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Improving growth, flower yield, and water relations of snapdragon (*Antirhinum majus* L.) plants grown under well-watered and water-stress conditions using arbuscular mycorrhizal fungi

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Abstract

The influence of arbuscular mycorrhizal (AM) fungus *Glomus deserticola* (Trappe and John) on plant growth, nutrition, flower yield, water relations, chlorophyll (Chl) contents and water-use efficiency (WUE) of snapdragon (*Antirhinum majus* cv. butterfly) plants were studied in potted culture under well-watered (WW) and water-stress (WS) conditions. The imposed water stress condition significantly reduced all growth parameters, nutrient contents, flower yield, water relations, and Chl pigment content and increased the electrolyte leakage of the plants comparing to those of nonstressed plants. Regardless of the WS level, the mycorrhizal snapdragon plants had significantly higher shoot and root dry mass (DM), WUE, flower yield, nutrient (P, N, K, Mg, and Ca) and Chl contents than those nonmycorrhizal plants grown both under WW or WS conditions. Under WS conditions, the AM colonization had greatly improved the leaf water potential (Ψ_w), leaf relative water content (RWC) and reduced the leaf electrolyte leakage (EL) of the plants. Although the WS conditions had markedly increased the proline content of the leaves, this increase was significantly higher in nonmycorrhizal than in mycorrhizal plants. This suggests that AM colonization enhances the host plant WS tolerance. Values of benefit and potential dry matter for AM-root associations were highest when plants were stressed and reduced under WW conditions. As a result, the snapdragon plants showed a high degree of dependency on AM fungi which improve plant growth, flower yield, water relations particularly under WS conditions, and these improvements were increased as WS level had increased. This study confirms that AM colonization can mitigate the deleterious effect of water stress on growth and flower yield of the snapdragon ornamental plant.

Additional key words: arbuscular mycorrhiza; flower yield; snapdragon; water relations; water stress.

Introduction

In many arid and semiarid regions, water stress affects physiological and biochemical processes of plants (Hanson and Hitz 1982, Al-Karaki and Al-Raddad 1997, Maggio *et al*. 2000, Asrar and Elhindi 2011), resulting in altering growth, yield and water relations (Abdel-Fattah *et al*. 2002, Wu and Xia 2006, Ibrahim *et al*. 2011) and metabolic pathways (Subramanian and Charest 1995). Reduction in plant growth under water stress is due to osmotic stress resulted from decreasing soil water potential and uptake of nutrients (Auge 2001, Lee *et al*. 2007). In this connection, many studies have pointed that the cell membranes were the initial sites of the stress injury and were damaged drastically by an environmental stress (Munns *et al*. 2006). Commonly, changes in electrolyte leakage have been measured to detect stress injury of the cell membrane. Leakage will vary in relation to the

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Abbreviations: AM – arbuscular mycorrhizal; AMF – arbuscular mycorrhizal fungi; DM – dry mass; DM_m – dry mass of mycorrhizal plants; DM_{nm} – dry mass of nonmycorrhizal plants; EC_1 – initial electrical conductivity; EC_2 – final electrical conductivity; FN – flower number; FFM – flower fresh mass; FDM – flower dry mass; FM – fresh mass; EL – electrolyte leakage; *G*. – *Glomus*; LA – leaf area; LN – leaf number; LDM – leaf dry mass; LFM – leaf fresh mass; LTM – leaf turgid mass; MD – mycorrhizal dependency; RWC – relative water content; SM – leaf saturated mass; non-AMF – nonarbuscular mycorrhizal fungi; Sd – stem diameter; SH – shoot height; SN – spike numbers; WS – water-stress; WW – well-watered; WUE – water-use efficiency; WUE_m – water-use efficiency of mycorrhizal plants; WUE_{nm} – water-use efficiency of nonmycorrhizal plants; Ψ_w - leaf water potential.
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membrane's abilities to take up and retain solutes and thus will reduce changes in both membrane potentials and membrane permeability (Hoque and Arima 2000, Liu *et al*. 2006).

The arbuscular mycorrhizal (AM) fungi can affect water balances of both amply watered and drought host plants (Auge 2001). AM fungal symbiosis has proved its capability to improve water relations and enhance plant resistance to water stress (Auge *et al*. 1987, Cho *et al*. 2006, Stevens *et al*. 2011, Zhang *et al*. 2011, Abdel-Fattah and Asrar 2012). However, the mechanisms of the AMF to improve drought resistance and water flow through plants are still unclear. The possible mechanisms could include the following: (*1*) extensive absorption of water by external hyphae (Ruiz-Lozano and Azcon 1995, Cho *et al*. 2006), (*2*) stomatal regulation through hormonal signals (Goicoechea *et al*. 1996), (*3*) a greater osmotic adjustment in mycorrhizal plants which promotes turgor maintenance even through at low tissue water potential (Auge *et al*. 1986, Porcel and Ruiz-Lozano 2004), and (*4*) an indirect effect of including photosynthetic activity, proline accumulation and increased nutritional status in the mycorrhizal plants (Abdel-Fattah 2001, Wu and Xia 2006, Asrar and Elhindi 2011). These mechanisms may be essential in adaptation of the mycorrhizal plants to drought conditions.

Snapdragon (*Antirhinum majus* L.) is one of the important ornamental bedding and cut flower plants, used worldwide in landscaping of gardens, parks, streets, and borders. Flowers and leaves of snapdragon have been known to have medical properties (Bulir 2009). Increasing growth and productivity of the ornamental plants such as snapdragon per unit area as well as expanding the cultivated area, particularly in soil subjected to water stress is the major concern of the decision markers. AM plants have a greater osmotic adjustment than non-AM plants (Subramanian *et al*. 2006, Wu and Xia 2006, Wu *et al*. 2008). However, the application of AM inoculation to mitigate the adverse effect of water stress on growth, flower yield, and quality of ornamental plants is still unexplored. The strategy for managing water stress *via* improving WUE and nutrient uptake can be achieved by inoculating soils with appropriate AM fungi. Therefore, this study was conducted to evaluate the effects of the arbuscular mycorrhizal fungus, *Glomus deserticola* (Trappe and John) on growth, water relations, flower yield, nutrition, Chl and proline contents and WUE of snapdragon plants grown either under WW or WS conditions.

