

Response of snapdragon (*Antirrhinum majus* L.) to blended water irrigation and arbuscular mycorrhizal fungi inoculation: uptake of minerals and leaf water relations

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Abstract

A greenhouse study was performed in order to investigate the effects of three arbuscular mycorrhizal fungi (AMF) species on vegetative growth, water relations, and mineral composition parameters of snapdragon (*Antirrhinum majus* cv. Bells white) under irrigation from different water sources. Five irrigation treatments included using purely desalinized (fresh) water (DW), as a control, three different blends of DW with saline ground water from a well with increasing salinity, and one with 100% of saline well water. Inoculation with AMF enhanced growth rates and a relative water content of snapdragon plants grown under well-water irrigation. AMF also improved the leaf water potential and increased water-use efficiency of the plants. Shoot and root dry masses were higher in the AMF-treated plants than those in AMF-free plants. In both shoots and roots, concentrations of total P, Ca²⁺, N, Mg²⁺, and K⁺ were higher in the AMF-treated plants compared with AMF-free plants under salt-stress conditions. Shoot Cl⁻ and Na⁺ concentrations were lower in the AMF-treated plants than those in the AMF-free plants grown under well-water irrigation. Snapdragon plants exhibited a high degree of dependency on AMF; it improved plant growth rates and leaf water relations, particularly, with increasing salinity of irrigation water.

Additional key words: blending water; leaf water relations; mineral composition; mycorrhizal fungi; salt water.

Introduction

Snapdragon (*Antirrhinum majus* L.), a member of the Scrophulariaceae native to the Mediterranean region, is a perennial flowering plant that is often treated as an annual by horticulturalists (Carter and Grieve 2008). It has irregularly shaped flowers of various colors that occur in terminal racemes, and is considered to have valuable medicinal properties for its use in pharmaceutical industry (Bulir 2009).

Soil salinization is a wide-spread problem worldwide; approximately 7% of the global land surface is covered with saline soil (Ruiz-Lozano *et al.* 1996, Sheng *et al.* 2008). Water stress limits crop productions throughout the world (Kramer and Boyer 1995, Kaya 2003, Augé 2004) and, in contrast to other factors (*e.g.*, acidity, alkalinity, salinity), water availability is highly variable within a

given growing season and between growing seasons (Gutierrez-Boem and Thomas 1999, Kaya 2003). As such, a control of salinity levels is often a major target of irrigation management (Dehayer and Gordon 2004). Currently, there are two primary water-management strategies of using saline water for the irrigation of flower crops: blending (mixture of saline with nonsaline water at different ratios) and cyclic use (alternation of saline and nonsaline water) (Ragab *et al.* 2005).

The use of biological tools as a practical way of alleviating soil stresses, including salinity, on plant growth has received considerable attention over the past decade (Al-Khaliel 2010). Arbuscular mycorrhizal fungi (AMF), including *Glomales* species, are ubiquitous in agriculture soils and establish mutual relationship with over 90%

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Abbreviations: AMF – arbuscular mycorrhizal fungi; DM – dry mass; DW – desalinized water; FM – fresh mass; Pt – plant tolerance; RWC – relative water content; WW – well water; WUE – water-use efficiency; ψ_w – leaf water potential.

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of vascular plant species (Smith and Read 2008, Kumar *et al.* 2012). In such species, photosynthates are exchanged for water and mineral sources that are assimilated by the fungi from soils (Wu and Zou 2010). However, it is often extremely difficult to distinguish between direct and plant-mediated effects of salinity on AMF biology because of a close AMF-plant relationship. Presumably, any environmental factor affecting physiology of a host plant is expected to influence its fungal symbiont (Wu and Zou 2010, Çekiç *et al.* 2012). Several studies have shown that AMF can enhance plant growth and nutrient uptakes, improve leaf water relations, and reduce yield losses under saline conditions (Al-Khalil 2010). In addition, degradation of reactive oxygen species in arbuscular mycorrhizas (AM) may be an efficient mechanism for attenuating the activation of plant defense (Rahmaty and

Khara 2011). Nevertheless, the mechanism by which AMF improves salt resistance remains unclear (Kaya *et al.* 2009), and only several studies have focused on the effects of the interaction of AMF and saline water blends on alleviating salt stress in herbal species including snapdragon. Therefore, the current study was conducted in order to determine effects of two different water treatments: (1) desalinated water and underground well water on snapdragon plant growth; (2) to evaluate the effects of AMF mix *Glomus mosseae*, *G. constrictum*, and *G. fasciculatum* on snapdragon growth under highly blended irrigation water; (3) to determine the effects of saline irrigation water and AMF inoculation on leaf water relation and chemical composition of snapdragon under greenhouse conditions.

