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The effects of Glomus mosseae on growth and physiology of Acacia albida Del. seedlings under drought stress

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Abstract

The effects of Glomus mosseae on morphology (basal diameter, seedling height, root length, root volume, leaf surface, root and shoot dry weight, root length) and physiology (transpiration rate, stomatal conductance, photosynthetic rate, chlorophyll content and proline) of Acacia albida Del. seedlings were studied under drought stress. Seedlings were grown in pot with drought stress (25%, 50%, 75% and 100% of soil water content) for 8 months, following 2 weeks of non-drought stress pretreatment in the greenhouse. Under drought stress, mycorrhizal *Acacia albida* seedling had higher shoot and root dry weight, basal diameter, height and leaf area surface. Results also showed that AMF symbiosis increased, root volume of seedlings but didn't effect on root length. Based on this study, the arbuscular mycorrhiza fungi had a positive effect on plant development under drought conditions, so it could be offered that for the best accomplishment with the afforestation in dry regions, the Acacia seedlings had better to be pre-colonized with Glomus mosseae fungi.

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Introduction

Drought is the most common stress affecting plant growth in arid and semiarid regions. Thus, it is necessary to improve the level of efficiency in plant capture and use of water and nutrients (*Marulanda et al.*, 2009). The arid regions of Iran are characterized by hard climate conditions such as a scarce and irregular rainfall, long dry season and hot summer (*Heshmati*, 2007). The lack of natural plant cover and consequently low organic matter is the most important factor that limit plant establishment. This multiple stress situation, together with uncontrolled man-mediated activities may promote desertification in these ecosystems in near future.

Using natural species such as *Acacia albida* in reforestation programs for desert region of Iran has been encouraged by forestry organization. However the most of these programs failure and this forest area decreasing yearly because of high cost of irrigation and also low knowledge of reforestation involving these species. So using techniques and methods that increase seedling establishment is very useful. According to Allen (1989), in semiarid and disturbed soils, the establishment of plants can be improved by colonization with arbuscular mycorrhiza fungi.

A mycorrrhiza is a symbiotic association between plant and fungus localized in root or root–like structure in which energy moves primarily from plant to fungus and inorganic resources from fungus to plant (Cho *et al.*, 2006). The most common type is formed by the arbuscular mycorrhizal fungi (AMF), which are formed by approximately 80% of vascular plant species in all major terrestrial biomes (Smith *et al.*, 2010). AMF can influence the growth of plant species and consequently plant community composition. Arbuscular mycorrhizal fungal symbiosis affecting many aspects of the host plant morphology including seedling height, collar diameter, terminal growth, root length, leaf number, leaf surface, fresh shoot and root weight, dry shoot and root weight and root volume (Pasqualini *et al.*, 2007; Fan *et al.*, 2008; Yao *et al.*, 2008) and physiology, including transpiration rate, leaf surface temperature, stomatal conductance, photosynthetic rate, chlorophyll concentration (Fan *et al.*, 2008; Borkowska, 2002). However there are a few studies about the effects of arbuscular mycorrhiza fungi on growth and physiology of forest trees grown in dry regions such as *Acacia albida*. So, this study aims to investigate the effects of *Glomus mosseae* on growth and physiology of *Acacia albida* seedling in different drought stress conditions.

Materials and Methods

AM fungal inocula

inocula that used in this experiment consisted of *Glomus mosseae* (Nicol & Gerd) Gerdemann. This inocula was propagated on maize (*Zea mays* L.), grown in an autoclaved (121 8C for 2 h on 2 successive days) sandy soil for 5 months long. The inocula consisted of a mixture of rhizospheric soil from pure pot culture containing spores, hyphae and mycorrhizal root fragments that were air-dried and sieved (2 mm).

Plant material and Greenhouse experiment

Seeds of *Acacia albida* were transplanted into pots with a 2: 1 v/v mixture of autoclaved native soil and sand (Table1.). The corresponding arbuscular mycorrhizal inoculum was applied at a rate of 10% (v/v). The same amount of autoclaved mixture of the inocula was added to control plants. After transplanting, seedlings watered to field capacity every three days for two week and arranged in a greenhouse. The experimental design was a two-way ANOVA with four levels of irrigation (25%, 50%, 75%, 100% of soil water content) and two levels of mycorrhizal inoculums (AM and non-AM). Treatments were randomly arranged in 20 replicates of each treatment. Inoculated and non-inoculated seedlings were grown for 8 months under greenhouse conditions.