Materials and methods

Experimental design: The experiment was conducted using a 2 × 4 randomized complete block design with two arbuscular mycorrhizal treatments [*Glomus deserticola* (AMF) and noninoculated (non-AMF)] and four soil water regimes [one WW (near field capacity) and three levels of water stresses)]. These eight treatments were replicated seven times (one plant per pot) to give a total of 56 pots. WS treatments started three weeks after sowing (to allow time for mycorrhizal colonization to be establish in roots of the snapdragon seedlings). At the same time, WW pots were controlled with 80% of soil water content (–0.075 MPa), and three levels of water stress [(WS_1), 60% of soil water content (–0.14 MPa); (WS_2), 40% of soil water content (–0.38 MPa) and (WS_3), 20% of soil water content (–0.55 MPa)]. The soil water potential was measured by a pressure plate apparatus (*Shimadzu, CL-800*, Kyoto, Japan). WS treatments were imposed by withholding water from pots until soil water potential was achieved. Therefore, the water status in the soil was determined by weighing pots and the amount of water loss was supplied to each pot to keep the intended soil water contents. Pots with WW plants were kept at soil water content near the field capacity. The amount of water added to each pot was recorded to determine evapotranspired water. Pots were arranged in a complete randomized blocks design in a greenhouse under controlled conditions of 225 μmol m^{-2} s^{-1} light intensity, 27/20ºC day/night temperatures, 70–80% relative humidity and 16-h photoperiod. During the experiment, soil water potential was checked in three WW and three of each WS pots without plants.

Mycorrhizal fungus inoculums: The mycorrhizal fungus inoculums, consisting of spores, soil, hyphae, and infected root fragments of sudangrass plants (*Sorghum halepense* L.) from a stock culture of *Glomus deserticola* (Trappe and John), were provided by the stock mycorrhizal cultures (isolated from water stressed sites) of the Experimental Station of Plant Production Department, College of Food and Agriculture Sciences, King Saud University. The mycorrhizal inoculated dosage consisting of 15 g of rhizosphere soil (approx. 850 spores) and 0.5 g of infected sudangrass root fragments with an infection level of 85.7% were then inoculated to each pot. The mycorrhizal inoculums were placed 5 cm deep below the snapdragon seedlings at transplanting time.

Plant and growth conditions: Seeds of snapdragon (*Antirhinum majus* cv. butterfly) were surface-sterilized in 7% sodium hypochlorite for 10 min, subsequently rinsed with sterilized distilled water and left to germinate for 5 days on moistened filter paper in dark at 25ºC. Uniform germinated seedlings were transplanted into 8 × 8 × 9 cm plastic pots containing moist-autoclaved vermiculite soil and left to grow in a greenhouse under controlled conditions (as described before). Two weeks later, plants

were sown in plastic pots of 30 cm diameter (one seedling per pot) containing a mixture of autoclaved sandy loamy soil (4.5 kg soil pot^{-1}). The soil used in this study was collected from the top layer (0–20 cm) of the Experimental Agricultural Research Station at Dirab, Riyadh region, Saudi Arabia. Soil characteristics were pH = 7.65 (soil:water ratio of 1:5, w/v); an organic matter (0.63%); an available nitrogen (26.8 mg kg^{-1}); an available phosphorus (8.12 mg kg^{-1}); a potassium (82 mg kg^{-1}) and EC (0.61 dS m^{-1}). Plants were carefully watered as needed with tap water to maintain soil moisture near field capacity (75–80 %) for three weeks. Then, plants were subjected to four water stresses as described earlier. Regarding the mycorrhizal treatments, half of WW and WS pots were inoculated with the mycorrhizal fungus, *Glomus deserticola* (15 g of soil stock culture and 0.5 g of infected root of sudangrass per pot). Mycorrhizal inoculums were placed 5 cm below the seedlings at transplanting time. The nonmycorrhizal treatments were supplied with filtered washings of an equal amount of the mycorrhizal soil inoculum to provide the same microflora without mycorrhizal fungi. Plants were fed once weekly with 25 ml of Hoagland nutrient solution minus phosphorus per pot, where high accumulation of phosphorus in soil inhibits the growth of mycorrhizal fungi in root tissues (De Miranda and Harris 1994, Abdel-Fattah 1997). Plants were kept under controlled conditions in a greenhouse (as described above) and harvested, seven replicate plants from each treatment, after 10 weeks from transplanting.

Growth and flower parameters: At harvest, the shoot height and diameter were scaled with tape and caliper. Leaf numbers per plant were recorded. Shoots and roots were oven-dried at 80ºC for 24 h and weighted. Leaf area was measured using a leaf area meter (*Li-Cor*, Lincoln, NE, USA). Number of branches and flowers were also recorded. Flower fresh and dry masses in each treatment were determined. Potential shoot DM of nonmycorrhizal (nm) plants grown under the same WS level as mycorrhizal (m) plants were calculated according to a formula adopted from Raju *et al.* (1990) as ($WUE_{nm}/WUF_m \times DM_{nm}$), where WUE is water-use efficiency [g(DM) kg^{-1} (evapotranspired water)] and benefit of AMF-root association with plants was determined as $DM_m - DM_{nm}$.

The mycorrhizal dependency (MD) was defined as a percentage of a plant growth subjected to AMF application, and calculated using the following formula (Menge *et al*. 1978) as (mycorrhizal plant DM/nonmycorrhizal plant DM) × 100.

Leaf water status determination: The third full leaf from the apices of the snapdragon plants was used to measure the relative water content (RWC), water potential (Ψ_w) and electrolyte leakage (EL). RWC was determined in leaf discs, and calculated according to the equation of Levitt (1980) as (LFM – LDM)/(LTM – LDM) × 100, where LFM is a leaf fresh mass, LTM is a leaf turgid mass (determined after floating discs for 3 h on distilled water at 5°C) and LDM is a leaf DM (determined after drying at 80°C for 48 h).

