were used for each treatment). When seedlings were transplanted, irrigation was given uniformly to all pots. Marigold seedlings were exposed to four levels of drought stresses (100% (D<sub>0</sub>), 75% (D<sub>1</sub>), 50% (D<sub>2</sub>), and 25% (D<sub>3</sub>) according to water holding capacity of the soil. Plants were fertilized twice weekly with 13 N–0.9 P–10.8 K soluble fertilizer prepared to supply N and K at approximately 200 mg kg<sup>-1</sup> N, and P at 16 mg kg<sup>-1</sup>. Each pot received approximately 100 ml of the fertilizer solution to ensure thought-out saturation of the medium. Growth measurements, pigment analysis, and determination of phosphorus content in both the leaves and the flowers were carried out after 6 weeks from transplantation.

### 2.4. Seedling measurements

The following parameters were examined at the end of the experiment (8 weeks old):

1. Plant height (cm).
2. Shoot fresh and dry weights per plant (g).

3. Flower fresh and dry weights (g) and diameter (cm) per plant.
4. Mineral analysis: oven-dried shoots (leaves and flowers) and samples were ground to pass through 0.5 mm sieve and then analyzed for P content according to method of Allen (1989).
5. Estimation of photosynthetic pigments content: the photosynthetic pigments (chlorophylls *a*, *b* and carotenoids) were extracted and determined in fresh leaves of marigold plants according to the spectrophotometric method recommended by Metzner et al. (1965).

### 2.5. Statistical analysis

A randomized complete block design with three replicates was used. Data were subjected to statistical analysis according to (Steel and Torrie, 1980). The treatment means were compared using the least significant differences (LSD) test at 0.05% level.

## 3. Results and discussion

### 3.1. Growth parameters and plant productivity

Under well-watered conditions, mycorrhizal fungus significantly increased all the growth attributes such as plant height, shoot dry weight, flower diameter, flower fresh, and dry

weights of marigold plants comparing to non-mycorrhizal plants (Table 1). The drought stress treatments significantly reduced the height, shoot dry weight, flower diameter, flower fresh, and dry weights of both the mycorrhizal and the non-mycorrhizal plants. This reduction was greatly offset by the *G. constrictum* stimulation of growth response of the treated plants comparing to the non-mycorrhizal treated plants (Fig. 1). Generally, under drought stress, mycorrhizal fungus stimulated greater growth criteria and flower parameters of treated plants than those of the non-mycorrhizal plants (Fig. 2). The enhanced dry weights of AM inoculated plants were 5.29, 4.12, 3.06, and 2.62 g, while of non-AM inoculated plants were 4.44, 3.28, 2.89, and 2 g at different levels of water-stressed conditions (well-watered, mild, moderate and severe drought-stressed treatments, respectively). Such pronounced growth response to mycorrhizal colonization was observed by Wu and Xia (2006) and Wu et al. (2008) who noticed that the mycorrhizal (AM) seedlings of *Citrus tangerine* and *Poncirus trifoliata* had significantly higher shoot and root dry weights, plant height, leaf area, leaf number per plant, and stem diameter under well-watered and water-stressed conditions than the corresponding non-AM seedlings. Similar results have been reported for other plant species (Kaya et al., 2003; Wu and Xia, 2006). The positive effect was likely attributed to the improvement of phosphorus nutrition (Bethlenfalvay et al., 1988) and uptake of water by hyphae (Faber

**Table 1** Growth parameters of marigold plants in response to mycorrhizal inoculation grown under different levels of drought stress (D<sub>0</sub> no drought, D<sub>1</sub> mild, D<sub>2</sub> moderate and D<sub>3</sub> severe drought).

Treatments		Plant height (cm)	Shoot dry weight (g)	Flower diameter (cm)	Flowers fresh weight (g)	Flowers dry weight (g)
Drought	Mycorrhizal status					
D <sub>0</sub>	M+	58.6	5.29	5.22	9.01	1.64
	M-	54.4	4.44	4.62	8.14	1.15
D <sub>1</sub>	M+	53.3	4.12	4.23	7.14	1.02
	M-	50.8	3.28	4.01	6.91	0.93
D <sub>2</sub>	M+	47.6	3.06	4.02	6.33	0.83
	M-	45.1	2.89	3.79	5.75	0.75
D <sub>3</sub>	M+	36.5	2.62	3.34	5.24	0.67
	M-	31.4	2.01	2.86	4.84	0.55
LSD 5%		1.5*	0.31*	0.22*	0.19*	0.11*

Where M+ = mycorrhizal treatment and M- = non-mycorrhizal treatment.

