Iran Agricultural Research (2016) **35(2)** 19-26

Shiraz University

The effects of arbuscular mycorrhizal fungus and water stress on some antioxidant enzymes activities and nutrients uptake of two citrus rootstocks

M. Zarei*, Z. Paymaneh, A. Ronaghi

Department of Soil Science, College of Agriculture, Shiraz University, Shiraz, I. R. Iran

* Corresponding Author: Mehdizarei@shirazu.ac.ir

ARTICLE INFO

***Article history*:**
Received **20 July 2014**
Accepted **9 December 2014**
Available online **28 June 2016**

***Keywords*:**
Citrus
Nutrients uptake
Antioxidant enzymes
Water stress
Glomus mosseae.

ABSTRACT- Water stress is the main cause for crop yield reduction in the majority of agricultural regions of the world because it affects almost all plant functions. The effects of *Glomus mosseae* on growth, nutrients uptake, and antioxidant enzymes of sour orange (*Citrus aurantium*) and rough lemon (*Citrus jambheri*) rootstocks were assessed in sterilized soil under greenhouse conditions. A three-factor experiment was set up in a completely randomized design with three replicates of each treatment. Treatments consisted of water stress at four levels (irrigation intervals of 2, 4, 6, and 8 days) and mycorrhizal treatments at two levels (inoculation with *G. mosseae* and non-mycorrhizal control). Mycorrhizal seedlings of two citrus rootstocks were successfully infected by *G. mosseae*. As water stress increased, root colonization, shoot dry weight, shoot N, P, Mn, Cu, and Fe uptake of two citrus rootstocks significantly decreased but shoot Zn uptake and the antioxidant enzymes [superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (G-POD) and ascorbate peroxidase (APX)] activities of two citrus rootstocks leaves increased. With inoculation of seedlings by *G. mosseae* compared with control, shoot dry weight, N and P uptake, and antioxidant enzymes activities increased. It may be concluded that mycorrhizal inoculation notably influenced shoot nutrients uptake and leaves antioxidant enzymes activities in citrus and an increase in these parameters alleviated water stress.

INTRODUCTION

In Iran, water stress is one of the important environmental factors that limits the distribution and productivity of major crops because more than 82 % of Iran's regions are located in the arid and semi- arid zones with average rainfall about 250 mm, which is less than 1/3 of the average rainfall in the world (860 mm) (Amiri et al., 2010). Water stress is caused by reducing the availability of external water, which limits the ability of the plant's roots to take up nutrients and induces cellular and photo-oxidative damages, through the increased accumulations of reactive oxygen species (ROS) such as superoxide, hydrogen peroxide and hydroxyl radicles (Wu et al., 2007). The photosynthetic electron transport system is the major source of active oxygen in plant tissues, having potential to generate singlet oxygen, O_2 and superoxide, O_2^-. The production of active oxygen is an unavoidable consequence of the operation of the photosynthetic electron transport chain in an oxygen atmosphere (Arora et al., 2001). Citrus is one of the largest and most important groups of fruits of tropical and subtropical regions (Wutscher, 1989). Citrus plants are cultivated on an area of 235000 hectares with annual production of 3712000 tons in the North and South of Iran. Citrus has number one position in Iran and is grown on an area of 290 thousand hectares (FAO, 2011). Most citrus species such as sour orange and rough lemon have rare and short root hairs and are moderately dependent on mycorrhizal symbiosis especially most of *Glomus* species (Wu and Xia, 2006). The vegetative stage of citrus plants is more sensitive to drought stress (Syvertsen, 1985). Mycorrhizal symbiosis involves a complex interaction among plant, soil and mycorrhizal fungi. Arbuscular mycorrhizal (AM) associations' relationships are rather important in horticultural crops because they are believed to increase nutrients uptake, improve plant fitness, and plant water relations and thus increase the drought resistance of host plants (Augé, 2001). AM symbiosis improves water relations of plants in part due to increases in antioxidant enzymes (Wu et al., 2007). Wu et al. (2007) studied the efficacy of five *Glomus* species, *Glomus mosseae*, *G. geosporum*, *G. versiforme*, *G. etunicatum* and *G. diaphanum* for the ability to improve water relations and antioxidant enzymes activities of leaves. The more efficient fungus in *C. tangerine* was *G. mosseae* or *G. geosporum* and the least was *G. etunicatum* under both

well water and water stress conditions (Wu et al., 2007). This study is to evaluate the effects of *Glomus mosseae* and water stress on nutrients uptake and activities of some antioxidant enzymes in sour orange (*C. aurantium L.*) and rough lemon (*Citrus jambhiri*) in a calcareous soil.

