RESEARCH PAPER

Symbiosis of AMF with growth modulation and antioxidant capacity of Caucasian Hackberry (*Celtis Caucasica* L.) seedlings under drought stress

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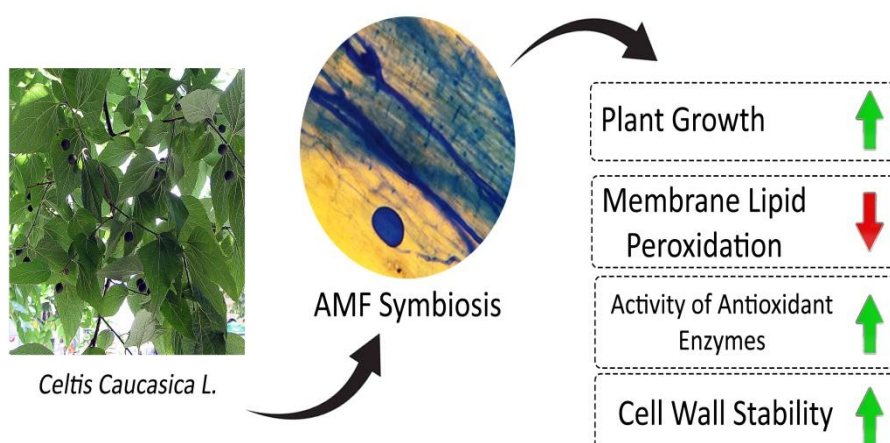[1] Department of Forestry, Faculty of Natural Resources, University of Tehran, Karaj, Iran
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Highlights

• Arbuscular Mycorrhiza fungi had significant impact on plant growth parameters.
• Mycorrhiza symbiosis illustrate positive effect on plant tolerance to water deficiencies.
• Symbiosis decreased H2O2 and malondialdehyde (MDA) content in leaves, while the activity of antioxidant enzymes catalase and superoxide dismutase raised in the host mycorrhiza-inoculated seedlings.

Graphical Abstract

Celtis Caucasica L.

AMF Symbiosis

Plant Growth ⬆
Membrane Lipid Peroxidation ⬇
Activity of Antioxidant Enzymes ⬆
Cell Wall Stability ⬆

Article Info

Abstract

Beside climate changes, drought stress has become a serious limitated factor for plant production and seedling growth. Arbuscular mycorrhizal fungi (AMF) symbiosis has proposed to improve the growth and water efficiency under limited-water condition. For this purpose, Caucasian Hackberry (Celtis Caucasica L.) seedlings inoculate with mycorrhizal fungi Rhizophagus intraradices and Funneliformis mosseae under well-watered and water deficient conditions. The mycorrhizal and non-mycorrhizal seedlings were treated under 75 % FC (as control), 50 and 25 % FC for 90-days. The Result showed that the plant growth parameters dry shoot weight, leaf area, seedling height, dry root weight, length of root, number of secondary root, and chlorophyll content were greater in mycorrhizal seedlings in comparison with non-inoculated seedlings under normal irrigation and drought treatments. AMF symbiosis decreased H2O2 and malondialdehyde (MDA) content in leaves. The positive correlation was observed between colonization rate and plant growth as well as antioxidant enzymes activity, remarkably. These results suggest that AMF symbiosis is a potential tool to alleviating the detriment created by drought stress on young seedling by elevating plant growth, reducing membrane lipid peroxidation, raising cell wall stability and increasing the activity of antioxidant enzymes.

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1. Introduction

Reforestation programs are vital in semi-arid areas where the main cause of soil degradation has been the loss of natural vegetal cover. In this areas, water deficit as mainly limiting factor can cause improper growth of plants and trees (Requena et al., 2001; Medrano et al., 2015). Here, soil degradation, lack of nutrients and adverse microclimate conditions lead to limited establishment and reproduction of plants (Fageria, 2016). Caucasian Hackberry (*Celtis Caucasica* L.) is a broad-leaved tree species from Celtis genus of Celtidaceae family that has considerable growth and resistance to water deficit under diverse environments, thus could be used for afforestation, reforestation and avoid of soil disturbance in semi-arid forests (Benhiba et al., 2015).

Environmental stresses such as water deficiencies, salinity, plant diseases negatively affects several aspects of plant physiology. For instance, it uncouples photosynthesis, disorders the structure of enzymes, diminishes nutrient uptake and/or transport to the shoot, therefore prompting a hormonal and nutritional imbalance in the plant. In addition, drought stress results in osmotic stress that can lead to turgor loss, thereby, leading to inhibition in plant growth and development. Drought stress also induces the production of reactive oxygen species (ROS), resulting in oxidative damage to carbohydrates, protein synthesis, lipid metabolisms, and alternatively leading to the membrane damage and cell death in plant tissues (Sohrabi et al., 2012) Drought stress as one of the substantial abiotic factors limiting the plant growth and ecosystem production throughout the world (Xiao et al., 2009). Diminution in the growth of plants is the effect of lack of moisture in whole-plant level that is due to the reduction of leaf size, stem extension and root propagation, as well as disturbs plant water relations and reduces water use efficiency (Farooq et al., 2009). At cellular levels, CO_2 absorption decline by leaves is through stomatal closure, membrane damage and disturbed activity of various enzymes (Farooq et al., 2009).

The generation of ROS results in lipid peroxidation, which in turn increases membrane permeability (Dhindsa et al., 1981; Gill and Tuteja, 2010; Hossain et al., 2015). The accumulation of reactive oxygen species (ROS) is the initial effects of drought stress (Nath et al., 2017). At high concentrations, ROS production can cause oxidative damage to the photosynthetic system and other essential functions of cells by destroying oxidative lipids, proteins and nucleic acids (Golldack et al., 2014). Malondialdehyde (MDA) is the final product of peroxidation of unsaturated lipids in the cell. Therefore, it is used as an appropriate biomarker to determine the amount of lipid peroxidation caused by oxidation stress in the cell (Sofo et al., 2004; Campo et al., 2014; Gharibi et al., 2016). To drought-resistance, plants exhibit a wide range of responses at the whole plant, cellular and molecular levels as complex phenomenon (Blum, 2011; Chaves et al., 2003; Fang and Xiong, 2015). The ROS-scavenging enzymatic system utilizing catalase (CAT), superoxide dismutase (SOD), peroxidase (POD) and ascorbate peroxidase (APX) is a common mechanism for detoxifying synthesized ROS during stress response (Mittler et al., 2004; Bailey-Serres and Mittler, 2006; Nath et al., 2017).