Materials and methods

Plant material and growth conditions: Snapdragon (*Antirrhinum majus* cv. Bells white from *GOLDSMITH Seeds Company*, CA, USA) was grown in a greenhouse at the Experimental Station of Plant Production Department, College of Food and Agriculture Sciences of King Saud University in Riyadh, Saudi Arabia, during the 2013 growing season. The seeds were sterilized by immersion in 70% alcohol for 5 min and then rinsed four times with distilled water. Seeds were sown in plastic trays on 27 January. One-month-old seedlings, healthy, and uniform in size (10 cm) were transplanted into 15-cm diameter plastic pots (one seedling per pot) containing per pot 2.9 kg of autoclaved sandy soil, 79% sand, 12% silt, and 9% clay, pH 7.69, EC 1.69 dS m⁻¹, 0.34% organic matter. Available nitrogen (N), phosphorus (P), and potassium (K) corresponded to 31.8, 8.64, and 93 mg kg⁻¹, respectively. One week after transplantation, plants were carefully watered as needed with tap water to establish soil moisture close to field capacity (75–80%, v/w) in order to facilitate root-system development. The plants were then subjected to five different irrigation-water treatments. Average temperature of 22/18°C day/night, 70–80% of relative air humidity, and a photoperiod of 14 h were maintained throughout the growth stages.

Inoculation with mycorrhizal fungi: The mycorrhizal fungi mix was added to the soil during the transplantation process. The mycorrhizal fungi inocula consisted of

spores, soil, hyphae, and infected root fragments of Sudan-grass plants (*Sorghum halepense* L.) from a stock culture mix of *G. mosseae*, *G. constrictum*, and *G. fasciculatum*. We followed the procedures described by El-Nashar (2014) for injection of the mycorrhizal mix into soils.

Blending of irrigation water: Two main types of irrigation water were used in this experiment, *i.e.*, saline ground (well, WW) and desalinated (fresh, DW) water. The desalinated water was obtained from Desalination Water Station at College of Food and Agriculture Sciences, King Saud University:

Blended irrigation water treatments. DW – desalinated water; WW – well water; ECw – electrical conductivity of irrigation water.

| Treatments | Blended irrigation water [%] | ECw [dS m ⁻¹] |
|------------|------------------------------|---------------------------|
| T1 | 100 DW | 0.5 |
| T2 | 75 DW + 25 WW | 1.2 |
| T3 | 50 DW + 50 WW | 2.2 |
| T4 | 25 DW + 75 WW | 2.8 |
| T5 | 100 WW | 3.6 |

The irrigation by water with different salinities were applied for 80 d (irrigation period). The irrigation treatments were applied three times weekly using the same amount of water for each treatment:

Chemical composition of two sources of irrigation water. DW – desalinated water; WW – well water.

| Irrigation sources | EC [dS m ⁻¹] | pH [%] | Ca ²⁺ [meq L ⁻¹] | Mg ²⁺ [meq L ⁻¹] | Na ⁺ [meq L ⁻¹] | K ⁺ [meq L ⁻¹] | HCO ₃ ⁻ [meq L ⁻¹] | Cl ⁻ [meq L ⁻¹] | SO ₄ ²⁻ [meq L ⁻¹] | NO ₃ ⁻ [ppm] |
|--------------------|--------------------------|--------|---|---|--|---------------------------------------|--|--|--|------------------------------------|
| WW | 3.6 | 7.6 | 11.1 | 10.6 | 14.65 | 0.55 | 4.60 | 12.7 | 14.2 | 5.30 |
| DW | 0.5 | 7.2 | 0.74 | 0.16 | 03.60 | 0.10 | 0.32 | 01.84 | 00.9 | 2.86 |

Data collection: Plant material was collected after harvesting (115 d after planting). Shoot and root fresh and dry masses (FM and DM, respectively) were recorded; DM values were obtained by oven-drying fresh shoot and root material at 70°C for 48 h until a constant mass was attained. Plant tolerance (Pt) was calculated following Hatimi (1999):

$$Pt [\%] = 100 \times (DMSP/DMNSP)$$

where DMSP represents the dry mass of stressed plant and DMNSP represents the dry mass of the nonstressed plants.