Table 1. Analytical characteristics of the soil used in the greenhouse experiment.

pH (1:2.5)	7.5	Mg (ppm)	25
Electrical conductivity EC (1.5 mmhos/cm)	392.7	Ca (1:100 ppm)	810
Total organic C (%)	0.533	Sand (%)	50
Total N (%)	0.231	Silt (%)	26
Total K (ppm)	53	Clay (%)	24
Total P (ppm)	25		

Measurements and analysis

Root and shoot biomass were determined after oven drying at 70°C for 90 h. Basal diameter, shoot height, terminal growth, root length, leaf surface, shoot dry weight, root dry weight and root volume measured at the end of experiment (After 8 month of sowing). Relative chlorophyll concentration was measured using SPAD-100 chlorophyll meter. Gas exchange parameters (including photosynthetic rate, stomatal conductance, and transpiration rate) were determined using an LCA-4-type open system infrared gas analyzer (ADC Co. Ltd). For studying root and shoot free proline, used .5 g of fresh organ and *sulfosalicylic acid* (3%). Proline was estimated by spectrophotometric analysis at 520 nm absorption.

Statistical Analysis

Two-way analysis of variance was used to test differences and, when appropriate, Duncan Tests were used for comparison of means. Statistical procedures were carried out with the software package SPSS 16.0 for Windows.

Results

Morphological parameters

Mycorrhizal colonisation of *Acacia albida* were 51.15%. Our study showed that colonization rate in this species decreased with increasing drought stress (Table 3.).

Also, the result showed that at the end of growth period (after 8 month) mycorrhiza-inoculated seedlings were larger than non-inoculated ones, so that shoot height, Basal diameter and leaf surface of inoculated seedlings were significantly higher than non-inoculated ones regardless of drought stress condition and decreased while irrigation decreased (Table 3.).

Biomass measurement demonstrated that AM fungal inoculation exerted differential influences on the shoot and root biomass (Table 2.). In general, AM fungi promoted the shoot biomass and the root biomass except for root fresh weight of *Acacia albida* and root dry weight of *Acacia albida* (Table 2.). Root measurement showed that root volume of plants was significantly higher in mycorrhizal than non-mycorrhizal plants but AM fungal inoculation had no effect on root length (Table 4.).

Table 2. Two factors ANOVA (mycorrhizal inoculation and drought stress) for growth and morphological parameters studied in *Acacia albida*. F values (significance level).

	Mycorrhizal (M)	Drought stress (DS)	Interaction (M.DS)
Shoot height (cm)	15.5(.000) **	3.47(.027) *	2.34(.091)
Basal diameter(mm)	15.4(.000) **	5.24(.005) **	1.1(.363)
Root length (cm)	.55(.462)	.87(.463)	.108(.955)
Leaf surface (cm²)	19.6(.000) **	38.5(.000) **	1.45(.245)
Shoot dry weight(g)	15.2(.000) **	4.67(.008) **	1.78(.170)
Root dry weight(g)	4.84(.035) *	4.54(.009) **	.43(.727)
Shoot fresh weight	16.6(.000) **	8.47(.000) **	2.41(.084)
Root fresh weight	3.95(.055)	8.41(.000) **	.22(.881)
Root volume	15.3(.000) **	8.2(.000) **	.22(.878)

Table 3. Effects of mycorrhizal (M) inoculation on basal diameter, shoot height, shoot biomass and root biomass of *Acacia albida* under different soil water content (SWC).

Treatment	Root colonization (%)	Shoot height (cm)	Basal diameter (mm)	Shoot weight (g)		Root weight (g)	
				Fresh	Dry	Fresh	Dry
M- 25% SWC	40.2 b	9 bc	2.64 bcd	.98 d	.65 c	2.15 bc	.61 bc
N- 25% SWC		6.66 c	2.15 d	.96 d	.56 c	1.63 c	.5 c
M- 50% SWC	46.8 b	17.42 ab	3.4 ab	3.51 bc	2.01 ab	5.3 a	1.45 ab
N- 50% SWC		13 bc	2.41 cd	1.9 cd	1.12 bc	4.22 ab	1.16 abc
M- 75% SWC	50.4 b	17.5 ab	3.78 a	4.62 ab	2.32 a	6.05 a	1.8 a
N- 75% SWC		8.8 bc	3.28 abc	2.2 cd	.96 bc	5.14 a	1.22 abc
M- 100% SWC	67.2 a	24.75 a	4.1 a	6.07 a	2.92 a	5.77 a	1.65 a
N- 100% SWC		8.5 bc	2.69 bcd	2.5 cd	1.11 bc	4.01 ab	.98 abc

Table 4. Effects of mycorrhizal (M) inoculation on root volume, leaf surface, leaf temp and root length of *Acacia albida* under different soil water content (SWC).