\* Significant difference at  $p \leq 0.05$  (Steel and Torrie, 1980).



**Figure 1** Effects of mycorrhizal fungus (*Glomus constrictum*) on the vegetative growth of marigold plants grown either under well-watered (left) or under drought stress condition (right), where M = treated and NM = non-treated mycorrhizal plants.



**Figure 2** Effects of mycorrhizal fungus (*Glomus constrictum*) on the flowering growth of marigold plants grown either under well-watered (left) or under drought stress condition (right), where M = treated, NM = non-treated mycorrhizal plants.

et al., 1991). Dell-Amico et al. (2002) also found that the inoculation of tomato plants with *G. clarum* encouraged higher growth rates of plants in both well-watered and stressed conditions. Drought-stressed non-mycorrhizal plants produced lower products than mycorrhizal ones at all drought treatments (Table 1). These results are in good agreement with Al-Karaki et al. (2004), who observed that the inoculation with AM fungi provided an important enhancement to yield of two wheat cultivars. Sorial (2001) also observed increases in straw and grain yield of wheat plants subjected to different levels of water stress and inoculated with arbuscular mycorrhiza when compared to non-mycorrhizal plants. The enhancement in marigold growth and biomass yields due to AM fungi might be attributed to higher mineral and water uptake. Al-Karaki and Clark (1998) stated that the enhanced plant growth as well as yield following AM inoculation were due to the improved uptake of P and Cu, especially under water-stressed conditions. Whereas, M+ plants were taller than M- plants regardless of levels of drought stress, and the mycorrhizal response was more pronounced in severe drought treatment (D<sub>4</sub>).

### 3.2. Photosynthetic pigments

The content of photosynthetic pigments (chlorophylls *a*, *b* in leaves and carotenoids in flowers) of mycorrhizal and non-mycorrhizal marigold plants are presented in Table 2 and Fig. 3. In general, with all treatments, the contents of chlorophylls *a*, *b* and carotenoids in mycorrhizal plants were significantly greater than those of non-mycorrhizal ones at all stages of plant growth. The total photosynthetic pigments increased due to mycorrhizal colonization by 60% at well-watered conditions. These results also indicated that the deleterious effect of drought treatment on total pigments was at the severe drought stress of the plants.

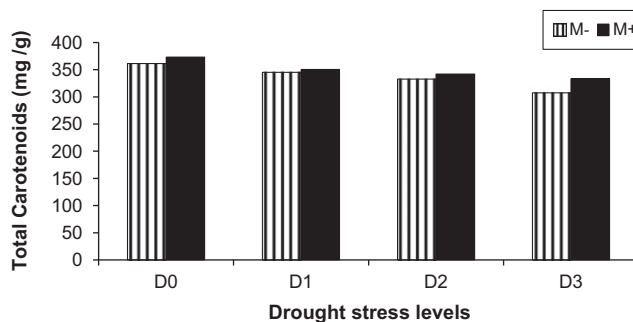
The decrease of chlorophyll content in marigold plants as a result of water deficit has also been reported by several authors (Dhanda et al., 2004; Shao et al., 2007) and citrus (Wu and Xia, 2006). Shao et al. (2007) reported that chlorophyll was the substantial basis for wheat photosynthesis, so the content of chlorophyll could be one of the indexes for evaluating photosynthesis. Many reports showed that drought could lead

**Table 2** Chlorophylls *a* and *b* for mycorrhizal (M+) and non-mycorrhizal (M-) marigold plants exposed to different levels of drought stress (D<sub>0</sub> no drought, D<sub>1</sub> mild, D<sub>2</sub> moderate and D<sub>3</sub> severe).

Treatments		Chlorophylls (mg/g fresh weight)	
Drought	Mycorrhizal	Chlorophyll <i>a</i>	Chlorophyll <i>b</i>
D <sub>0</sub>	M+	0.343	0.145
	M-	0.335	0.136
D <sub>1</sub>	M+	0.323	0.127
	M-	0.313	0.117
D <sub>2</sub>	M+	0.303	0.112
	M-	0.295	0.104
D <sub>3</sub>	M+	0.284	0.101
	M-	0.270	0.095
LSD 5%		0.004*	0.004*

Where M+ = mycorrhizal treatment and M- = non-mycorrhizal treatment.