Leaf water relations: Relative water content (RWC) was determined using the method described by Kramer and Boyer (1995). Ten uniform leaves from three randomly chosen plants were selected and FM was determined immediately. Then leaves were floated on distilled water in closed Petri dishes at room temperature of about 25°C in order to obtain the turgid mass (TM). The DM was determined after over-drying at 80°C for 24 h.

$$RWC = [(FM - DM)/(TM - DM) \times 100]$$

The leaf water potential (ψ_w) was measured 70 d after the irrigation treatments using a Scholander pressure chamber (*Model 600, PMS Instruments, Corvallis, USA*) (Vanaja *et al.* 2011). Measurements were consistently performed around 11:00 h, the time of day at which light intensity was maximal, and thus when the plant's water content was at its lowest and the leaf water potential was at its highest values.

Water-use efficiency (WUE) values were used to compare the effects of the different irrigation treatments. WUE was calculated from the total fresh flower yield and the total water use as follows:

$$WUE = \text{total fresh yield/total water applied (Lovelli *et al.* 2007).$$

Proline concentration: The proline content of leaves was extracted following the techniques described by Bates *et al.* (1973). Leaf samples (0.1 g) were homogenized in 10 ml of 3% aqueous sulfosalicylic acid, with the resulting homogenate passed through filter paper. Two milliliters of

the filtered extract were mixed with 2 mL of acid ninhydrin and 2 mL of glacial acetic acid in a test tube for 1 h at 100°C, and the reaction was terminated by placing the test tube directly on ice. The reaction mixture was then extracted with 4 mL of toluene and then mixed. Chromophore containing toluene were aspirated from the aqueous phase and warmed to room temperature, with absorbance determined *via* a spectrophotometer (*UV-160, Shimadzu, Japan*) at 520 nm using toluene as a blank. The proline concentration was approximated using a standard curve with L-proline (*Sigma-Aldrich Chemie, Germany*).

Mineral composition: Leaf (the fifth leaf from the apex) and root samples from randomly selected plants were washed, dried to a constant mass, and ashed at 550°C, after being grinded. Then, ash was extracted with HNO₃ up to constant volume (Kaya and Higgs 2002). Total N was measured in samples of 0.1 g of DM using the Kjeldahl method (Nelson and Sommers 1973). All chemical elements were determined in the sample solution. Phosphorus was analyzed using the vanadate-molybdate method (Chapman and Pratt 1961). Chloride concentrations were measured with an ion chromatograph (*761 Compact IC Metrohm Ltd, Herisau, Switzerland*). Sodium (Na⁺) and potassium (K⁺) concentrations were assayed *via* a flame photometer (*Corning 400, UK*). Calcium (Ca²⁺) and magnesium (Mg²⁺) were determined using atomic absorption (*PerkinElmer, Model 2380, USA*) following the method described by Allen (1989).

Experimental layout and statistical analysis: The experimental layout was split-plot in a randomized complete block design, with three replicates (beginning from 30 d after seeding). Five irrigation treatments were randomly allocated to the main plots and the two AMF-inoculation treatments were assigned to the sub-plots. Each plot contained three pots in each replicate, for a total of 90 pots.

The collected data were statistically analyzed using *SAS (SAS version 9.2, Cary, NC)* software. Analysis of variance (*ANOVA*) was used to analyze the data. Differences among means were subjected to a revised least significant difference (LSD) test at the 0.05 level (Steel and Torrie 1980).

Results

Effects of blending-irrigation water and mycorrhizal fungi inoculation treatments: Analysis of variance revealed significant differences (0.05% LSD) between several plant growth traits as a result of using different irrigation treatments and AMF inoculation (Table 1). Thus, shoot DM and total DM were both affected by saline-water treatments (T2-T5), and both shoot FM and DM traits were higher in the AMF-treated plants compared to the AMF-untreated plants (Table 1).

Interaction effects between blends of irrigation water and AMF inoculation: Both AMF and salinity level in the substrate affected snapdragon growth traits (Table 1). The AMF treatment resulted in elevated shoot FM in the T1 plants followed by, in order, T3 and T5 plants, in enhanced shoot DM in the T3 plants, and increased root FM and root DM in the T4 plants. Interactions between the treatments had also a significant effect on some plant growth traits; *e.g.*, higher salinity levels reduced plant growth. The