The plant–organism symbioses represent a vital ecological element in assistance better plant development in abused ecosystems (Requena et al., 2001). Recently, essential roles of Arbuscular mycorrhizal fungi (AMF) have been investigated in the vine, citrus, apple, peach, strawberry, etc. (Ruiz-Lozano et al., 2001; Krishna et al., 2010; Wu et al., 2012). As well as, some studies have shown the activation of antioxidant enzymes of host plants by AMF symbiosis may due to growth stimulation effects of mycorrhization under drought stress (García-Garrido and Ocampo, 2002; Wu et al., 2006; Latef and Chaoxing, 2011). The AMF are ubiquitous soil microorganisms and obligate symbionts, which confer a direct link between soil and roots (Lehmann et al., 2017; Manchanda et al., 2017). AMF cause enhancing plant mineral nutrition, water acquisition and resistance to biotic and abiotic stresses (Göhre and Paszkowski, 2006; Latef and Chaoxing, 2011). However, the mycorrhizal relationship is a strategy, which has been widely increased in recent years among reforestation programs (Smith et al., 2011). The affirmative impact of mycorrhizal fungi on plants tolerance to abiotic stress such as drought and salinity as well as biotic stress, such as pathogens have been known (Berruti., 2014; Seki et al., 2002).

The soil water content can affect spore germination strongly; therefore, disadjust mycorrhizal formation and further development (Nasim, 2010). Although limited water resources in soil had a negative impact on the amount of AMF hyphae, the AMFs still increased nutrients uptake of host plants (Van Der Heijden et al., 2003;

Neumann et al., 2009). The response of root colonization to drought stress depends on the severity and periodicity of drought (Aroca et al., 2008). In an experiment, exposed to short-term (up-to-6 days) soil water deficit causes a non-significant reduction of root mycorrhizal colonization by Glomus versiforme in Citrus sinensis Osbeck grafted on Poncirus trifoliata (Wu and Zou, 2009). The role of mycorrhiza in drought stress attenuation has been frequently recognized, and improved nutritional status and reduced damage of water deficit are among the most recognized benefits for host plants (Subramanian and Charest, 1995; Al-Karaki et al., 2004; Rapparini and Peñuelas, 2014; Ruiz-Lozano et al., 2012). The objectives of present study were to investigate effect of AMF-symbiosis on morphology and enzymatic antioxidant scavenging system of Celtis Caucasica seedlings under water-limited condition.

2. Materials and methods

2.1. AMF inoculation and water-stress application

Mature seeds of Caucasian were collected from Zagros region in Shar Kord province (Char Tagh Ardal), Iran (N 31° 49′ 29″ E 50° 51′ 33″). Seeds were scarified by chilling at 4°C in the refrigerator for three months. Two types of more common fungi, *Rhizophagus intraradices,* and *Funneliformis mosseae* were used to investigate the effect of AM-symbiosis on Caucasian seedlings. So that, 100 g inoculation materials containing 100 spores were applied with scarified seeds. Then, pots were irrigated for 90-days in a normal conditioning spring, after that they were treated under drought stress for three months subsequently. For perform drought stress, the first step, field capacity of 100g soil was measured using pressure plate apparatus, then it calculated for per pot under 3 treats include 75% FC as control, 50 and 25% as drought stress. To keep the target water regime, soil moisture in each pot was measured daily and the sum of water lost was replaced (Faghire et al., 2010).

2.2. AMF colonization and growth parameters measurement

Ninety days after drought stress application, Caucasian Hackberry seedlings were harvested and AMF colonization rate, plant height, root length, leaf area, dry shoot weight, dry root weight, Number of secondary roots were determined as growth parameters. Caucasian Hackberry's root samples were cleaned and stained as described by Phillips and Hayman (1970). A hundred root fragments (1 cm) per plant were cleared with 10 % KOH at 90 °C for 15 min, bleached in alkaline hydrogen peroxide for 20 min, acidified in 1% HCl for 5 min, and then stained with trypan blue (0.05%) for 5 min. Light microscopy was used to observe the degree of root colonization by AMF and the percentage of mycorrhizal root colonization was determined by visual observation of fungal colonization. Percentage of AMF colonization was calculated with the following equation:

$$Colonization\ (\%) = \frac{Number\ of\ mycorrhizal\ root\ pieces}{Total\ number\ of\ observed\ root\ pieces} \times 100 \tag{1}$$

2.3. Chlorophyll determination

Before extracting the chlorophyll content, fresh leaf scavenged with deionized water to remove any surface contamination. Extraction was carried out using 1 g leaf sample ground in 80% acetone in a pestle and mortar. The absorbance measured using UV/Visible spectrophotometer (Shimadzu UV-160) and the concentration of total chlorophyll was calculated.

$$Chl.total = [(20.2 \times A_{645}) + (8.02 \times A_{663})] \times V / 1000 \times W \tag{2}$$

Where (A_{480}), (A_{663}), (A_{645}) represent absorbance values read in 480, 663, 645 nm wavelengths, respectively.

2.4. Measurement of H2O2 and MDA

The concentration of H_2O_2 was measured. So that 0.35 g of leaves was homogenized with 5 ml 0.1% (w/v) trichloroacetic acid (TCA) in an ice bath. The homogenate was centrifuged at 12,000×g during 15 min and 0.5 ml of supernatant was transferred to a new tube and mixed 0.5 ml 10 mM phosphate buffer (pH 7.0), and 1 ml 1 M

KI. The assay mixture was kept in dark for 1 h and absorbance was read at 390 nm. The concentration of H_2O_2 was determined from a standard curve and expressed as µmol H_2O_2 g^{-1} fresh weight. By measuring the concentration of malondialdehyde (MDA), Lipid peroxidation in leave was detected. So that, 0.2 g of fresh leaves, powdered with 5 ml 0.1 % trichloroacetic acid (TCA). The solution was centrifuged at 8,000 ×g for 5 min, 1 ml of supernatant transferred to a new tube then added 4.5 ml 20% TCA. The solution was centrifuged at 4,000×g for 10 min, the absorbance was read at 532 nm. The extinction coefficient of 155mM^{-1} cm^{-1} was used for determine of MDA concentration.