MATERIALS AND METHODS

Fresh fruits of sour orange (*C. aurantium* L.) and Rough lemon (*Citrus jambhiri*) were provided from Narenjestan Museum of Shiraz and Agricultural Research Center of Darab, Fars Province, Iran, respectively. Fresh fruits were washed with water, surface sterilized with 5% hypochlorite sodium and then dried. Seeds were taken out and sterilized by immersing in 70% alcohol for 5 min, rinsed four times with sterile distilled water, and planted into plastic pots containing sterilized mixture of field soil, humus and sand (1:1:1, v/v/v). After 90 days, the same size seedlings were transferred to plastic pots containing sterilized soil. The fungus was prepared from Iranian soils, because they are abundant in these soils (Aliasgharzadeh et al., 2001, Kariman et al., 2005; Zarei et al., 2008).*Glomus mossae* inoculum was prepared through the trap culture of maize (*Zea mays* L.). Trap culture medium was composed of autoclaved soil/quartz-sand (<1 mm) (1: 4, v/v). Simultaneously, some pots were kept without any spore inoculation for preserving the naturally-occurring microbial associations and used for control treatments. After 4.5 months, at the beginning of the reproductive period, shoots were removed and the contents of pots (mycorrhizal roots plus soil possessing fungal spores and mycelia) were maintained in polyethylene bags at 4°C. For extraction and counting of spores, and evaluation of root colonization, inoculants potential (spore numbers of 10 spores g^{-1} substrate and root colonization of 75%) was measured based on the described methods (Zarei et al., 2008). For determining water stress levels, moisture content of pots containing 3 kg of the used soil was kept at field capacity (FC) (FC and permanent wilting point were 23% and 9.8%, respectively based on method of Klute (1986)) and total weight was recorded. Pots were weighed daily at the specified time for 15 days. Daily moisture reduction was recorded by Sepaskhah and Yarami equation (2009). Then soil moisture retention curve was plotted by moisture contents which was obtained during 15 days (Moisture content at the vertical axis and time at the horizontal axis were shown). Afterwards, by using this curve, four irrigation intervals or soil drying intervals of 2, 4, 6, and 8 days were determined. Soil sample wascollected from depth of 0-30 cm (fine mixed mesic calcixerollic Xerochrept) in Bajgah Agricultural Station of Shiraz University, Shiraz, Iran. The soil sample was air-dried, passed through a 2 mm sieve, and mixed uniformly. Some physical and chemical properties of the studied soil were measured using standard methods (Olsen et al., 1954; Chapman, 1965; Lindsay et al., 1978; Nelson and Sommers, 1996; Rhoades, 1996; Thomas, 1996) and are presented in Table 1. The experiment was a completely randomized design with three replications. Treatments consisted of four water stress levels (2, 4, 6, and 8 days irrigation intervals equivalent to 100%, 75%, 50% and 25% FC, respectively), mycorrhizal treatments at [*G. mosseae* and non-mycorrhizal treatment (NM) (control)] and two rootstock citrus [sour orange (*C. aurantium L.*) and rough lemon (*Citrus jambhiri*)]. Each pot was filled with 5 kg of sterilized soil (autoclaved for 1 h at 121°C under 1.1atm of pressure) (Al-Khaliel, 2010). Nitrogen (100 mg kg^{-1} of soil), Fe, Zn, Cu, and Mn (5 mg kg^{-1} of soil) were applied to all pots as urea (46%), $FeSO_4.7H_2O$, $ZnSO_4.7H_2O$, $CuSO_4.5H_2O$, and $MnSO_4.H_2O$, respectively (Koo, 1980). In mycorrhizal treatments, 70 g of fungus inoculum was spread as a thin layer below the soil surface before planting. In control (NM treatments), 70g of pots that were kept in the trap culture were added. Two seedlings were transferred to each pot in the main culture. Water treatments were applied after one month of sowing. Pots were placed in greenhouse conditions at 15-28˚C, with a 16/8 h light/dark periods.