Ψ_w was measured by the method of Li (2000) using a pressure chamber model 600 (*PMS Instruments*, Corvallis, USA). The fully mature leaf was immediately wrapped in a plastic bag filled with breathing air and reading after 3 min. Electrolyte leakage (EL) was measured by an electrical conductivity meter using the following formula (Dionisio-Sese and Tobita 1998) as $EL = EC_1/EC_2 \times 100$, where EC_1 is the initial electrical conductivity (fresh tissues) and EC_2 is the final electrical conductivity (121ºC killed tissue).

Estimation of Chl contents: Leaf contents of Chl *a* and Chl *b* were assayed according to Hiscox and Israelstam (1979). The extraction was made from a 100 mg of fresh sample placed in 15 ml acetone (80%) in the dark at the room temperature and was measured at 646 and 664 nm with a *UV/VIS* spectrophotometer (*Shimadzu, UV-160*, Kyoto, Japan).

Nutrient analysis: Leaf samples from the four randomly chosen plants (third leaf from the apex) were detached, washed, dried to a constant mass, ashed at 550ºC, acid-extracted and then, the extract mad up into a constant volume (Kaya and Higgs 2002). All chemical elements except N were determined in the sample solution. Phosphorus (P) was analyzed by the vanadate-molybdate method (Chapman and Pratt 1961). Potassium (K) was assayed using a flame photometer (*Corning 400*, UK). Calcium (Ca) and magnesium (Mg) were determined using atomic absorption (*PerkinElmer*, *Model 2380*, USA) according to the method of Allen (1989). Total nitrogen (N) was measured in samples of 0.1 g of dry mass using Kjeldahl method (Nelson and Sommers 1973).

Estimation the levels of mycorrhizal colonization: Immediately after harvest, part of the root system was washed carefully in tap water to remove the adhering soil particles, cut into 0.5–1 cm fragments and thoroughly mixed. Subsamples of root pieces were cleared in 10% KOH solution and stained with 0.05% trypan blue in lactophenol (Phillips and Hayman 1970). Mycorrhizal colonization levels [the intensity of colonization (M) and rate of arbuscular development (A)] of the stained roots were estimated by the method of Trouvelot *et al*. (1986).

Proline was determined following the ninhydrin method as described by Sadasivam and Manikam (1996). Dry sample of leaf tissue was extracted in 2 ml of 3% sulfosalicyclic acid, placed in a boiling water bath for 10 min and filtered through filter paper. Two milliliters of the extract were added to 6 ml (final volume) of the assay media containing 2 ml of 1% ninhydrin solution and 2 ml of glacial acetic acid. The reaction mixture was incubated

in a water bath at 100ºC for 30 min, then rapidly cooled and portioned against 1 ml of toluene. After centrifuging at 3,000 × *g* for 5 min the organic phase was collected and absorbance was read by spectrophotometer (*Shimadzu, UV-160*, Kyoto, Japan) at 520 nm using toluene as a blank. Proline concentration was determined against a standard curve with L-proline (*Sigma-Aldrich Chemie*, Steinheim, Germany).

Statistical analysis: Data were subjected to statistical analysis using two-factor analysis of variance (*ANOVA*). Means were separated by *Duncan*'s multiple range tests by the least significant difference (LSD, $P \leq 0.05$) method using the *Costat* software (*Cohort*, Berkeley, CA, USA). All of the measurements were performed four times for each treatment, and the means and calculated standard errors (SE) are reported.

Results

Changes in growth parameters: Shoot and root DM, shoot height, leaf number, stem diameter, and leaf area of both mycorrhizal (AMF) and nonmycorrhizal (non-AMF) snapdragon plants grown under WS conditions were significantly lower than those plants grown under WW conditions (Table 1). However, the reductions in most growth criteria were more distinct in nonmycorrhizal than in mycorrhizal snapdragon plants. AMF plants had significantly higher shoot and root DM, shoot height, leaf area, leaf number per plant and stem diameter than the non-AMF plants regardless the WS level. The differences in shoot height, leaf number, and leaf area were not significant between WW non-AMF and water-stressed (WS_1) AMF snapdragon plants.

Table 1. Growth parameters of mycorrhizal (AMF) and nonmycorrhizal (non-AMF) snapdragon plants grown under either well-watered (WW) or water-stress (WS) conditions. DM – dry mass; SH – shoot height; LN – leaf number; Sd – stem diameter; LA – leaf area. Values in each column followed by *the same letter(s)* are not significantly different at $P \leq 0.05$ (*Duncan*'s multiple range test), where WW, WS_1, WS_2, and WS_3 are WS treatments at 80%, 60%, 40%, and 20% soil water content, respectively. Each value represents the mean of four replicates ± SE.

Treatments		DM [g plant^{-1}]		SH [cm plant^{-1}]	LN [plant^{-1}]	Sd [cm plant^{-1}]	LA [cm^2 plant^{-1}]
Water status	AMF status	Shoot	Root				
WW	Non-AMF	3.20 ± 0.66^{Bc}	2.00 ± 0.19^{B}	39.1 ± 3.83^{B}	88 ± 5.99^{B}	1.38 ± 0.08^{D}	30.37 ± 2.33^{B}
	AMF	3.89 ± 0.62^{A}	2.55 ± 0.37^{A}	46.4 ± 4.11^{A}	105 ± 6.60^{A}	2.41 ± 0.33^{A}	36.48 ± 2.90^{A}
WS_1	Non-AMF	2.75 ± 0.48^{Cd}	1.88 ± 0.25^{B}	38.3 ± 3.98^{Bc}	74 ± 4.11^{C}	1.58 ± 0.18^{C}	23.81 ± 1.24^{C}
	AMF	3.58 ± 0.59^{Ab}	2.45 ± 0.39^{A}	40.6 ± 4.04^{B}	86 ± 5.80^{B}	1.83 ± 0.23^{B}	29.30 ± 1.95^{B}
WS_2	Non-AMF	2.05 ± 0.33^{D}	1.11 ± 0.16^{D}	31.2 ± 3.12^{E}	53 ± 4.66^{E}	1.37 ± 0.20^{D}	18.80 ± 1.08^{D}
	AMF	3.04 ± 0.54^{B}	1.70 ± 0.22^{Bc}	35.1 ± 3.35^{D}	65 ± 4.02^{D}	1.55 ± 0.28^{C}	22.68 ± 1.32^{C}
WS_3	Non-AMF	1.29 ± 0.18^{E}	1.01 ± 0.12^{D}	28.5 ± 2.18^{F}	37 ± 2.99^{F}	1.06 ± 0.04^{E}	14.33 ± 0.98^{E}
	AMF	2.39 ± 0.39^{D}	1.44 ± 0.28^{C}	31.3 ± 2.88^{E}	47 ± 3.85^{E}	1.42 ± 0.21^{D}	17.40 ± 1.10^{D}
LSD (0.05)		0.564	0.302	2.049	5.94	0.054	3.01