2.5. Protein content and antioxidant enzyme activity

Before extracting the proteins content, fresh leaf samples were taken randomly from different seedling heights and cleaned with deionized water to remove any surface contamination. The extraction of total soluble protein was performed for using Bovine Serum Albumin V as a standard. Leaf samples were ground to a fine powder and 50 nM phosphate buffer (pH 7.0) used for extraction. The solutions were centrifuged at 4 °C during 30 min at 20000×g and after that supernatant was collected and used for protein content assay and enzyme activities. The activity of catalase enzyme was measured using Aebi (1984). The reaction mixture contained 100 µL crude extracts, 50 µL 30% H_2O_2, and 3000 µL 50 mM potassium phosphate buffer. The reduction in the absorbance was noted at 240 nm during 1 min by a spectrophotometer apparatus. Catalase activity of the extract was expressed as ΔOD mg^{-1} protein min-1. The activity of superoxide dismutase was measured by calculating the ability of the enzyme extract to inhibit the reduction of nitroblue tetrazolium compound using method of (Dhindsa et al., 1981). The reaction buffer containing 100 mM phosphate buffer (pH 8.7), 12 mM Methionine, 75 mM nitroblue tetrazolium, 100 µM EDTA, and 0-100 ml of enzyme extract was added 2 mM riboflavin. The reaction was stayed for 15 min, then absorbance read at 560 nm. The amount of superoxide dismutase which caused 50% inhibition of photochemical reduction of nitroblue tetrazolium considered as one unit of this enzyme. The activity of superoxide dismutase was defined as ΔOD mg^{-1} protein min^{-1}.

2.6. Experimental design

The effects of mycorrhizal fungi (*Rhizophagus intraradices* and *Funneliformis mosseae*) and water deficit (optimal irrigation (75, 50, and 25% FC) evaluated on Caucasian Hackberry seedlings as factorial according to a completely randomized design (CRD) with ten replications.

2.7. Statistical analysis

Data were analyzed using two-way ANOVA (SAS 9.2). AMF and irrigation were used as first and second factor, respectively. In order to determine the significance of means, Duncan's multiple-range test (DMRT) was performed using SAS 9.2 software at P≤ 0.05. Correlation coefficient was calculated by SPSS v.22 to determine the relationship between studied traits. A principal component analysis (PCA) was conducted to assess the distribution of water and AMF treatments across a biplot figure by Past software. As well as, cluster analysis was performed using d3heatmap, dendextend, and gplots packages in R according to the Ward method to classify traits and applied treatments.

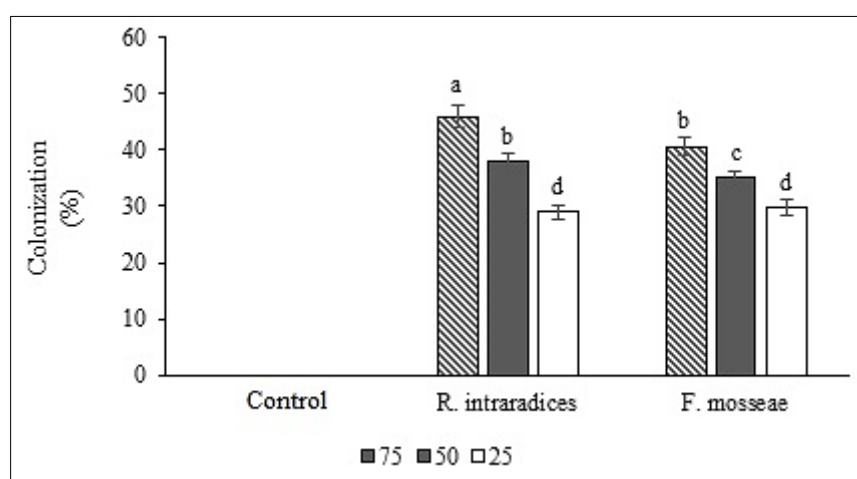## 3. Results and discussion

3.1. Mycorrhizal colonization

The morphological and biochemical parameters of Caucasian Hackberry seedlings were greatly influenced by AMF inoculation and drought stress (Table 1). Although, the effect of AMF was not significant on the root length of inoculated seedling, dry root weight, and secondary root number influenced by AMF considerably. Non-inoculated Caucasian Hackberry seedlings did not show any colonization of AMF in roots. Colonization rate in inoculated roots decreased significantly by drought stress (Fig. 1). The rate of colonization in *R. intraradices*-inoculated was more than *F. mosseae*-inoculated seedlings at all three irrigation levels. Fig. 2 presents the development of AMF in Caucasian roots.
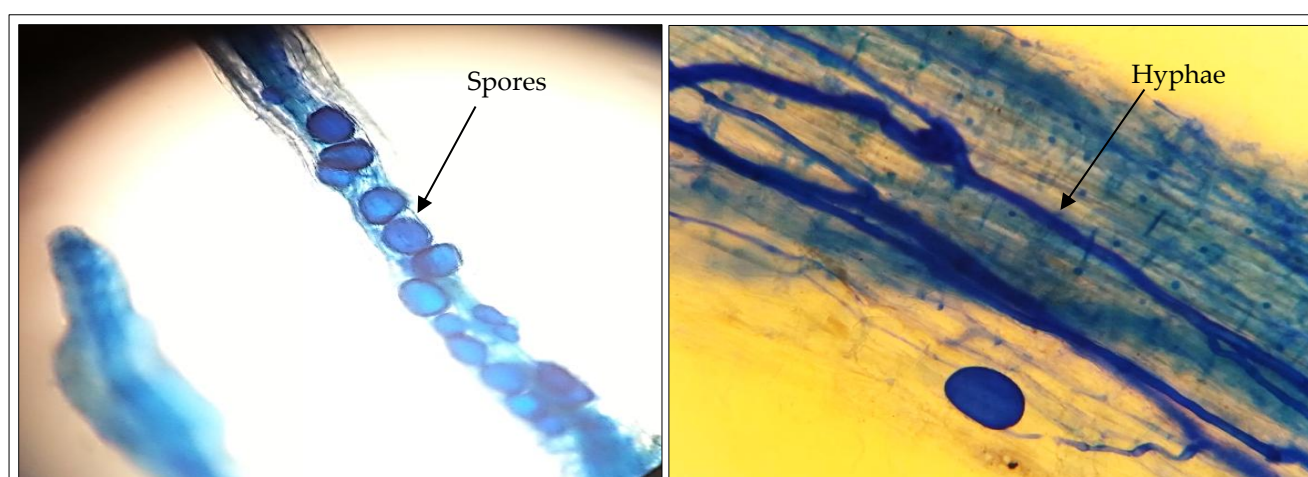
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Table 1. Effect of AMF and drought stress on plant growth, H_2O_2, MDA and antioxidant enzymes.