After 6 months, all plants were harvested. Shoots were separated, oven-dried at 65°C for 72 h, and weighed. The percentage of root colonization was determined using the grid-line intersect method, after clear washing the roots in 10% KOH and staining with 0.01% acid fuchsin in lactoglycerol (Kormanik and McGraw, 1982). Shoots samples were ashed at 500-600 ^oC and the residues were dissolved in 2 M HCl. The estimation of Fe, Zn, Mn and Cu was carried out in the dry-ashed sample solution byatomic absorption spectrophotometer (Shimadzu AA-670, Kyoto, Japan) and P concentration was measured (Cottenie, 1980). Shoot N was determined by the Kjeldahl method (Bremner, 1965).

Table 1. Some physical and chemical properties of the studied soi

Properties		Characteristics	
Sand (%)	47.1	$NaHCO_3$- extractable P (mg kg^{-1})	9
Silt (%)	28	DTPA-extractable Fe (mg kg^{-1})	2.7
Clay (%)	24.9	DTPA-extractable Cu (mg kg^{-1})	1.5
pH (1:1)	7.9	DTPA-extractable Mn (mg kg^{-1})	4.3
EC(dS m^{-1})	0.3	DTPA-extractable Zn (mg kg^{-1})	1
Organic Matter (%)	0.9		
CEC ($cmol^+ kg^{-1}$)	24		

EC: Soil electrical conductivity, CEC: Cation exchange capacity.

Fresh leaves samples were separated and kept at -80 ^0C, then homogenized in 2 mL of phosphate buffer (pH= 8), centrifuged at 13000×*g* for 15 min at 4°C, and the supernatant was used for superoxide dismutase (SOD), catalase (CAT), guaiacol peroxidase (G-POD)

and ascorbate peroxidase (APX) activities. The SOD activity was determined and expressed as Unit g^{-1} fresh weight (fwt.). One SOD unit was defined as the amount of enzyme that inhibited 50% nitroblue tetrazolium by light (10). The CAT activity was assayed by measuring the rate of disappearance of H_2O_2 (17). The G-POD activity was determined by Chance and Maehly method (14). The APX activity was measured by a decrease in absorbance at 290 nm for 1 min (Nakano and Asada, 1981). Data were analyzed with SAS 9.1 statistical software. Means were compared by least significant difference (LSD) test.

RESULTS AND DISCUSSION

There was no mycorrhizal colonization in roots of non-mycorrhizal (NM) control seedlings at all levels of water stress but inoculated seedlings were successfully infected by *G. mosseae* in two citrus rootstocks (Fig. 1A). Root colonization notably decreased with increased levels of water stress. Root colonization of seedlings by *G. mosseae* was 39.36- 76.14% and 46.55-86.48% in the sour orange and rough lemon, respectively (Fig.1A). Root colonization of rough lemon was significantly higher than sour orange in well water conditions (irrigation interval of 2 days), whereas there was no significant difference between them and other treatments. Tommerup (Tommerup, 1984) showed that the rate of spore hydration and hyphal growth (two phases of spore germination) decreased when the matric potential decreased. The hydration and hyphal growth required higher water for growth (Tommerup, 1984). Abiotic stresses can change the quantity and quality of host root exudates and soil extracts (Daniels et al., 1980; Sangtarash et al., 2009). Host root exudates and specifically, flavonoid compounds are stimulator for mycorrhizal symbiosis (Gianinazzi-Pearson, 1989; Hassan and Mathesius, 2012).

Water stress significantly decreased shoot dry weight in two citrus rootstocks (Fig. 1B). Shoot dry weight was significantly higher in *G. mosseae* mycorrhizal citrus rootstocks than control at all levels of water stress except for 4 days irrigation interval in sour orange. In mycorrhizal and NM citrus rootstocks, shoot dry weight of rough lemon was significantly higher than that of sour orange up to 6 days irrigation interval (Fig. 1B). Wu and Zou (Wu and Zou, 2009a; Wu and Zou, 2009b) showed that water stress caused a significant reduction in shoot and root dry and fresh weights. Others reported that plant growth could be reduced by changes in the elastic properties of cell walls (Nonami and Boyer, 1990) or by production of abscisic acid in the roots (Saab et al., 1990) under water stress conditions. Moreover, soil water availability and nutrients uptake reduction cause decrease in plant dry weight (Marschner, 1986).