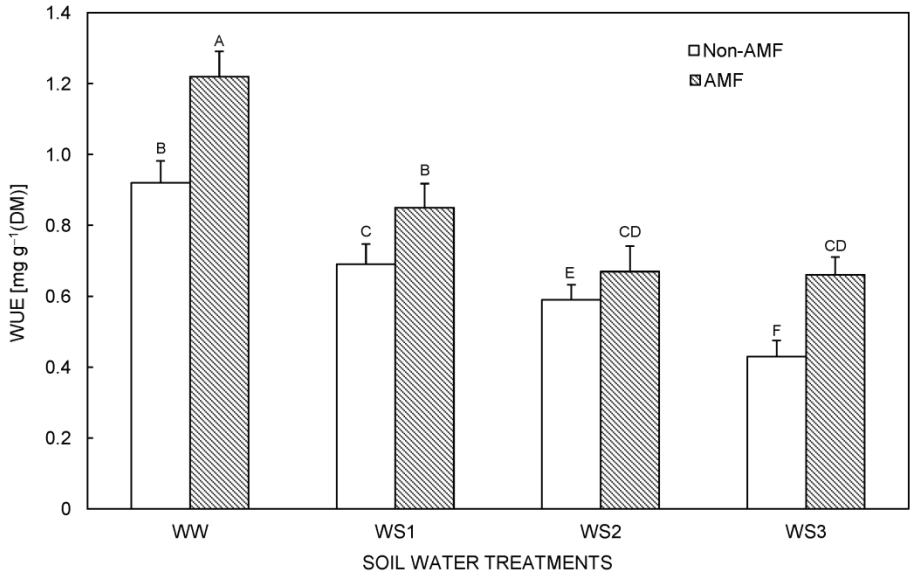

Fig. 1. Water-use efficiency (WUE) of mycorrhizal (AMF) and nonmycorrhizal (non-AMF) snapdragon plants grown under either well-watered (WW) or water-stress (WS) conditions. Values in each bar followed by *the same letter* are not significantly different at $P \leq 0.05$ (*Duncan*'s multiple range test). Values shown are means ± SE of four replicates.

Table 2. The leaf chlorophyll (Chl) contents of mycorrhizal (AMF) and nonmycorrhizal (non-AMF) snapdragon plants grown under either well-watered (WW) or water-stress (WS) conditions. Values in each column followed by *the same letter(s)* are not significantly different at $P \leq 0.05$ (*Duncan*'s multiple range test), where WW, WS_1, WS_2, and WS_3 are WS treatments at 80%, 60%, 40%, and 20% soil water content, respectively. Each value represents the mean of four replicates ± SE. FM – fresh mass.

Treatments		Chl content [mg g^{-1}(FM)]			
Water status	AMF status	*a*	*b*	Total	*a*/*b*
WW	Non-AMF	$0.387 \pm 0.03^{B*}$	0.139 ± 0.011^{Bc}	0.526 ± 0.04^{B}	2.784 ± 0.37^{A}
	AMF	0.425 ± 0.039^{A}	0.154 ± 0.019^{A}	0.579 ± 0.05^{A}	2.759 ± 0.38^{A}
WS_1	Non-AMF	0.342 ± 0.035^{D}	0.120 ± 0.009^{De}	0.466 ± 0.035^{C}	2.758 ± 0.35^{A}
	AMF	0.370 ± 0.04^{C}	0.144 ± 0.015^{B}	0.515 ± 0.044^{B}	2.619 ± 0.39^{A}
WS_2	Non-AMF	0.277 ± 0.03^{F}	0.125 ± 0.01^{D}	0.402 ± 0.032^{D}	2.216 ± 0.298^{De}
	AMF	0.323 ± 0.038^{E}	0.132 ± 0.013^{Cd}	0.455 ± 0.039^{C}	2.446 ± 0.30^{Bc}
WS_3	Non-AMF	0.228 ± 0.025^{H}	0.088 ± 0.005^{F}	0.316 ± 0.028^{F}	2.591 ± 0.30^{B}
	AMF	0.263 ± 0.029^{G}	0.115 ± 0.009^{E}	0.378 ± 0.029^{E}	2.287 ± 0.25^{D}
LSD (0.05)		0.005	0.011	0.013	0.145

Table 3. Water potential (Ψ_w), relative water content (RWC) and electrolyte leakage (EL) in leaves of mycorrhizal (AMF) and nonmycorrhizal (non-AMF) snapdragon plants grown under either well-watered (WW) or water-stress (WS) conditions. Values in each column followed by *the same letter(s)* are not significantly different at $P \leq 0.05$ (*Duncan*'s multiple range test), where WW, WS_1, WS_2, and WS_3 are WS treatments at 80%, 60%, 40%, and 20% soil water content, respectively. Each value represents the mean of four replicates ± SE.