Table 1. Vegetative growth and leaf water relations parameters of mycorrhizal (+AMF) and nonmycorrhizal (-AMF) snapdragon plants grown under well-watered (WW) and desalinated water (DW). Values in each column followed by the *different letter(s)* are significantly different at $P \leq 0.05$. T1: 100% desalinated water (DW), T2: blinding 25% well water (WW) and 75% DW, T3: 50% WW and 50% DW, T4: 75% WW and 25% DW, T5: 100% WW. Each value represents the mean of four replicates \pm SE. ** – highly significant; * – significant; ns – not significant; DM – dry mass; FM – fresh mass; Pt – plant tolerance; WUE – water-use efficiency; ψ_w – water potential; RWC – relative water content.

| Irrigation treatments | Mycorrhizal inoculation | Shoot FM per plant [g] | Shoot DM per plant [g] | Root FM per plant [g] | Root DM per plant [g] | Plant total DM per plant [g] | Pt [%] | WUE [%] | ψ_w [MPa] | RWC [%] |
|-----------------------|-------------------------|---------------------------------|--------------------------------|--------------------------------|-------------------------------|--------------------------------|--------|--------------------------------|--------------------------------|--------------------------------|
| T1 | -AMF | 10.98 \pm 0.15 ^D | 2.99 \pm 0.09 ^{AB} | 5.13 \pm 0.49 ^{A-C} | 1.45 \pm 0.18 ^{AB} | 4.44 \pm 0.46 ^{AB} | | 1.23 \pm 0.05 ^{BC} | -1.05 \pm 0.11 ^F | 82.34 \pm 8.11 ^{AB} |
| | +AMF | 16.09 \pm 1.26 ^A | 3.03 \pm 0.45 ^{AB} | 6.54 \pm 0.57 ^{AB} | 1.97 \pm 0.20 ^A | 5.00 \pm 0.58 ^A | | 1.43 \pm 0.12 ^A | -0.96 \pm 0.08 ^F | 85.29 \pm 6.23 ^A |
| T2 | -AMF | 15.42 \pm 1.40 ^{A-C} | 3.01 \pm 0.13 ^{AB} | 4.40 \pm 0.44 ^{B-D} | 1.15 \pm 0.12 ^{BC} | 4.16 \pm 0.45 ^{ABC} | 93.6 | 1.06 \pm 0.06 ^D | -1.20 \pm 0.12 ^{EF} | 84.31 \pm 6.89 ^{AB} |
| | +AMF | 17.19 \pm 1.07 ^A | 3.37 \pm 0.24 ^A | 6.05 \pm 0.46 ^{A-C} | 1.49 \pm 0.17 ^{AB} | 4.86 \pm 0.53 ^A | 97.2 | 1.14 \pm 0.18 ^{BCD} | -1.14 \pm 0.09 ^{EF} | 87.42 \pm 11.82 ^A |
| T3 | -AMF | 13.18 \pm 1.12 ^{B-D} | 2.09 \pm 0.04 ^C | 4.22 \pm 0.21 ^{B-D} | 0.96 \pm 0.19 ^{BC} | 3.05 \pm 0.38 ^{CDE} | 68.6 | 1.11 \pm 0.11 ^{CD} | -1.39 \pm 0.10 ^{DE} | 84.01 \pm 8.26 ^{AB} |
| | +AMF | 17.22 \pm 1.69 ^A | 3.06 \pm 0.31 ^{AB} | 6.12 \pm 0.51 ^{A-C} | 1.51 \pm 0.13 ^{AB} | 4.57 \pm 0.42 ^{AB} | 91.4 | 1.25 \pm 0.15 ^B | -1.21 \pm 0.07 ^{EF} | 87.38 \pm 5.68 ^A |
| T4 | -AMF | 15.29 \pm 1.17 ^{A-C} | 2.59 \pm 0.24 ^{BC} | 3.89 \pm 0.32 ^{CD} | 0.86 \pm 0.09 ^C | 3.45 \pm 0.33 ^{BCD} | 77.7 | 0.93 \pm 0.09 ^{EF} | -1.76 \pm 0.09 ^C | 77.67 \pm 6.35 ^C |
| | +AMF | 17.80 \pm 1.20 ^A | 3.07 \pm 0.26 ^{AB} | 7.35 \pm 0.53 ^A | 1.84 \pm 0.16 ^A | 4.91 \pm 0.40 ^A | 98.2 | 1.04 \pm 0.11 ^{DE} | -1.53 \pm 0.09 ^C | 86.34 \pm 10.41 ^A |
| T5 | -AMF | 12.45 \pm 1.52 ^{CD} | 2.07 \pm 0.18 ^C | 3.32 \pm 0.30 ^D | 0.82 \pm 0.11 ^C | 2.89 \pm 0.13 ^D | 65.1 | 0.81 \pm 0.08 ^F | -2.41 \pm 0.13 ^A | 72.65 \pm 8.95 ^D |
| | +AMF | 16.87 \pm 0.64 ^A | 2.78 \pm 0.25 ^{A-C} | 3.58 \pm 0.32 ^{CD} | 0.91 \pm 0.10 ^C | 3.69 \pm 0.21 ^{BCD} | 73.8 | 0.89 \pm 0.11 ^F | -2.03 \pm 0.12 ^B | 79.69 \pm 8.74 ^{BC} |
| Irrigation | | ns | * | ns | ns | * | | * | ** | ** |
| AM Inoculation | | ** | ** | ns | ns | ns | | ** | ns | * |