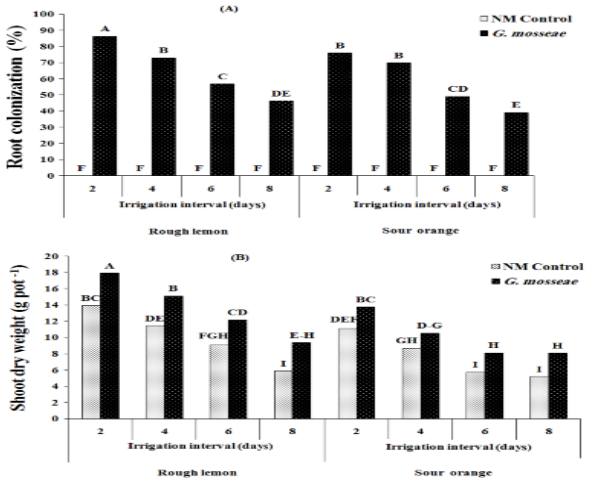

Fig. 1. Root colonization (A) and shoot dry weight (B) of sour orange and rough lemon seedlings affected by water stress and *Glomusmosseae.* NM Control=Non-Mycorrhizal Control.

Arbuscular mycorrhizal fungi promoted plant growth in the well-water and water stress conditions via different mechanisms. An increase in plant growth following mycorrhizal fungal inoculation was related to improving the exploration of the soil pore space, soil aggregation, uptake of nutrients and water by external hyphae, antioxidants production and plant growth promoting factors (especially under water stress conditions) (Augé, 2001;Wuand Zou, 2009b). Safiret al. (Safir et al., 1972) demonstrated that hyphae access smaller pore spaces than plant and provide additional surface area in the root system. By increasing water stress from 2 to 8 days, shoot N, P, Mn, Cu, and Fe uptake of two citrus rootstocks decreased, but shoot Zn uptake increased (Table 2). Other investigators stated that soil moisture affects the movement of nutrients and water in the soil. Decrease in soil water availability affects transpiration, diffusive flux of plant nutrients, the composition and concentration of soil solution and plant nutrients. Under drought stress conditions, the number of root hairs reduced and root morphology and branching were damaged. Over a period of water stress, a marked decrease in nutrients uptake has been reported through decreasing the transfer of ions to roots (Marschner, 1986; Nahar and Gretzmacher, 2002). In our experiments, the increase in shoot Zn uptake may be due to its interactions with other micronutrients. Micronutrients may compete for the same sites for absorption into the plant root (Mousavi et al., 2012).

Shoot N and P uptakes were significantly higher in mycorrhizal seedlings than NM seedlings of two citrus rootstocks, whereas there were no significant differences in shoot Mn, Cu, Zn and Fe uptake of two citrus root stocks at all irrigation intervals (Table 2).

Table 2. Mean comparison of water stress and *Glomu smosseae* (GM**)** interaction on nutrients uptake (N, P, Fe, Mn, Zn (mg pot^{-1}) and Cu (µg pot^{-1})) of shoot in rough lemon and sour orange seedlings

	2 days		4 days		6 days		8 days	
	Sour orange	Roughlemon	Sour orange	Rough lemon	Sour orange	Rough lemon	Sour orange	Rough lemon
N								
control	491.99 bc	581.48 b*	326.68 ef	360.98 de	159.47 hi	232.06 fgh	110.20 i	98.97 i
GM	705.16 a	772.41 a	452.78 cd	510.31 bc	258.70 fg	371.04 de	224 fgh	239.10 fgh
P								
control	25.46 b	27.80 b	19.05 cd	15.44 def	10.13 fg	11.73 efg	7.23 g	6.74 g
GM	35.03 a	37.68 a	25.09 b	27.70 b	16.83 de	21.59 bcd	15.89 def	15.41 def
Mn								
control	1.02 b-e	1.74 ab	0.86cde	1.44 abc	0.55 de	1.37abc	0.29 e	0.99 b-e
GM	1.49 abc	1.85 a	1.07 a-e	1.66 abc	1.06 a-e	1.49 abc	0.41 e	1.27 a-d
Cu								
control	202.43 c-g	296.00 abc	148.04 fg	240.96 b-f	125.86 g	205.44 c-g	113.98 g	169.2 d-g
GM	259.08 a-e	350.23 a	212.74 c-g	328.62 ab	150.47 efg	276.58 a-d	121.90 g	193.28 c-g
Zn								
control	0.74 d	0.85 cd	0.84 cd	1.18 bcd	0.94 cd	1.36 a-d	1.15 bcd	1.48 abc
GM	0.86 cd	1.32 bcd	0.85 cd	1.45 abc	1.07 bcd	1.69 ab	1.20 bcd	1.10 bcd
Fe								
control	2.11 a-d	2.40 abc	1.86 a-d	1.65 a-d	1.55 a-d	1.37 bcd	0.93 d	0.99 cdf
GM	2.56 ab	2.83 a	2.01 a-d	2.07 a-b	1.63 a-d	1.47 bcd	1.56 a-d	1.09 bcd