Treatments		Leaf water relations		
Water status	AMF status	Ψ_w [MPa]	RWC [%]	EL [%]
WW	Non-AMF	$-0.69 \pm 0.02^{B*}$	88 ± 7.5^{B}	25 ± 2.10^{F}
	AMF	-0.58 ± 0.029^{A}	92 ± 8.7^{A}	22 ± 2.5^{G}
WS_1	Non-AMF	-0.84 ± 0.04^{C}	83 ± 9.0^{C}	34 ± 2.9^{D}
	AMF	-0.78 ± 0.05^{B}	87 ± 10.0^{B}	31 ± 3.5^{E}
WS_2	Non-AMF	-1.12 ± 0.09^{E}	81 ± 6.8^{C}	58 ± 4.0^{B}
	AMF	-0.93 ± 0.08^{D}	86 ± 9.0^{B}	54 ± 4.5^{C}
WS_3	Non-AMF	-1.38 ± 0.14^{F}	78 ± 5.9^{D}	65 ± 5.0^{A}
	AMF	-1.18 ± 0.09^{E}	84 ± 11.0^{Bc}	59 ± 5.1^{B}
LSD (0.05)		0.095	2.481	1.359

WUE: The results in Fig. 1 clearly indicated that mycorrhizal plants used less water to produce one unit of shoot DM (*i.e*. higher WUE) than nonmycorrhizal plants. Both mycorrhizal and nonmycorrhizal plants had higher WUE when grown under WW than under WS conditions.

Chl content: Water stress significantly decreased the content of Chl *a* and Chl *b* in leaves of snapdragon mycorrhizal and nonmycorrhizal plants (Table 2). The total Chl content in leaves of AMF plants was significantly higher than that in leaves of non-AMF plants, regardless of water treatments. No significant differences were observed in ratios of Chl *a*/Chl *b* between the mycorrhizal and nonmycorrhizal plants grown both under WW conditions. However, there were no constant trends in these ratios between the mycorrhizal and nonmycorrhizal plants grown under three levels of water stress. Comparing to that in non-AMF plants, the total Chl content of leaves increased by 10% in AMF plants under WW condition and increased by 11%, 13%, and 19% under the three levels of water stress conditions (WS_1, WS_2, and WS_3), respectively.

Leaf water status and electrolyte leakage: Ψ_w, RWC and EL in leaves of snapdragon plants were highly affected by the AMF inoculation and application of water stress (Table 3). The RWC and Ψ_w in AMF and in non-AMF plants were markedly decreased by water stress. Moreover, AMF plants had significantly higher RWC and Ψ_w than non-AMF plants regardless of water treatments. Yet, the beneficial effects of AMF were more pronounced in plants grown under high levels of water stress.

Water stress markedly increased EL of snapdragon leaves comparing to WW plants (Table 3). This effect was increased as WS levels enlarged. AMF inoculation significantly reduced the EL of snapdragon leaves when compared with non-AMF plants grown under either WW or WS conditions. Reduction in EL of snapdragon leaves was more pronounced in mycorrhizal plants grown under highest water stress level.

Table 4. Flower yield of mycorrhizal (AMF) and nonmycorrhizal (non-AMF) snapdragon plants grown under either well-watered (WW) or water-stress (WS) conditions. Dependence of AMF = 100 × (flower dry mass of AMF plants/flower dry mass of non-AMF plants). Values in each column followed by *the same letter(s)* are not significantly different at $P \leq 0.05$ (*Duncan*'s multiple range test), where WW, WS_1, WS_2, and WS_3 are WS treatments at 80%, 60%, 40%, and 20% soil water content, respectively. Each value represents the mean of four replicates ± SE. SN – spike numbers; FN – flower number; FFM – flower fresh mass; FDM – flower dry mass.

Treatments		Flower yield				Dependence on AMF [%]
Water status	AMF status	SN	FN	FFM [g plant $^{-1}$]	(FDM) [g plant $^{-1}$]	
WW	Non-AMF	$3.83 \pm 0.87^{C*}$	11.5 ± 1.07^{B}	4.09 ± 0.80^{B}	0.88 ± 0.07^{B}	121 ± 18.5^{C}
	AMF	5.03 ± 0.95^{A}	13.7 ± 1.09^{A}	5.35 ± 0.85^{A}	1.06 ± 0.09^{A}	-
WS_1	Non-AMF	3.00 ± 0.66^{Cd}	9.70 ± 1.08^{C}	3.84 ± 0.64^{B}	0.81 ± 0.07^{B}	126 ± 16.4^{C}
	AMF	4.13 ± 0.85^{B}	11.3 ± 1.07^{B}	5.11 ± 0.95^{A}	1.02 ± 0.08^{A}	-
WS_2	Non-AMF	2.09 ± 0.45^{Ef}	6.30 ± 1.03^{E}	3.10 ± 0.50^{C}	0.66 ± 0.06^{C}	135 ± 20.8^{B}
	AMF	2.67 ± 0.54^{Cd}	8.35 ± 1.05^{D}	4.16 ± 0.75^{B}	0.89 ± 0.07^{B}	-
WS_3	Non-AMF	1.66 ± 0.35^{F}	4.30 ± 0.88^{F}	2.60 ± 0.45^{C}	0.41 ± 0.05^{D}	142 ± 22.0^{A}
	AMF	2.33 ± 0.50^{De}	6.93 ± 1.05^{E}	2.96 ± 0.50^{C}	0.58 ± 0.06^{C}	-
LSD (0.05)		0.865	1.20	0.951	0.08	5.18

Table 5. Intensity of mycorrhizal colonization (M) and arbuscular frequency (A) in the root tissues of mycorrhizal (AMF) and nonmycorrhizal (non-AMF) snapdragon plants grown under either well-watered (WW) or water-stress (WS) conditions. Values in each column followed by *the same letter(s)* are not significantly different at $P \leq 0.05$ (*Duncan*'s multiple range test), where WW, WS_1, WS_2, and WS_3 are WS treatments at 80%, 60%, 40%, and 20% soil water content, respectively. Each value represents the mean of four replicates ± SE.