Traits	Drought	AMF	Drought × AMF	CV
Colonization	0.011 *	0.122 **	0.045 *	16.53
Shoot dry weight	12.89 **	34.19 **	15.62 *	17.61
Leaf area	2.01 *	97.42 **	1.06 *	14.12
Height	8.56 **	1418.34 **	45.42 **	15.25
Root dry weight	0.65 *	12.81 *	0.38 *	21.99
Length of root	3.23 *	14.23 ns	138.2 *	18.51
Number of secondary root	52.48 ns	77.37 **	55.92 **	14.65
Chlorophyll content	1.09 *	0.046 *	0.042 ns	19.32
H_2O_2	1.32 **	2.14 *	3.03 *	6.7
MDA	3.78 **	4.8 *	9.54 **	10.01
CAT	0.054 **	0.079 **	0.067 *	14.43
SOD	0.38 **	0.076 *	0.064 *	16.53

ANOVA: ns not significant. *, ** Significant at P≤ 0.05 and P≤ 0.01 respectively.

Fig. 1. The root colonization rate of Caucasian Hackberry seedlings treated to three different irrigation levels.

Fig. 2. Symptoms of the establishment symbiotic relationship between mycorrhizal fungi and the rootstock of seedlings.

3.2. Plant growth parameters

Data presented in Table 2 shown the effect of AMF on the morphological and physiological parameters of Caucasian Hackberrys seedling under three levels of irrigation at 75, 50, and 25% FC which showed a significant increase for all traits include shoot dry weight, leaf area, seedling height, root dry weight, length of

root, number of secondary roots and total chlorophyll content, comparing with non-inoculated seedling in normal irrigation or drought treatment. The highest values in almost all growth parameters were observed in inoculated seedling with *R. intraradices* and *F. mosseae* under 75% FC. However, these traits showed a significant reduction with increasing drought stress, interestingly the rate of depletion in inoculated seedlings was lower than non-inoculated ones. AMF-inoculated seedlings maintained their leaf area and leaf number during raising water deficit, which indicated the positive effect of these fungi. As well as, there was a considerable difference between control and inoculated plants in term of the dry weight of root. Generally, shoot and root morphological traits in AMF-inoculated seedlings were higher than control plants (Fig. 3).

Even though water limited condition results in a steady reduction in chlorophyll content of all seedlings, the leaf of inoculated seedlings was contained more chlorophyll pigments in comparison to control plants. However, AMF-inoculated seedling remained green under drought stress condition according to the morphological investigation (Fig. 3) and the result of chlorophyll measurement (Fig. 4).

Table 2. Effect of drought and AMF on growth, leaf area and chlorophyll content Caucasian Hackberrys seedling. Mean pairs followed by different letters are significantly different (P< 0.05) by Duncan's test.

Drought	AMF	Shoot DW (g/plant)	Leaf area (cm²/plant)	Leaf number	Height (cm)	Root DW (g/plant)	Length of root (cm)	Number of secondary root
	Control	7.54±0.51 ab	5±0.84 d	11±3.61 c	20.6±1.34 c	1.36±0.11 d	42.33±1.2 ab	30±0.67 c
75% FC	*R. intraradices*	9.14±0.69 a	10.9±0.42 ab	17.33±4.5 ab	43.5±1.2 ab	3.74±0.018 a	45±1.07 ab	39±1.2 a
	F. mosseae	8.95±0.78 a	11.3±0.74 a	18±1.73 a	45.66±1.83 a	2.81±0.29 b	51.66±0.51 a	34.67±1.17 b
	Control	5.12±0.74 b	4.7±0.26 e	10.33±4.16 c	20±1.07 c	0.85±0.087 e	48.33±1.17ab	26±0.33 d
50% FC	*R. intraradices*	7.23±0.49 ab	11±0.5 a	16±2 b	38.17±1.2 b	3.59±0.29 a	48.67±1.83 ab	35±1.76 b
	F.mosseae	7.69±0.94 ab	10.3±0.19 b	17.67±2.88 a	38.17±1.38 b	3.25±0.27 ab	36.27±1.74 c	31.67±1.01 c
	Control	2.35±0.28 d	6.7±0.55 c	10±4.35 c	15.6±1.2 d	1.16±0.19	40.17±1.39 b	29.33±1.21 c
25% FC	*R. intraradices*	5.36±0.86 c	11.2±0.54 a	16±2 b	38.17±1.56 b	3.04±0.16 ab	43.8±2.08 ab	25.67±0.69 d
	F. mosseae	5.65±0.73 c	11±0.32 a	17±2.65 ab	38.17±1.64 b	2.21±0.49 c	49.33±1.37 ab	35±1.78 b

Shoot DW: shoot dry weight, Root DW: root dry weight. Values are means (n= 3–6). Within each parameter data followed by the same letter indicate that values are similar (P≤ 0.05).