Means with the same letter are not significantly different at the 0.05 level using LSD test

Changes in the host physiology because of mycorrhizal formation and mycorrhizosphere may influence nutrients uptake (Wu and Zou, 2009b). Mycorrhizal symbiosis can enhance the resistance of plants to abiotic stresses probably by maintaining higher nutrients uptake including P and N and other elements (Augé, 2001) because mycorrhizal plants enhanced root growth and created a greater nutrients absorption surface area. Soil water availability influenced mobility of nutrients in the soil–root system, fungal mycelia extended from the root surface into the soil and increased the surface areas and water by the roots and thus acquired more nutrients beyond the depletion zone (Schnepf et al., 2011). It has also been reported that the effect ofArbuscular mycorrhizal symbiosis on plant drought tolerance may be because of physical, nutritional, physiological, and cellular effects (Augé, 2001). Rough lemon had significantly higher shoot N, Mn, Zn, and Cu uptake than sour orange, whereas there was no significant difference in shoot P and Fe uptake of two citrus root stocks (Data not shown).

The highest leaves catalase (CAT) activity was observed in sour orange seedlings colonized by *G. mosseae* and irrigation interval of 8 days, although they showed no significant difference with other treatments. The lowest leaves CAT activity was observed in NM rough lemon seedlings and irrigation interval of 2 days. The highest superoxide dismutase (SOD) activity of leaves was observed in mycorrhizal seedlings of sour orange at irrigation interval of 8 days. By increasing water stress, the leaves SOD activities of sour orange seedlings increased and there was a significant difference between low (2 and 4 days) and high (6 and 8 days) irrigation intervals. At each level of water stress, there were no significant differences between the SOD activities of leaves in mycorrhizal and NM seedlings of sour orange and rough lemon. Among different treatments of two citrus root stocks, the SOD activity of leaves in mycorrhizal seedlings of sour orange was significantly higher than that of mycorrhizal seedlings of rough lemon at 8 days irrigation interval, while no significant differences were observed between other treatments (Fig. 2B).

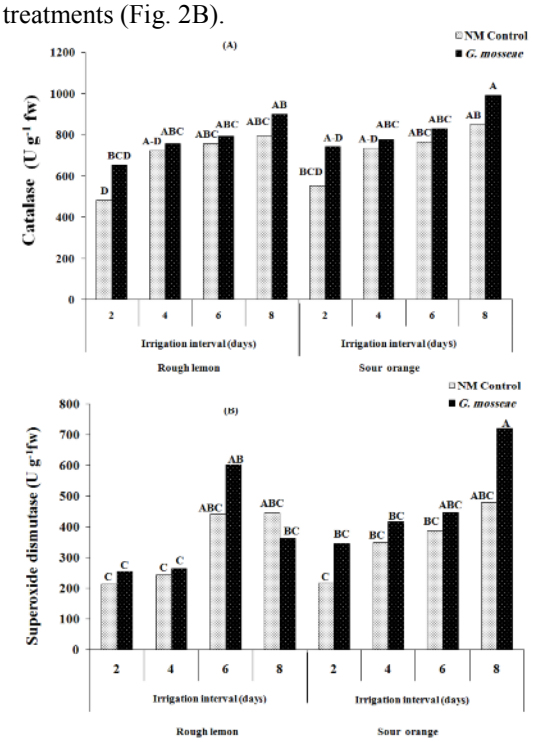

Fig. 2. Catalase (CAT) (A) and superoxide dismutase (SOD) (B) activities of leaves in sour orange and rough lemon seedlings affected by water stress and *Glomusmosseae*.