Treatments		Mycorrhizal colonization levels [%]	
Water status	AMF status	M	A
WW	Non-AMF	0.0^{D}	0.0^{E}
	AMF	85.5 ± 9.0^{A}	64.7 ± 7.2^{A}
WS_1	Non-AMF	0.0^{D}	0.0^{E}
	AMF	81.5 ± 9.5^{A}	58.0 ± 7.0^{B}
WS_2	Non-AMF	0.0^{D}	0.0^{E}
	AMF	72.8 ± 8.2^{B}	52.6 ± 6.8^{C}
WS_3	Non-AMF	0.0^{D}	0.0^{E}
	AMF	65.0 ± 8.0^{C}	48.4 ± 6.8^{C}
LSD (0.05)		6.11	5.02

Flower yield: Flower fresh mass (FM) and DM, spike and flower numbers of water stress mycorrhizal and nonmycorrhizal snapdragon plants were significantly lower than those of WW plants (Table 4), but reductions in flower yield parameters due to water stress were more pronounced in nonmycorrhizal than in mycorrhizal plants. On the other hand, AMF plants had higher flower FM and DM and spike numbers than non-AMF plants regardless of the water treatments. The differences in flower FM and DM were not significant between WW non-AMF and WS AMF plants (WS_2). The mycorrhizal dependency values (flower DM) of snapdragon plants in responding to AMF inoculation were significantly higher under WS than WW conditions (Table 4) and these values were increased as WS level increased.

Mycorrhizal colonization levels: Both intensity of mycorrhizal colonization (M) and arbuscular frequency (A) were significantly decreased in snapdragon root tissues with WS than with WW plants, and this effect was more elicited with the highest water stress level (Table 5). However, no significant differences were observed in the rate of arbuscular frequency between AMF plants grown under WS_2 and WS_3 conditions. No mycorrhizal colonization was observed in the noninoculated plants.

Leaf nutrients content: Leaves of AMF snapdragon plants had higher contents of N, P, K, Ca, and Mg contents than those of non-AMF plants, regardless of water treatments (Table 6). However, both mycorrhizal and nonmycorrhizal WW plants had higher P, N, and K contents than WS plants had. Reduction in leaf nutrient contents as a result of water stress was more pronounced in nonmycorrhizal than in mycorrhizal plants.

Proline content: The proline content in mycorrhizal and nonmycorrhizal snapdragon leaves was increased by increasing the WS level (Fig. 2). However, AMF plants

Table 6. Nutrients (N, P, K, Ca, and Mg) contents in leaves of mycorrhizal (AMF) and nonmycorrhizal (non-AMF) snapdragon plants grown under either well-watered (WW) or water-stress (WS) conditions. Values in each column followed by *the same letter(s)* are not significantly different at $P \leq 0.05$ (*Duncan*'s multiple range test), where WW, WS_1, WS_2, and WS_3 are WS treatments at 80%, 60%, 40%, and 20% soil water content, respectively. Each value represents the mean of four replicates ± SE. DM – dry mass.

Treatments		Nutrients content [mg g^{-1}(DM)]				
Water status	AMF status	N	P	K	Ca	Mg
WW	Non-AMF	$3.65 \pm 0.68^{B*}$	1.41 ± 0.12^{C}	6.87 ± 1.05^{B}	3.21 ± 0.54^{B}	1.63 ± 0.05^{B}
	AMF	3.88 ± 0.65^{A}	1.56 ± 0.15^{A}	8.97 ± 1.15^{A}	5.19 ± 0.88^{A}	1.96 ± 0.08^{A}
WS_1	Non-AMF	3.36 ± 0.55^{C}	1.36 ± 0.13^{De}	5.95 ± 1.05^{D}	3.01 ± 0.52^{B}	1.44 ± 0.06^{D}
	AMF	3.59 ± 0.60^{B}	1.46 ± 0.15^{B}	7.76 ± 1.20^{C}	5.08 ± 0.79^{A}	1.65 ± 0.05^{B}
WS_2	Non-AMF	2.76 ± 0.50^{E}	1.33 ± 0.13^{Ef}	4.89 ± 1.02^{E}	2.88 ± 0.40^{D}	1.38 ± 0.04^{E}
	AMF	3.01 ± 0.52^{D}	1.39 ± 0.16^{Cd}	6.33 ± 1.18^{B}	4.70 ± 0.65^{C}	1.48 ± 0.05^{C}
WS_3	Non-AMF	2.05 ± 0.42^{F}	1.22 ± 0.10^{G}	4.03 ± 0.09^{E}	2.62 ± 0.52^{D}	1.26 ± 0.04^{F}
	AMF	2.60 ± 0.48^{G}	1.29 ± 0.13^{F}	5.71 ± 1.05^{D}	3.87 ± 0.58^{E}	1.39 ± 0.04^{De}
LSD (0.05)		0.065	0.043	1.081	0.285	0.046

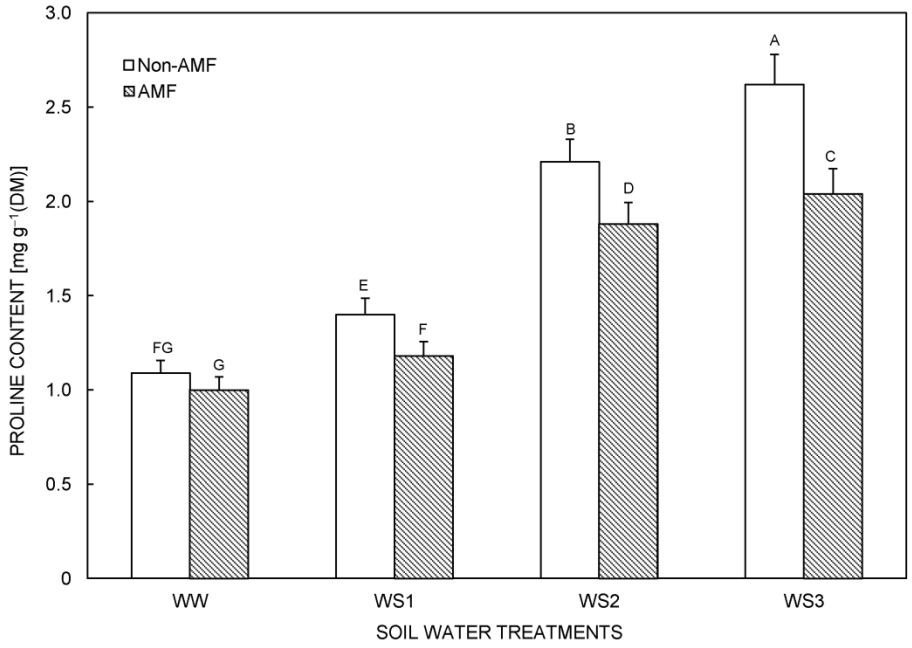

Fig. 2. Proline content in leaves of mycorrhizal (AMF) and nonmycorrhizal (non-AMF) snapdragon plants grown under either well-watered (WW) or water-stress (WS) conditions. Values in each bar followed by *the same letter* are not significantly different at $P \leq 0.05$ (*Duncan*'s multiple range test). Values shown are means ± SE of four replicates.