Fig. 3. A comparison between inoculated and non-inoculated seedlings in term of growth parameters and root density.
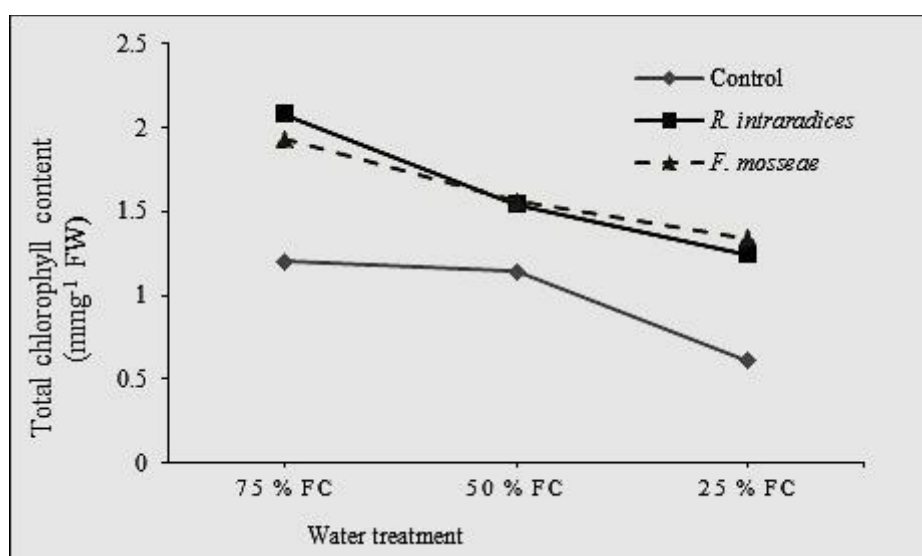
Fig. 4. Effect of water treatments on chlorophyll content of AMF-inoculated seedlings.

3.3. H2O2 and MDA content

H_2O_2 and MDA content in the leaves was lower in mycorrhizal than in non-mycorrhizal seedlings at all drought treatments (Fig. 5A). Indeed, the difference of H_2O_2 concentration between mycorrhizal and non-mycorrhizal plants was significant at 50 and 25% FC. The difference of MDA content between mycorrhizal and non-mycorrhizal plants was significant at 75 and 25% FC. As the drought stress was increased, the H_2O_2 and MDA content in the leaves of both AMF-inoculated and non-inoculated plants increased (Fig. 5B).
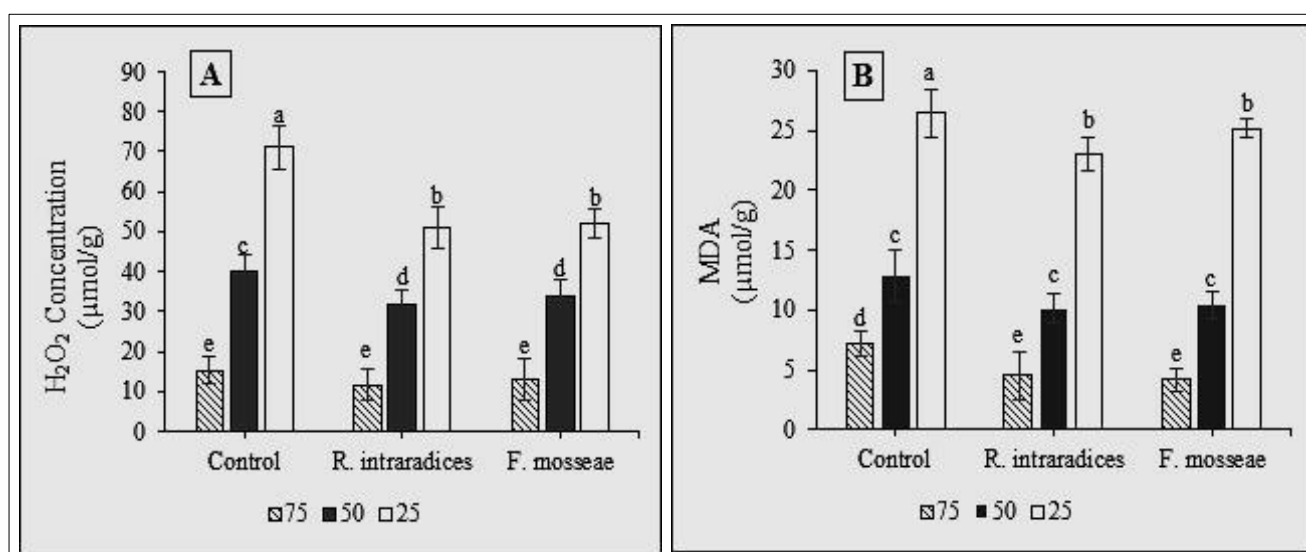

Fig. 5. The concentration of H_2O_2 (A) and MDA (B) in the leaves of Caucasian Hackberry's seedling inoculated with *R. intraradices* and *F. mosseae* under three water treatments. Mean pairs followed by different letters are significantly different (P< 0.05) by Duncan's test.