When plants are subjected to stress, the first reactive oxygen species (ROS) scavenging enzyme active in the enzymatic mechanism is SODwhich plays a key role in cellular defenses against ROS (Scandalios, 1993). The increase of SOD activity in leaves is closely related to a higher ability to scavenge active oxygen radicals under water stress. The increase in SOD activity in citrus leaves at irrigation interval of 6 days occurred faster than the increase in irrigation interval of 8 days in rough lemon (Fig. 2B). Other researchers have also reported that activities of this anti-oxidative enzyme increased when plants were exposed to water stress (Singh et al., 2009; Abedi and Pakniyat, 2010).

Results showed that antioxidant enzymes activities in citrus leaves increased with increasing water stress. The highest leaves APX activity was observed in mycorrhizal seedlings of rough lemon at irrigation interval of 8 days. However, no significant differences were observed between this treatment and other ones (Fig. 3A). At each level of water stress, there were no significant differences between leaves APX activity in mycorrhizal and control seedlings of sour orange and rough lemon (Fig. 3A).

By increasing water stress, leaves guaiacol peroxidase (G-POD) activities in mycorrhizal and control seedlings of rough lemon and control seedlings of sour orange were not significantly changed, but it significantly increased in mycorrhizal seedlings of sour orange (Fig. 3B). There was no significant difference between the G-POD activities of leaves of mycorrhizal and control seedlings of rough lemon at all levels of water stress and for sour orange at 2 and 4 days irrigation intervals. The G-POD activity of leaves in mycorrhizal seedlings of sour orange was significantly higher than that of control at irrigation intervals of 6 and 8 days. Generally, leaves G-POD activity was significantly higher in sour orange seedlings than those in rough lemon seedlings (Fig. 3A). The increase in G-POD activity might be responsible for the elimination of H_2O_2 from the cytosol. An increase in G-POD activity as affected by water stress had been reported by Wu et al. (Wu and Zou, 2009a).

Water stress can reduce the production of crops by inducing generation and accumulation of ROS such as superoxide anion radical, hydrogen peroxide, hydroxyl radicals, and singlet oxygen that results in disturbance of cellular homeostasis and induces oxidative stress (Neill et al., 2002). The antioxidant enzymes SOD, CAT, and APX activities effectively scavenged ROS. Our experimental findings on antioxidant system indicated that two citrus rootstock acted similarly under normal and water stress conditions. Defense system against ROS under stress conditions and enzymatic free radical processing systems include: SOD, catalyzing the dismutation of superoxide into H_2O_2, O_2 and those involved in the detoxification of H_2O_2 include: CAT and APX activities. Increase in CAT and SOD activities promote water stress tolerance (Sairam et al., 2000). Protection against stresses was achieved as a result of higher activities of SOD, CAT, and APX under water stress conditions in the leaves of *Zea mays* (Helal et al., 2008).

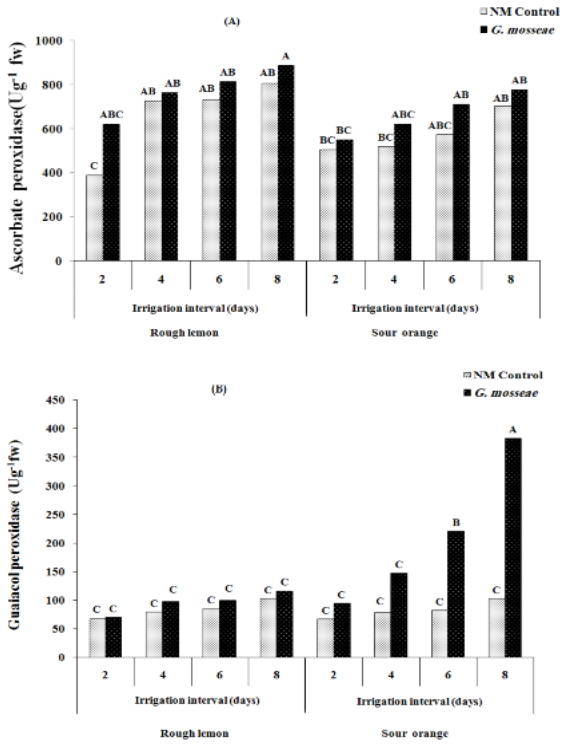

Fig. 3. Ascorbate peroxidase (APX) (A) and guaiacol peroxidase (G-POD) (B) activities of leaves in sour orange and rough lemon seedlings affected by water stress and *Glomusmosseae*.