\* Significant difference at  $p \leq 0.05$  (Steel and Torrie, 1980).



**Figure 3** Total carotenoids in flowers of mycorrhizal M+ (filled bars) and non-mycorrhizal M- (empty bars) marigold plants grown under different levels of drought stress.

to lower photosynthesis and efficiency (Dhanda et al., 2004). However, Moran et al. (1994) stated that the decrease in chlorophyll or protein concentrations would be a typical symptom of oxidative stress that had been observed in drought-stressed

plants. The increase of photosynthetic pigments as a result of mycorrhizal colonization was also supported by Aboul-Nasr (1996) and Wu and Xia (2006). The present results indicate that AM application assist plants to counter photoinhibition and photodestruction of pigments under stressed conditions by increasing the content of carotenoids. It is well known that carotenoids are involved in protecting photosynthetic apparatus against the photoinhibitory damage by the single oxygen. Therefore, carotenoids can directly deactivate, and can also quench the excited triple state of chlorophyll (Foyer and Harbinson, 1994). Moreover, it has been mentioned that the higher chlorophyll content in AM than in non-AM plants has sometimes been associated with a higher rate of photosynthesis, or with the increase in nitrogen and magnesium contents (major components of chlorophyll molecules) of mycorrhizal plants (Mathur and Vyas, 1995).

### 3.3. Phosphorus content

Drought stress levels significantly reduced the phosphorus content of mycorrhizal and non-mycorrhizal marigold plants comparing to well-watered plants (Fig. 4). However, the rate of reduction was higher in non-mycorrhizal than in mycorrhizal plants. These results agreed with the findings of Al-Karaki et al. (2004) on wheat plants; Augé et al. (2007) on sorghum and squash; Wu and Xia (2006) on citrus. Moreover, Subramanian et al. (2006) also observed that the mycorrhizal tomato plants had significantly higher phosphorus uptake in both roots and shoots of the plants regardless of the drought stress intensities.

Phosphorus concentration may affect host water balance. For instance, stomatal conductance can be influenced by P starvation. Koide (2000) suggested that the increased stomatal conductance and transpiration rate in AM plants could be due to P-mediated improvement in photosynthetic capacity. Phosphorous concentrations in leaves may affect stomatal response to environmental perturbations, perhaps by affecting the energetic involved in guard cell osmotic potential or wall stiffening governing stomatal movements (Weyers and Meidner, 1990). Wu and Xia (2006) reported that under water stress conditions, higher plants accumulate some small molecules including organic solutes and inorganic ions. The accumulation of these molecules in AM seedlings resulted in a greater osmotic adjustment, and allowed AM seedlings to accumulate more carbohydrate and thus higher plant biomass. Davies et al. (1992) found that external hyphal development and soil aggregation of

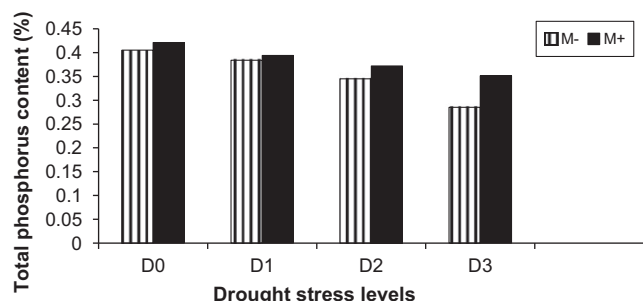
mycorrhizal plants were enhanced by drought acclimation. However, O'Keefe and Sylvia (1993) observed that external hyphae adhere to soil particles, and improve contact with soil solution. Furthermore, they mentioned that hyphae access smaller pore spaces better than plant roots and root hairs.

## 4. Conclusion

The results of this study indicated that marigold plants grown under semiarid habitats were affected greatly by water-stressed conditions. The data showed that the mycorrhizal colonization improved drought resistance of the marigold plants as a consequence of enhancing nutritional status, especially P and water status of the plants. This enhances plant growth, phosphorous uptake, and plant productivity. Therefore, this study recommends farmers in new reclaimed lands to not withhold irrigation during heading stage of marigold plants, and suggests adding arbuscular mycorrhizal fungi to arid farms, or to farms suffering from withholding irrigation water at critical growth stages.

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**Figure 4** Phosphorus content in leaves of mycorrhizal M+ (filled bars) and non-mycorrhizal M- (empty bars) marigold plants grown under different levels of drought stress.

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