3.4. Antioxidant enzymes activity

Drought stress at 50% FC increased the activity of CAT and SOD in mycorrhizal and non-mycorrhizal plants (Figs. 6A and 6B) then a significant decrease in the activity of both antioxidant enzymes was recorded at the highest level of drought in non-mycorrhizal plants. Meanwhile, the activity of both enzymes in *Rhizophagus intraradices* and *Funneliformis mosseae* inoculated seedling was greater than control seedlings, significantly.
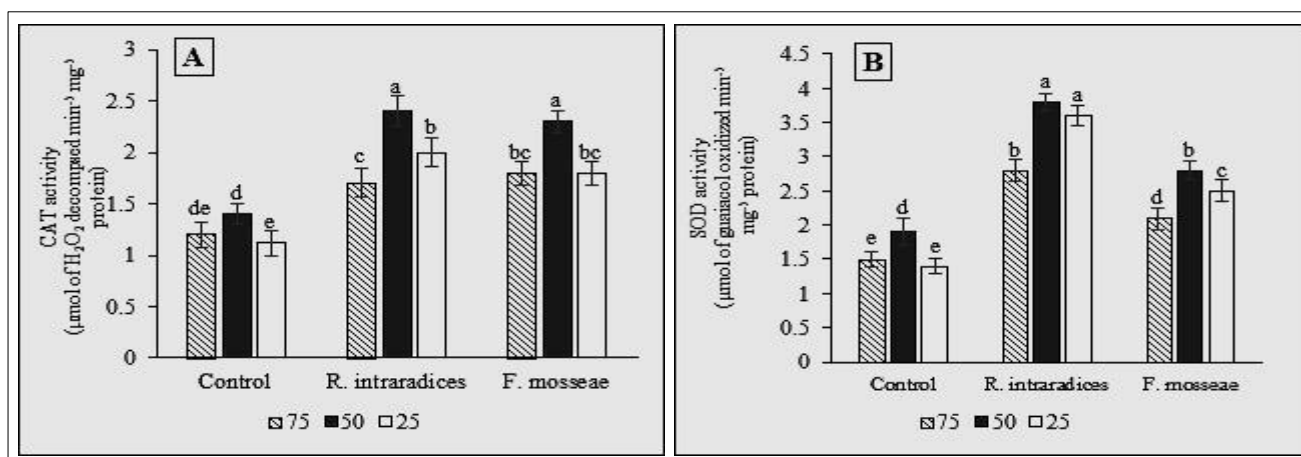
Fig. 6. The activity of antioxidant enzymes catalase (A) and superoxide dismutase (B) of Caucasian Hackberry's inoculated with *R. intraradices* and *F. mosseae* under three water treatments. Mean pairs followed by different letters are significantly different (P< 0.05) by Duncan's test.

3.5. Correlation

We observed a positive correlation between colonization and plant growth (Table 3). Correlation between H_2O_2, MDA and plant growth as well as, CAT and SOD was negative. Data showed increasing colonization rate cause decline in lipid peroxidation and generation of H_2O_2, also increase antioxidant enzymes. The highest correlation of colonization was observed with CAT activity (*r*=0.87**). Production of H_2O_2 impacted shoot and root dry weight negatively (r=-0.49*, and -0.26, respectively), while increasing in catalase and superoxide dismutase was associated with well growth condition of seedlings.

3.6. Principal component analysis (PCA)

An analysis of PCA conducted to investigate morphological and physiological traits distribution (Fig. 7). Biplot attained using two main components (PC1-2), where 78.37% of total variance explained by the first component. As well as, the second major component justified 17.38% of the total variation. The first component was correlated to colonization, height, leaf number, secondary root number, shoot and root dry weight. These results suggest that the high rate of root colonization resulted in longer seedlings, and more biomass for up and underground organs. Close relation between AMF-inoculated treatments and first component indicated the importance role of colonization in the improvement of growth parameters of Caucasian Hackberry. In contrast, a significant negative correlation observed between H_2O_2 concentration, MDA content and colonization rate. As well as, there were a positive correlation between chlorophyll content and activity of antioxidant enzymes. However, non-inoculated plants (control) had lower values for PC1 and PC2, which indicated a significant difference with colonized seedlings.
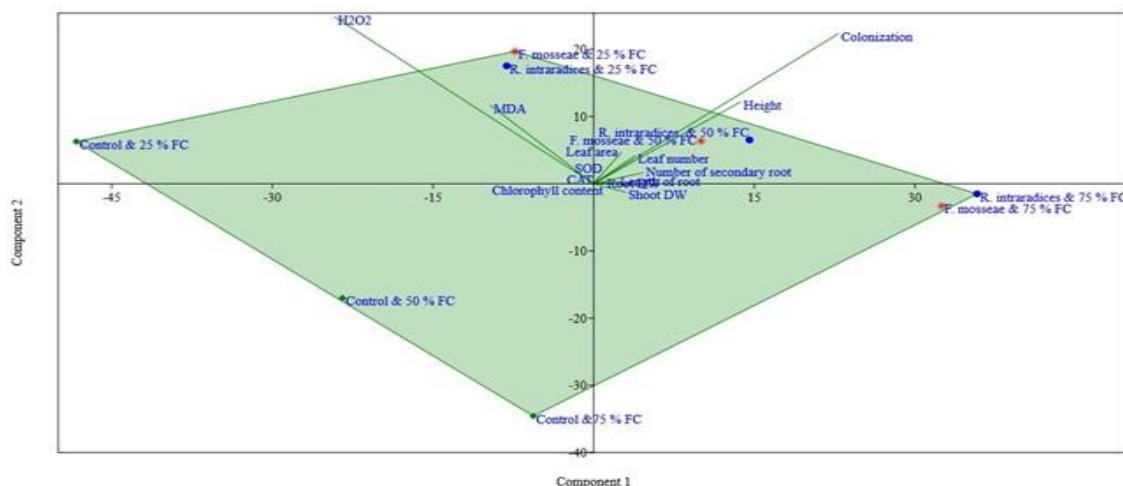

Fig. 7. Principal component analysis for morphological and physiological parameters of Caucasian Hackberry exposed to different water treatments.