reduction in both shoot FM and DM, and Pt were more distinct in the AMF-untreated plants than in the plants treated with AMF, particularly, for the plants irrigated with WW (T5) (Table 1). On the basis of total DM, Pt was higher in the AMF-treated plants (97.2, 91.4, 98.2, and 73.8% for T2, T3, T4, and T5, respectively) than that in the AMF-untreated plants (93.6, 68.6, 77.7, and 65.1%, respectively). The T4 plants exhibited the highest values of FM and DM for both shoots and roots. Table 1 shows that for plant DM, mycorrhizal dependency increased as WW contents increased. Generally, the AMF-treated T4 plants had higher shoot and root FM compared to other treatments.

Water-use efficiency (WUE): In general, WUE values were higher in the AMF-treated than those in the AMF-untreated plants grown under different salinities. However, T1 and T3 produced the highest WUE values in comparison between the AMF-treated and AMF-untreated plants (Table 1). On the other hand, both AMF-treated and AMF-untreated plants had higher WUE values when grown with DW than that with WW. Moreover, WUE decreased with increasing salinity level in both the AMF-treated and AMF-untreated plants (Table 1).

Leaf water relations (ψ_w and RWC): The leaf water potential (ψ_w) in both mycorrhizal and nonmycorrhizal plants was markedly reduced by water stress; moreover, the AMF-treated plants showed significantly higher ψ_w than

that in the AMF-untreated plants regardless of the irrigation treatment. Leaf RWC was relatively higher in the AMF-treated than that in AMF-untreated plants under saline water conditions. The lowest significant values for RWC were detected between T4 and T5 treatments (Table 1).

Proline concentration: The proline concentration in the AMF-inoculated and untreated snapdragon leaves increased in response to increasing water salinity (*i.e.*, increasing fraction of WW) (Fig. 1A). The increase in the proline concentration was related to the gradation of the mycorrhizal infection. However, the AMF-inoculated plants had relatively lower proline content than that of the AMF-untreated plants irrespective of the irrigation treatment.

Mineral composition: Contents of some elements in shoots and roots of snapdragon are shown in Figs. 1B–D. The concentrations of Na^+ , Ca^{2+} , total P, Mg^{2+} , N, K^+ , and Cl^- in leaf and root tissues were significantly influenced by the presence of AMF. Concentrations of Na^+ and Cl^- (Figs. 1B, 2D) in leaf tissues and in roots were significantly higher when the salinity was higher. The AMF-treated plants contained lower amounts of Na^+ and Cl^- in leaves and roots, but these values were lower in the roots compared to leaves. The contents of N and K^+ (Fig. 2B,C) were reduced by increasing salinity, although the AMF-treated plants showed the higher K^+ concentration than that of the AMF-untreated plants.