Treatment	Root volume (cm^3)	Root length (cm)	Leaf surface (cm^2)	Leaf surface temp (°c)
M- 25% SWC	3.85 bc	47.1 a	18.35 cd	24.37 b
N- 25% SWC	2.33 c	46.66a	12.83 d	25.84 a
M- 50% SWC	7.38 a	55.7 a	28.14 cd	23.97 bc
N- 50% SWC	4.8 bc	58 a	16.74 cd	23.19 de
M- 75% SWC	8 a	50 a	54 b	23.42 cd
N- 75% SWC	6 ab	56 a	30.5 c	22.72 e
M- 100% SWC	7.25 a	47.5 a	76 a	22.52 e
N- 100% SWC	4.5 bc	53.3 a	53.33 b	22.63 e

Physiological parameter

AMF inoculation had significant effect on net photosynthetic rate, while had no effect on stomatal conductance and leaf surface temperature (Table 5.). Photosynthetic rate was higher in inoculated plants than non-inoculated ones (Table 6.). The transpiration rate in mycorrhizal and non-mycorrhizal *Acacia albida* plants was similar, while drought stress had significance effects on transpiration rate, leaf temperature, stomatal conductance and photosynthetic (Table 5.). So that transpiration rate, stomatal conductance and photosynthetic unlike to leaf surface temperature were higher in well irrigated plants (100% SWC) than drought stressed (25 % SWC) ones for *Acacia* seedlings (Table 6.).

Chlorophyll measurement showed that unlike to drought stress, AM fungi had not significant effect on the Relative chlorophyll concentration. Based on this result, chlorophyll concentration in the leaves of both plants decreased as irrigation decreased (Fig .2.).

Proline

Drought stress had significant effects on root and shoots proline content, so that root proline increased in plants subjected to drought stress (Table5. And Table6.). AMF had significant effect on shoot proline, so that shoot proline was higher in non-mycorrhizal plants than mycorrhizal ones (Fig.1.).

Table5. Two factors ANOVA (mycorrhizal inoculation and drought stress) for physiological parameters studied in *Acacia albida* F values (significance level).

	Mycorrhizal (M)	Drought stress (DS)	Interaction (M.DS)
Transpiration rate	2.32(.138)	24.6(.000) **	.609(.614)
Leaf surface temp	.032(.858)	48.3(.000) **	10.7(.000) **
Stomatal conductance	1.72(.199)	16.9(.000) **	.29(.830)
Photosynthetic	9.2(.005) **	13.5(.000) **	5.84(.000) **
Chlorophyll content	.000(.985)	4.99(.012) *	0.982(.057)
shoot prolin	1.76(.041) *	12.68(.000) **	.931(.448)
Root prolin	.359(.557)	3.26(.049) *	.640(.600) **

Table 6. Effects of mycorrhizal (M) inoculation on Transpiration, Photosynthetic, Chlorophyll content and Stomatal conductance of *Acacia albida* under different soil water content (SWC).

Treatment	Transpiration (mmol $m^{-2}s^{-1}$)	Photosynthetic (umol $m^{-2}s^{-1}$)	Stomatal con (mol $m^{-2}s^{-1}$)	Root proline (μmolgfw^{-1})
M- 25% SWC	.26 d	.64 d	.005 d	.166 ab
N- 25% SWC	.18 d	.75 d	.004 d	.169 ab
M- 50% SWC	.50 cd	2.3 cd	.017 cd	.149 ab
N- 50% SWC	.53 cd	2.43 cd	.016 cd	.208 a
M- 75% SWC	.95 ab	6.49 a	.028 abc	.14 ab
N- 75% SWC	.68 bc	1.79 cd	.022 bc	.101 ab
M- 100% SWC	1.27 a	5.35 ab	.040 a	.049 b
N- 100% SWC	1.09 a	3.93 bc	.032 ab	.092 ab

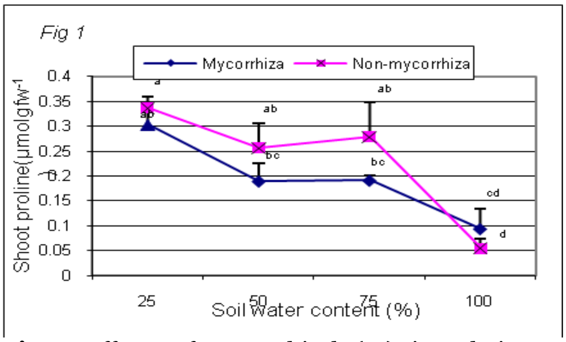

Fig .1. Effects of mycorrhizal (M) inoculation on shoot proline.