Table 3. Perrson correlation between plant growth, H_2O_2, MDA and antioxidant enzymes.
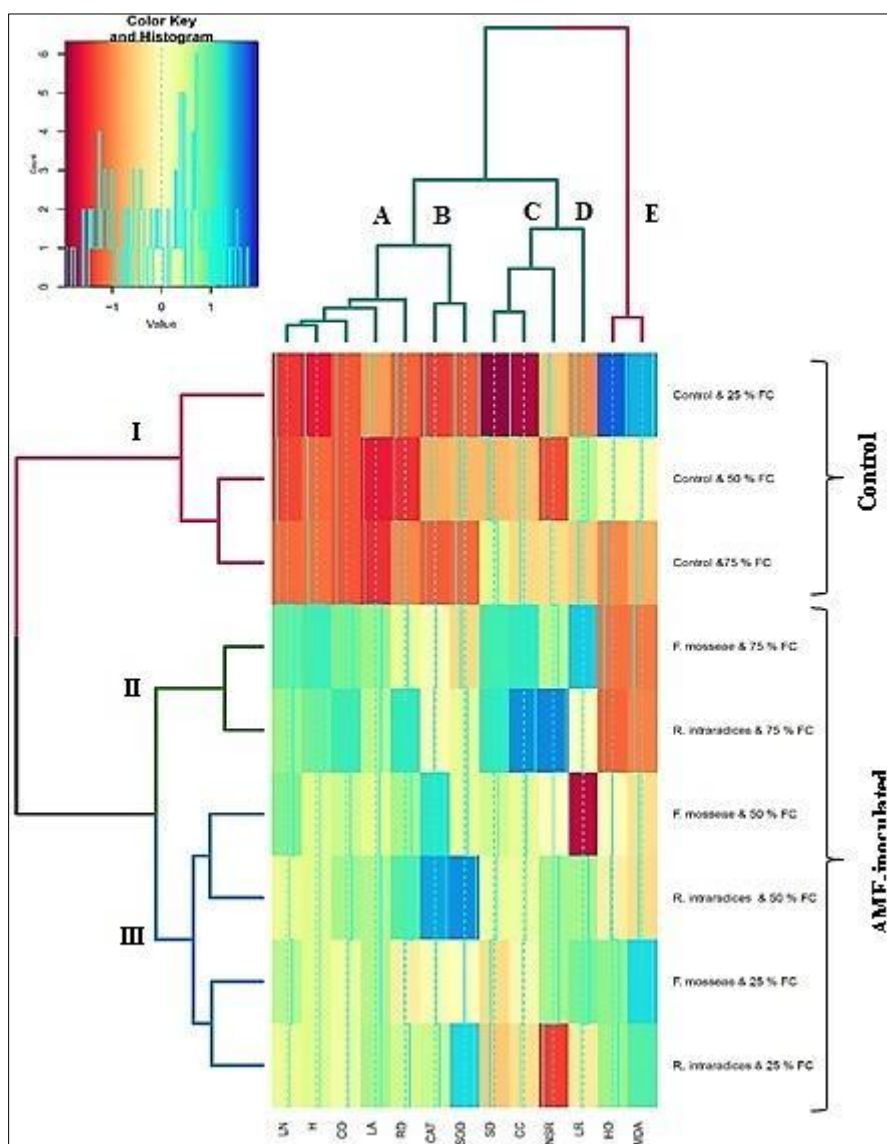
	Colonization	shoot DW	Leaf area	Height	root DW	Length of root	secondary root number	Chlorophyll content	H_2O_2	MDA	CAT	SOD
Colonization	1	0.788**	0.804**	0.769**	0.771**	0.204	0.558**	0.641**	-0.486*	-0.46**	0.87**	0.76**
Shoot DW		1	0.680**	0.837**	0.913**	0.050	0.642**	0.684**	-0.491*	-0.483*	0.764**	0.687**
Leaf area			1	0.748**	0.571**	0.041	0.33*	0.76**	-0.52*	-0.567*	0.672**	0.743**
Height				1	0.755**	0.08	0.645**	0.29	-0.33*	-0.342*	0.862**	0.595**
Root DW					1	0.145	0.649**	0.35*	-0.262	-0.239	0.749**	0.852**
Length of root						1	0.385*	0.36*	-0.238	-0.194	0.542*	0.675**
Secondary root number							1	0.29	-0.346*	-0.367*	0.435*	0.593**
Chlorophyll content								1	-0.372*	-0.341*	0.568**	0.54*
H_2O_2									1	0.97**	-0.786**	-0.689**
MDA										1	-0.715**	0.648**
CAT											1	0.953**
SOD												1

*, ** Significant at P≤ 0.05 and P≤ 0.01 respectively.

3.7. Cluster analysis

We applied a hierarchical cluster analysis to get the heatmap plot of growth and physiological parameters. Result demonstrated that all studied traits grouped into five clusters (Fig. 8). Growth parameters leaf number, height, colonization rate, leaf area and root dry weight clustered as a distinguished group (group A). Antioxidant enzymes CAT and SOD were classified as group B, shoot dry weight, chlorophyll content and number of secondary roots as group C. However, expectedly MDA, and H_2O_2 as indicators of drought stress damage were clustered into group E. Despite a positive correlation between length of roots and number of secondary root, they separated as different groups.

However, AMF and irrigation treatments classified into three different groups. Interestingly, non-inoculated seedlings clustered as group I, with a high correlation with MDA and H_2O_2. Whereas, inoculated seedlings by *R. intraradices* and *F. mosseae* exposed to 75% FC were classified as cluster II, and those treated with 25% FC distinguished as cluster III. As it clears from the color key of gained heatmap plot, AMF colonization reduced the negative effect of H_2O_2, by improving the plant ability to extend leaf area, leaf number, and root system to better water absorption (Fig. 8).

Fig. 8. Heatmap attained from cluster analysis for growth and physiological parameters of Caucasian Hackberry seedlings exposed to different water treatments. LN: leaf number, H: height, Co: colonization rate, LA: leaf area, RD: root dry weight, CAT: catalase, SOD: superoxide dismutase, SD: shoot dry weight, CC: chlorophyll content, NSR: number of secondary roots, LR: length of root, HO: H_2O_2, MDA: Malondialdehyde.

3.8. Biomass production and mycorrhizal colonization

Utilizing AMF symbiosis to improve plants growth has been raised among researchers during recent years (Huang et al., 2011; Cartmill et al., 2012; Mo et al., 2016). However, there are some studies which proved the positive effect of AMF on respiration, and growth parameters of tree seedling under severe environmental conditions (Fahey et al., 2016; Bachelot et al., 2017). In the current study, colonization rate declined with increasing drought stress level, indicating that drought suppressed the growth of AMF. It has been reported that increasing of water deficit inhibits hyphal growth with a subsequent decrease in the spread of mycorrhizal colonization (Lehto and Zwiazek, 2011; Mechri et al., 2014). The data reported in the present study clearly indicated that *R. intraradices* and *F. mosseae* inoculated seedling performed superior under different irrigation levels, in comparison to non-inoculated seedlings. Inoculated seedling exhibited higher biomass production and greater chlorophylls pigment than non-inoculated seedlings. Enhancing plant growth parameters in mycorrhizal plants has been stated by several researchers (Kohler et al., 2008; Doubková et al., 2013; Pedranzani et al., 2016). Liu et al., (2015) suggested that mycorrhizal inoculated seedlings of hybrid poplar had a greater rate of photosynthetic compare to non-mycorrhizal plants (Liu et al., 2015).