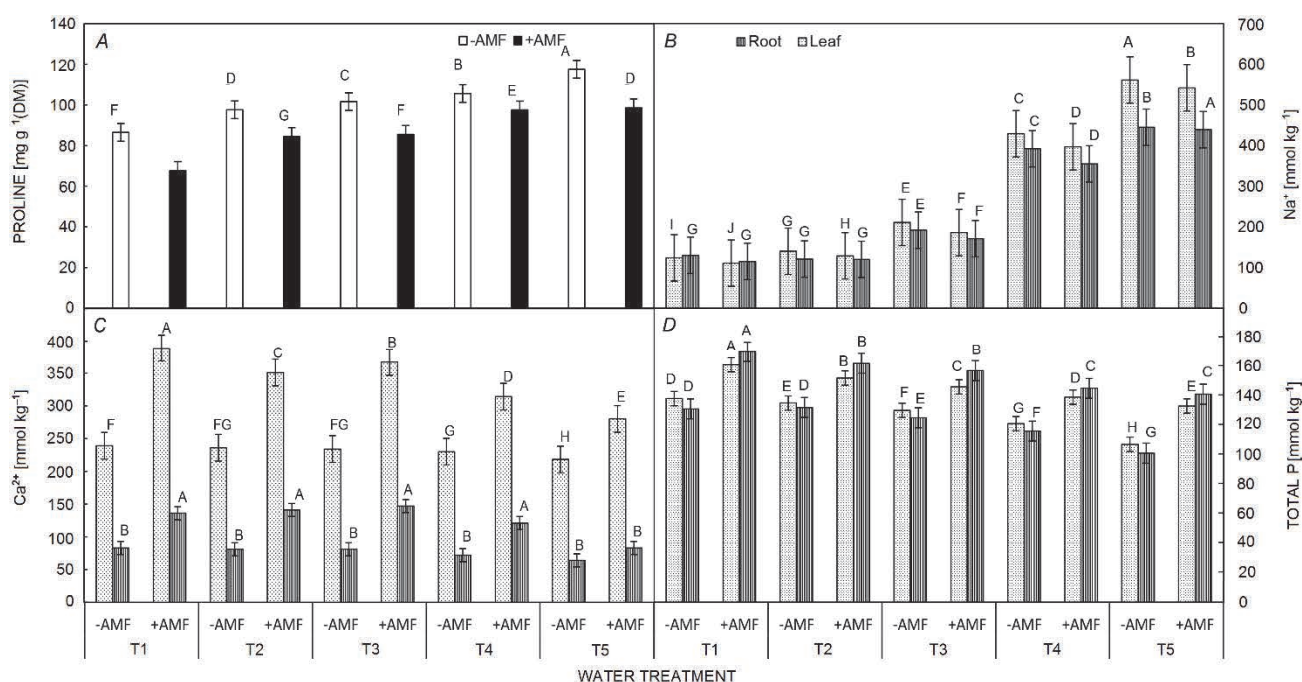


Fig. 1. Proline (A) and mineral concentrations of Na^+ (B), Ca^{2+} (C), and total P (D) of mycorrhizal (+AMF) and nonmycorrhizal (-AMF) snapdragon plants grown under well-watered (WW) and desalinized water (DW) conditions. T1, T2, T3, T4, and T5 are irrigation water treatments with 100% DW, 25% WW and 75% DW, 50% WW and 50% DW, 75% WW and 25% DW, and 100% WW, respectively. Values in each column followed by the different letter(s) are significantly different at $P \leq 0.05$.