Table 7. Potential shoot dry matter (DM), AMF benefit and mycorrhizal dependency (MD) of snapdragon plants grown under either well-watered (WW) or water-stress (WS) conditions. Values in each column followed by *the same letter(s)* are not significantly different at $P \leq 0.05$ (*Duncan*'s multiple range test), where WW, WS_1, WS_2, and WS_3 are WS treatments at 80%, 60%, 40%, and 20% soil water content, respectively. Each value represents the mean of four replicates ± SE.

Water status	Potential DM [g $plant^{-1}$]	Benefit [g $plant^{-1}$]	MD [%]
WW	$0.235 \pm 0.04^{C*}$	0.69 ± 0.07^{D}	122 ± 15.5^{D}
WS_1	0.295 ± 0.045^{C}	0.83 ± 0.08^{C}	131 ± 18.0^{C}
WS_2	0.431 ± 0.05^{B}	0.99 ± 0.07^{B}	148 ± 16.0^{B}
WS_3	0.505 ± 0.07^{A}	1.10 ± 0.09^{A}	185 ± 19.1^{A}
LSD (0.05)	0.062	0.081	15.8

had lower proline content in leaves than in non-AMF plants regardless of water treatments, and this difference was more pronounced under WS conditions. Moreover, and comparing to proline on non-AMF plants, the proline of AMF plants decreased by 9% and 28% under WW and the highest level of WS conditions (WS_3), respectively.

Potential DM, benefit and mycorrhizal dependency: Potential DM, calculated benefit and mycorrhizal dependency values of snapdragon plants in responding to AMF

inoculation were significantly higher under WS than under WW conditions (Table 7). These values were increased as WS levels increased. These results have suggested that the growth of snapdragon plants at high levels of water stress conditions depends highly on mycorrhizal fungi, and on the same time, the AMF protect the plants from the deleterious effect of WS conditions.

Discussion

Growth and mineral contents: Water stress is a world-wide problem that reduces plant growth and productivity. Since soils first begin drying, the shoot growth can be inhibited before any leaf dehydration occurs through a root-to-shoot nonhydraulic signaling mechanisms (Davies *et al*. 1994). In the present study, the arbuscular mycorrhizal inoculation enhanced growth parameters (DM, leaf area, shoot height, and number of leaves) of snapdragon plants grown under either WW or WS conditions comparing to nonmycorrhizal plants. The rate of growth in response to mycorrhizal colonization was more pronounced at higher levels of water stress. Enhanced growth effects on mycorrhizal plants are often related to improved P and other nutrients (K, N, Ca) acquisition (Table 6), as the availability of P in soils is reduced by soil drying (Auge 2001). This is in agreement with some previous findings which stated that the main mechanism for enhanced drought tolerance in mycorrhizal plants was the improvement of P nutrition (Abdel-Fattah *et al*. 2002, Al-Karaki 1998, Giri *et al*. 2007), although in some cases, the drought tolerance of the mycorrhizal plants appeared to be independent of plant P concentration (Feng *et al*. 2002). N, K, Mg, and Ca were significantly higher in leaves of WS mycorrhizal than those in nonmycorrhizal snapdragon plants. This finding supports the previous investigators who have mentioned that arbuscular mycorrhizal fungi absorb higher N, P, and Mg than nonmycorrhizal plants during drought stress (Tobar *et al*. 1994, Al-Karaki and Al-Raddad 1997, Asrar and Elhindi 2011). Furthermore, the protection of the mycorrhizal plants against drought stress was related to the mycorrhizal induction of leaf conductance (Auge *et al*. 2008) and transpiration (Auge 2001) as well as P, N, and K uptake. Potassium plays a key role in plant water stress and its cationic solute is responsible for stomatal movement as a result of changes in bulk leaf status (Ruiz-Lozano *et al.* 1995). In this study, the response of *G. deserticola* to protect snapdragon plants from harmful effect of water stress and the K content in plants were positively related.

Flower yield: In this study, water stress often caused reductions in flower responses of mycorrhizal and non-mycorrhizal snapdragon plants comparing to WW conditions. Plants colonized by *G. deserticola* had greater flower yield and tended to possess better flower number and diameter than the nonmycorrhizal plants grown under WS conditions. This is in agreement with the previous findings on other ornamental plants (Levy and Krikun 1980, Auge *et al.* 1987, Aboul-Nasr 1996, Linderman and Davis, 2004, Zandavalli *et al.* 2004) in which mycorrhizal plants had better flower yield than nonmycorrhizal plants. The same trend was apparent in the *G. deserticola*-colonized snapdragon plants (Tables 3, 6).

Water relations: The higher WUE in mycorrhizal than in nonmycorrhizal snapdragon plants grown under WS conditions could indicate that AMF increased the ability of root to absorb soil moisture. Enhanced water conductivity has been attributed to area increase for water uptake produced by AMF hyphae in soil (Auge 2001, Zhang *et al*. 2010). We have suggested that the mycorrhizal hyphae may enhance the ratio of below-ground absorptive surface to leaf area. Significant water uptake and transport by hyphae have been observed or computed in instances in which the AM symbiosis has also affected the stomatal behavior (Allen 1982, Ruiz-Lozano and Azcon 1995). Moreover, the mycorrhizal snapdragon plants in this study produced more root DM than nonmycorrhizal plants. This might partially explain why mycorrhizal plants had higher WUE than the nonmycorrhizal plants. The ability of AMF to increase root density is consistent with earlier investigations (Berta *et al*. 1993, Al-Karaki and Al-Raddad 1997, Al-Qarawi 2010).