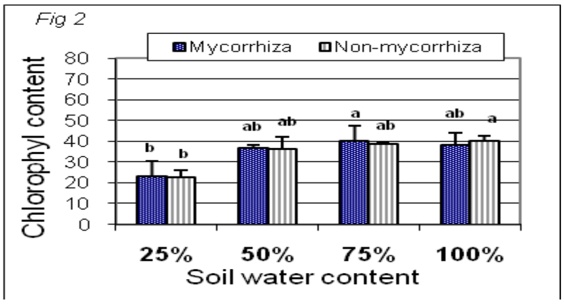

Fig .2. Chlorophyll content of *Acacia albida* under different soil water content (SWC).

Discussion

Mycorrhizal symbiosis is a key element in helping plants establishment and survive under stressful conditions (Augé *et al.*, 1992). Desertification generally reduces colonization rates, so re-vegetation must include the reconstitution of an appropriate mycosymbiont population (Caravaca *et al.*, 2002). Our results showed that the AM fungal colonization rate decreased with increasing drought stress. This is in agreement with the results of other researchers (Kaya *et al.*, 2003; Wu and Xia, 2006

and Sheng *et al.*, 2008; Kumar *et al.*, 2010 for salt stress. Based on Green *et al.*, 2005 and Auge (2001), root colonization more often increased than decreased under water stress conditions that it's not agreement with current study.

Results also showed that *Acacia albida* plants inoculated with AM fungi had higher shoot and root dry weight than non-mycorrhizal plants when being exposed to drought stress, which means mycorrhizal plants grow better than non-mycorrhizal plants under drought conditions. This is in agreement with many greenhouse studies on *Penicillium pinophilum* (Fan *et al.*, 2008), *Citrus tangerine* (Wu and Xia, 2006), *Jatropha curcas* (Kumar *et al.*, 2010), *Zea mays* (Sheng *et al.*, 2008), *Retama sphaerocarpa* (Caravaca *et al.*, 2003). Other morphological data strongly demonstrated that AM fungi significantly increased the seedling height, basal diameter and the leaf surface area. Positive effects of AMF on seedling height and basal diameter were recorded in *pistacia lentiscus* (Caravaca *et al.*, 2002), *Olea europae* and *Rhamnus lysiodis* (Palenzuela *et al.*, 2002), *Retama sphaerocarpa* (Caravaca *et al.*, 2003), *Citrus tangerine* (Wu and Xia, 2006) and seedling growth of *Myrtus communis* (Matosevic *et al.*, 1997), but Caravaca *et al.*, 2002, didn't observed significant effects of AMF symbiosis on basal diameter of *Pistacia lentiscus*.

In other hand we know that, plant roots are the key structure in contact with soil; therefore, the abiotic stresses such as drought in the soil environment can directly injure the roots. Thus we study the effects of drought stresses and AMF symbiosis on root length and root volume of seedlings. Result showed that, root volume of seedling decreased with increasing drought stress, while AMF symbiosis compensates this decreasing. This result isn't agreement with the results of Yao *et al.*, 2009 on *Poncirus trifoliata*. Our results also demonstrate that AMF symbiosis hadn't significant effects on root length of *Acacia albida* seedlings. Decreases in root length of mycorrhizal seedlings of *Pistacia lentiscus* (Green *et al.*, 2005) and *Poncirus trifoliata* (Yao *et al.*, 2009) were seen, while Kumar *et al.*, 2010 showed that AMF symbiosis increased root length of *Jatropha curcas* seedlings.

Stressful conditions, such as aridity and salinity, could disrupt components of plant's photosynthetic apparatus and further decrease photosynthetic capacity (Sheng *et al.*, 2008). Our data demonstrated that drought stress decreased photosynthesis rate and transpiration of *Acacia albida* seedlings, while AM fungi increased significantly photosynthesis rate of this species. Similar results have been reported for other plant species such as *Citrus tangerine* (Wu and Xia, 2006), Zea mays (Sheng *et al.*, 2008), *Penicillium pinophilum* (Fan *et al.*, 2008), *Pinus densiflora* (Choi *et al.*, 2005). Moreover, Results of this study showed that drought decreased stomatal conductance and chlorophyll content of seedlings, while AM fungi hadn't significant effects on stomatal conductance that it's not agreement with funding of Wu and Xia, 2006 on *Citrus tangerine* seedlings. Also, unlike to other studies on *Citrus tangerine* (Wu and Xia, 2006) and Zea mays (Sheng *et al.*, 2008), our study showed that AMF hadn't significant effects on chlorophyll content of seedlings.

Plants for resistance in drought condition will accumulate proline to regulate the osmotic potential of cells to aim them in absorption of water under drought stress. So that, our result showed that, root and shoot proline of seedlings were increased in severe drought stress condition. Proline accumulation in plant organs helps them to tolerate drought stress for a short period, if this condition last for a long time, proline accumulation decreased photosynthesis rate and consequently growth of plants.

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