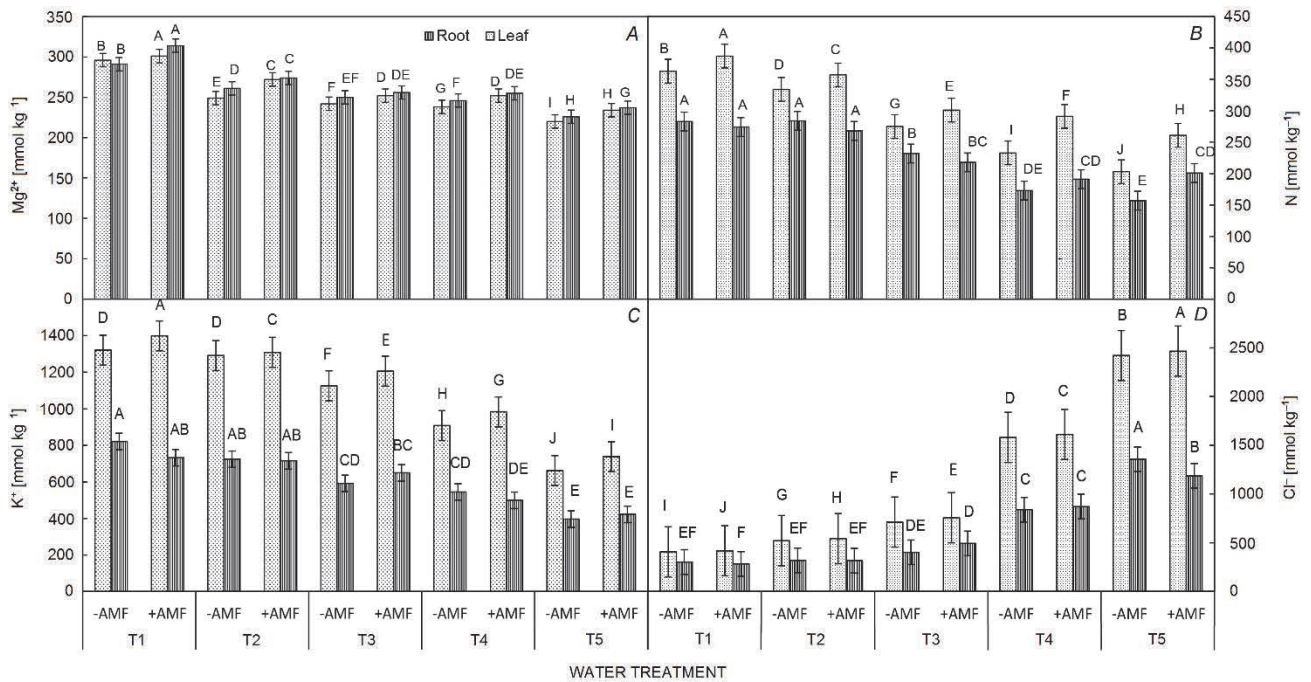


Fig. 2. Mineral concentrations, such as Mg^{2+} (A), N (B), K^+ (C), and Cl^- (D), of mycorrhizal (+AMF) and nonmycorrhizal (-AMF) snapdragon plants grown under well-watered (WW) and desalinated water (DW) conditions. T1, T2, T3, T4, and T5 are irrigation water treatments with 100% DW, 25% WW and 75% DW, 50% WW and 50% DW, 75% WW and 25% DW, and 100% WW, respectively. Values in each column followed by the different letter(s) are significantly different at $P \leq 0.05$.

Thus, AMF symbiosis improved K^+ uptake in snapdragon plants at all salinity levels in comparison with controls. Salinity reduced Ca^{2+} concentrations (Fig. 1C), but there were large differences between the AMF-treated and AMF-untreated plants. Total P and Mg^{2+} concentrations

(Figs. 1D, 2A) significantly decreased with the increasing salinity level, and the leaves and roots of the AMF-treated plants had higher concentrations of P and Mg^{2+} than that of the AMF-untreated plants.

Discussion

The results described here provide additional evidence for the role of AMF symbiosis in alleviating well-water stress in snapdragon (*Antirrhinum majus* L.). We found that mycorrhizal symbiosis decreased growth inhibition due to salt stress at all levels and had a beneficial effect of alleviating the negative osmotic stress caused by high saline concentrations (Rosendhal and Rosendhal 1991, Ruiz-Lozano 2003). Inoculation with AMF helps to mitigate the effects of salinity in various plant species, such as maize (*Zea mays*) and bajra (*Pennisetum glaucum*) (Sheng *et al.* 2008, Borde *et al.* 2011). This suggests that the symbiotic association between AMF and the snapdragon plants was strengthened in the saline environment once the association was established, perhaps due to greater allocation of carbohydrates to the shoot than to root tissues as a result of AMF inoculation (Shokri and Maadi 2009). Although shoot and root DM production decreased in both AMF-treated and AMF-untreated snapdragon plants as salinity increased, the AMF-treated plants generally displayed a higher level of salinity tolerance and exhibited the smallest declines in growth under salt-stress

conditions (Campanelli *et al.* 2013).

It is well documented that mycorrhizal symbiosis can improve the water status of host plants. Here, the AMF-treated plants had higher ψ_w than that of the AMF-untreated plants under salt-stress conditions (Porcel and Ruiz-Lozano 2004). Our study also showed that mycorrhizal symbiosis induced greater WUE in snapdragon plant under WW application. Moreover, mycorrhizal inoculation affected the RWC of snapdragon plants, with the mycorrhizal plants having higher RWCs than the AMF-untreated plants under WW conditions. There may be several reasons for AMF-treated plants having better water status than that of AMF-untreated plants (Zhu *et al.* 2012), including external hyphal extraction of soil water (Ruiz-Lozano *et al.* 1995), altered rates of water movement into, through, and out of the host plants, with consequent effects on tissue hydration and plant physiology (Ruiz-Lozano 2003), stomatal regulation through hormonal signals (Aroca *et al.* 2008), indirect and positive impacts that improve the uptake of phosphates and other nutrients (Subramanian and Charest 1997, Crespo 2015), greater

osmotic adjustment (Giri *et al.* 1999, Wu and Xia 2006), and higher root hydraulic conductivity (Augé 2004). Mycorrhizal-inoculated snapdragon maintained the higher leaf water content under saline conditions.