AM fungi can influence water-uptake ability and WUE in host plants (Allen 1982, Morte *et al.* 2000). In our study, water relations were significantly affected by *G. deserticola* inoculation in WS snapdragon plants. This suggests the presence of an adaptive effect of mycorrhizal symbiosis in arid conditions. The increase in water relations was more evident for mycorrhizal plants under WS than under WW conditions. Consequently, mycorrhization could increase water uptake by increasing the effectiveness of the root hydraulic conductivity (Safir *et al*. 1971). AM fungi were reported to enhance water uptake in sunflower and cowpea (Faber *et al*. 1991), lettuce (Ruiz-Lozano and Azcon 1995) and rose plants (Auge *et al*. 1986) but not in wheat and clover plants (George *et al*. 1992, Tarafdar 1995). It is interesting to note that AM fungal colonization greatly enhances water uptake in snapdragon plants grown under WW and WS conditions. These plants were able to maintain leaf water potentials and leaf water content in roots colonized by AMF. These results are in agreement with those reported by Davies *et al*. (1996) and Kaya *et al*. (2009) who mentioned that mycorrhizal colonization might increase root length density or alter root system morphology, enabling the colonized plants to explore more soil volume and extract more water than nonmycorrhizal plants during the drought conditions. Mycorrhizal fungi may also

directly enhance root water uptake, and thus increase water supply which would help sustain the physiological activity within plants (Allen 1982). Moreover, mycorrhizal colonization enhances the stomatal control in snapdragon plants and reduces the water loss during drought (Auge *et al*. 1986). The application of water stress markedly increased the electrolyte leakage of snapdragon leaves. AM inoculation mitigated the adverse effect of water stress on the electrolyte leakage of snapdragon plants. This effect of mycorrhiza could be due to its improvement of nutrient uptake and adjustment the osmotic pressure of plant cells (Auge 2001, Ibrahim *et al*. 2011).

Mycorrhizal colonization: Reductions in AMF colonization levels with increasing levels of water stress are consistent with previous field and greenhouse growth-room studies (Richert *et al*. 1994, Stevens *et al*. 2011, Asrar and Elhindi 2011). The AM colonization under WW conditions was 20% higher than that under WS conditions. The result is in accordance with the finding of Kaya *et al*. (2003), who reported that water stress significantly decreased the AM colonization by *Glomus clarum* in watermolen plants. In contrast, previous studies have shown that the percentage of root colonized by arbuscular mycorrhizal fungi was not affected by water stress (Davies *et al*. 1992, Morte *et al.* 2000, Auge 2001). The positive effects of AMF on growth and biomass of snapdragon plants grown under WS conditions were also observed in this study (Table 1). Similar results have been reported for other plant species (Ruiz-Lozano and Azcon 1995, Wu and Xia 2006). The positive effects are likely attributed to the improvement of phosphorus nutrition (Bethlenfalvay *et al*. 1988, Stevens *et al*. 2011), uptake of water by hyphae (Faber *et al*. 1991) and the increase of root length density (Bryla and Duniway 1997, Al-Qarawi 2010). In this connection, AM colonization can change specific root length, root architecture and root/shoot ratio (Berta *et al*. 1993). It has been suggested that extraradical hyphae of AM may enhance the root length and absorptive surface of leaf area, then affected on stomatal behavior (Allen 1982).

Chl and proline contents: The contents of Chl in the leaves of mycorrhizal and nonmycorrhizal snapdragon plants were reduced by increasing the level of water stress. In spite of this, all treatments of snapdragon plants colonized by AM fungi had greatly higher Chl contents than of these nonmycorrhizal plants. The overall reduction in Chl content with water stress may be due to the reduction in Mg and K concentrations (Auge 2001). The contents of these elements are usually higher in mycorrhizal than in nonmycorrhizal plants (Azcon-Aguilar *et al*. 1992). Osmotic adjustment is considered to be an important component of drought- and water-stress-tolerant mechanisms in higher plants. Under WS conditions, plants accumulate some small molecules including organic solutes like soluble sugars and proline. It seems likely that a high level of proline accumulation may play a role in drought tolerance and make plants survive short drought and and recover from stresses (Sanchez *et al*. 1998). However, our study indicated that AM snapdragon leaves had lower amount of proline, suggesting that AM colonization enhanced host plant WS tolerance and thus the plant were less stressed than the non-AMF plants (Wu and Xia 2006, Tang *et al*. 2009).

Potential and benefit of mycorrhizal fungi: Responses of the snapdragon plants and soil environment to AMF symbiosis may be useful criteria for selecting "efficient" plants for WS conditions. These responses have sometimes been discussed in terms of "potential and benefits" to plants (Koide and Elliott 1989, Zhang *et al*. 2010). For the AMF-root symbiosis to be beneficial plants colonized by AMF should have less biomass loss and produce more DM than nonmycorrhizal plants when grown under WS conditions (Ellis *et al*. 1985). The AMF absorb mineral nutrients and water which benefits the host plants. The calculated benefit and potential values of AMF on host plant DM were higher on snapdragon plants grown under WS than on those grown under WW conditions. The beneficial effect in response to AMF is enhanced as water stress level increases. The benefit and potential DM values may have increased in WS plants as a result of increased dependency of the snapdragon plant on AMF regarding growth, nutrition and water uptake. The plant water relation has improved due to AM colonization. Host plant yield stimulation resulted from AMF-root association under WS conditions have been reported (Auge 2001, Zhang *et al*. 2011).

Conclusion: Water stress has been shown to adversely affect growth, nutrition, and flower yield and water status of snapdragon plants. Mycorrhizal WS snapdragon plants had higher DM of shoots and roots, macronutrient enhancement, WUE, Ψ_w and RWC, and Chl contents than those in non-AM plants. Therefore, our results showed that AM colonization can protect plants against the deleterious effects of water stress. The results suggested that the benefits of AM colonization on snapdragon plants grown under WS conditions were due to the improvement of water relations, nutrients content, and Chl content of the plants.

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