The studies pointed out, mycorrhizal fungi have an important contribution toward accelerated acquisition of mineral nutrients such as P, Zn, and Cu in the host plant due to extraradical hyphae extend of plant rhizosphere to absorb and transport the nutrient elements to intracellular arbuscules in colonized cortical cells (Smith et al., 2011; Abbaspour et al., 2012). Therefore, nutrient deficiency under drought stress can improve by AMF inoculation that in turn leads to the better growth of the seedlings (Porras-Soriano et al., 2009; Hidri et al., 2016; Liu et al., 2017). Chlorophyll content was significantly reduced by drought treatments. Chlorophyll reduction is due to the suppression of specific enzymes that are responsible for the synthesis of photosynthetic pigments, as well as, reduction in the uptake of minerals (e.g. Mg and Fe) needed for chlorophyll biosynthesis also reduces the chlorophyll content in the leaf (Reddy et al., 2004; Ruiz-Lozano et al., 2012). As it clears from Table 2 chlorophyll content developed in mycorrhizal plants under all three irrigation levels. Higher chlorophyll content in leaves of mycorrhizal inoculated plants under drought stress has been reported by various authors (Sheng et al., 2008; Gong et al., 2013; Armada et al., 2015). Mycorrhizal fungi diminishes the plant's moisture obstacles and increase the photosynthetic pigmentation by enhancing the concentration of phosphorus and controlling stomata and supplying water with its hyphae network, which followed by increased photosynthesis and plant growth (Liu et al., 2015; Ouledali et al., 2018).

3.9. Oxidative damage

The production of ROS induced during water deficit condition, which followed by plant reaction employing an antioxidant and non-antioxidant defense system. Even though a molecular like H_2O_2 has a signaling role in the cell, extreme H_2O_2 concentration leads to an oxidative damage besides other reactive oxygen species (Noctor et al., 2014; Zou et al., 2015). Therefore, plants need to maintain ROS balance during environmental stresses in order to limit further more destruction. Although elevated H_2O_2 and MDA contents were observed in both mycorrhizal and non-mycorrhizal plants under drought stress conditions, this increase in mycorrhizal inoculated seedling was less than non-mycorrhizal seedlings. Previous studies also indicated that AMF cause reclines H_2O_2 and MDA in host plants under various stress (Yang et al., 2015; Pedranzani et al., 2016). The antioxidant capacity of the host plant can be activated by AMF symbiosis (Latef and Chaoxing, 2011).

3.10. Antioxidant enzymes activity

The activity of antioxidant enzymes CAT and SOD increased under drought stress in the Caucasian Hackberry's seedlings. In the current study, the activity of antioxidant defense enzymes was largely increased by AMF in the inoculated seedlings. Greater activity of antioxidant enzymes in mycorrhizal seedlings was associated with lower lipid peroxidation and H_2O_2 generation, indicating lower oxidative damage in the colonized seedlings. Previous research has also reported increases in SOD, CAT, and other antioxidant enzymes

activity in AMF inoculated plants exposed to drought stress (Huang et al., 2013; Pedranzani et al., 2016). Catalase and superoxide dismutase are both metalloenzymes which their activity highly influenced by available co-factors like Fe, Mn, and Cu (Alscher et al., 2002). As result of drought stress, the expression of antioxidant enzymes respective genes increases, while the availability of metallic co-factors is critical to correct activity (Bian and Jiang, 2009; Sharma et al., 2017). The extension of mycorrhizal hypha and secondary roots facilitate micronutrients transportation to plants, thus AMF-inoculated plants which exposed to long-term drought stress contain more active antioxidant enzymes in respect to non-mycorrhizal plants.

3.11. Multivariate analysis

Principal component analysis and correlation coefficient indicated the important role of colonization in growth improvement. There was a positive significant association between the rate of colonization and growth parameters like shoot height, root length, shoot and root dry weight. Although there are many studies indicated a positive effect of mycorrhizal fungi on plants (Liu et al., 2015; Sharma et al., 2017; Chaturvedi et al., 2018), the main involved molecular factors are known yet. However, according to cluster analysis AMF-inoculated seedlings were classified as the same group based on water treatments. As Fig. 8 shows non-mycorrhizal plants was heavily affected by drought stress and contained more H_2O_2, high lip peroxidation, along with lower growth. While H_2O_2 and MDA clustered as same group, morphological parameters of shoot and root showed more similarity and formed the other four groups. Nevertheless, cluster I and II demonstrated well growth condition and lower oxidative damage under drought stress condition. Although inoculated seedlings with *R. intraradices* and *F. mosseae* yielded a better performance under well-watered and drought stress condition than non-mycorrhizal plants, *R. intraradices* inoculated plants showed more capability to tolerate drought in term of better growth and antioxidant defense system.

4. Conclusion

As the drought season with less in annual average over recent years has risen in Zagros region and the forestlands have displayed sensitivity against this long period by showing oak decline and other significant trees. Therefore, select an intelligence approach for rehabilitate the Zagros ecosystem is the wisely way to save this valuable forest. Fortunately, mycorrhizal symbiosis is a ubiquitous plant–microbe interaction, plays an important role in nutrient cycling that helps alleviate the deleterious effects of drought conditions by promoting plant performance and seedling establishment. Also AM fungi is capable of diminishing the damages caused by drought stress and other environmental stresses on Caucasian Hackberry seedlings by increasing plant growth, reducing membrane lipid peroxidation and increasing antioxidant enzyme activity. AMF-symbiosis strategy leads to a better establishment of young and sensitive seedlings under severe environmental conditions which is very important in the future reforestation program particularly in semi-arid and arid areas. Consequently, this plant–fungus interaction has a great potential in an environmentally-friendly sustainable forestry

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How to cite this paper:
Sepahvand, T., Etemad, V., Matinizadeh, M., Shirvany, A., 2021. Symbiosis of AMF with growth modulation and antioxidant capacity of Caucasian Hackberry (*Celtis Caucasica* L.) seedlings under drought stress. *Cent. Asian J. Environ. Sci. Technol. Innov., **1**, 20-35.