Saline conditions modify the uptake of mineral nutrients and nutrient balance (Giri *et al.* 2007). Concentrations of Na⁺ and Cl⁻ were lower in the AMF-treated plants than that in AMF-untreated plants. Some researchers have hypothesized that lower Na⁺ and Cl⁻ concentrations in plant tissues may be due to the capability of the fungus to retain these ions in intraradical fungi hyphae or to compartmentalize them in the root cell vacuoles (Cantrell and Linderman 2001, Al-Karaki 2006). Mycorrhizal symbiosis improved plant vegetative growth and sustained plant physiology, and this resulted in a subsequent dilution of the ions in tissues (Al-Karaki 2000, Campanelli *et al.* 2012).

Giri *et al.* (2003) noted that the contribution of AMF to plant growth is not limited only to a nutrition improvement but that they also change the root biomass. The total P concentration in plant tissues rapidly decreases under salt stress because phosphate ions precipitate with Ca²⁺, Mg²⁺, and Zn²⁺ ions in salt-stressed soil and thus they become unavailable to plants (Munns 1993, Evelin *et al.* 2009, Evelin *et al.* 2012). AMF have been shown to positively affect the composition of mineral nutrients (especially low-mobility minerals such as P) in plants under salt stress conditions (Al-Karaki and Clark 1998, Evelin *et al.* 2009). Al-Karaki *et al.* (2001) showed that mycorrhizal tomato plants had higher P contents than non-mycorrhizal plants at all salinity levels (1.4, 4.9 and 7.1 dS m⁻¹). This may have occurred because of reduced P transport and uptake under these salinity levels. In some Gramineae species such as rice and barley, P uptake by plants decreased as irrigation water salinity levels increased due to the salinity stress conditions (Khan *et al.* 1992, Mohiuddinet *al.* 1997, Amer 1999, Noufalet *al.* 2000). Generally, plants grown under higher salinity levels may have lower H₂PO₄⁻ affinity (preferred phosphate ion for plant uptake) than under lower salinity levels (Sentenac and Grignon 1985, Al-Karaki 1997, Al-Karaki *et al.* 2001).

The present study confirmed that AMF symbiosis plays a vital role in improving the P nutrition of host plants under salt-stress conditions; our results are in accordance with

those of Evelin *et al.* (2009). It has been estimated that external hyphae of AMF can improve the supply of Ca²⁺ and Mg²⁺ uptake. This may also be influenced by the form of N available (NO₃⁻ or NH₄⁺), which strongly influences the accumulation of Na⁺ and other cations, particularly K⁺ (Giri and Mukerji 2004, Evelin *et al.* 2009).

In this study, the salt treatment led to decreased K⁺ and N concentrations in the plant tissues. The reduction in K⁺ and N uptake, caused by toxic Na⁺, is likely to be the result of the competitive intracellular influx of both ions (Morte *et al.* 2000, Colla *et al.* 2008). Mycorrhizae had a positive influence in helping to maintain K⁺ concentrations at all salinity levels. Potassium is a vital element for cell expansion, stomatal behavior, and water regulation, among other processes (Kaya *et al.* 2007); it also activates a range of enzymes, a role for which Na⁺ cannot act as a substitute (Giri *et al.* 2007). A high concentration of Na⁺ or a high Na⁺:K⁺ ratio can disrupt various enzymatic processes in the cytoplasm. It seems that greater K⁺ accumulation by AMF plants under salt-stress conditions may help in reducing the Na⁺:K⁺ ratio in the plants, thus limiting the metabolic toxicity of Na⁺ (Campanelli *et al.* 2013).

The water status of mycorrhizal plants was often associated with physiological indices, such as net photosynthesis and stomatal behavior, and transpiration fluxes. Augé (2001) noted that the leaf water potential is linked functionally: changes in one factor usually drive changes in the other. The higher RWC and WUE that occur as a result of AMF treatment may be beneficial for moving water through the plants to the evaporating surfaces and maintaining opened stomata in leaves. Moreover, higher turgor potential in AMF plants improves their water status (Nelsen and Safir 1982, Sheng *et al.* 2008). Thus, mycorrhizal symbiosis protects snapdragon plants against salt stress by improving water relations and chemical composition.

Conclusion: Here, we found that mycorrhizal treatment alleviated the deleterious effects of salt stress in snapdragon plants. Under saline conditions, snapdragon plants require mycorrhiza, not only for acclimation purposes, but also for continuing nutrient uptake during the growth stages.

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