Similarly, the SOD, APX and CAT activities increased in rice leaves under water stress (Gaber Gomaa et al., 2010). The activities of antioxidant enzymes increased in the presence of AM fungi (Wu et al., 2006). The CAT and G-POD activities increased in the presence of AM fungi under water stress conditions (Wu and Zou, 2009a). It seems that AM inoculation notably influences the activity of antioxidant enzymes in citrus leaves under water stress and an increase in the activity of antioxidant enzymes alleviates water stress (Asada, 1999). Resistance to oxidative stress may be closely related to water stress tolerance. These results are consistent with the results of other researchers (Alguacil et al., 2003; Lambais et al., 2003, Roldán et al., 2008; Wu and Zou, 2009a).

CONCLUSIONS

Seedlings were successfully infected by arbuscular mycorrhizal fungus but root colonization decreased with increased levels of water stress. Water stress significantly decreased shoot dry weight in two citrus rootstocks. Mycorrhizal rough lemon rootstocks had significantly higher shoot dry weight than the control at all levels of water stress. By increasing water stress, shoot N, P, Mn, Cu, and Fe uptake of two citrus rootstocks decreased, but shoot Zn uptake increased. Shoot N and P uptake were significantly higher in

mycorrhizal seedlings than NM seedlings of two citrus rootstocks, whereas there was no significant difference in shoot Mn, Cu, Zn and Fe uptake of two citrus root stocks at all levels of water stress. As water stress increased, SOD, CAT, G-POD and APX activities of two citrus rootstocks leaves increased. With inoculation of seedlings by *G. mosseae* compared with the control, antioxidant enzymes activities increased. The results suggested that the AM inoculation may play an important role in water stress tolerance.

ACKNOWLEDGEMENTS

The authors are grateful to Prof. A. A. Kamgar Haghighi and Dr. A. Shahsavar for useful comments and suggestions during the research. This research project (No. 335) has been financially supported by the National Drought Research Institute, College of Agriculture, Shiraz University, Ministry of Science, Research and Technology, Islamic Republic of Iran.

REFERENCES

Abedi, T., & Pakniyat, H. (2010). Antioxidant enzyme changes in response to drought stress in ten cultivars of oilseed rape (*Brassica napus* L.). *Czech Journal of Genetics and Plant Breeding*, 46(1), 27–34.

Alguacil, M.M., Hernandez, J. A., Caravaea, F., Portillo, B., & Roldan, A. (2003). Antioxidant enzyme activities in shoots from three mycorrhizal shrub species afforested in a degraded semi-arid soil. *Physiologia Plantarum*, 118, 562–570.

Aliasgharzadeh, N., SalehRastin, N., Towfighi, H., & Alizadeh, A. (2001). Occurrence of arbuscular mycorrhizal fungi in saline soils of the Tabriz Plain of Iran in relation to some physical and chemical properties of soil. *Mycorrhiza*, 11 (3), 119-122.

AlKhaliel, A.S. (2010). Effects of arbuscular mycorrhization in sterile and non-sterile soils. *Tropical Life Sciences Research*, 21(1), 55–70.

Amiri, M.J., & Eslamian, S.S. (2010). Investigation of climate change in Iran. *Journal of Environmental Science and Technology*, 3(4), 208-216.

Arora, A., Sairam, R.K., & Srivastava, G.C. (2001). Oxidative stress and antioxidative system in plants. *Current science*, 82 (10), 1227–1238

Asada, K. (1999). The water–water cycle in chloroplasts: scavenging of active oxygen and dissipation of excess photons. *Annual Review of Plant Physiology and Plant Molecular Biology*, 50, 601–39.

Augé, R.M. (2001). Water relations, drought and VA mycorrhizal symbiosis. *Mycorrhiza*, 11, 3–42.

Bates, L.W., Waldern, R., & Tearal, P. (1973). Rapid determination of free proline for salt water stress studies. *Plant and Soil*, 39, 205-207.

Beauchamcp, C., & Fridovich, I. (1971). Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. *Analytical Biochemistry*, 44, 276-287.

Bor, M., Ozdemir, F., & Turkan, I. (2003). The effect of salt stress on lipid peroxidation and antioxidants inleaves of sugar beet *Beta vulgaris* L. and wild beet *Beta maritima* L. *Plant Science*, 164, 77–84.

Bradford, M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principles of protein dye binding. *Analytical Biochemistry*, 72, 248-245.

Bremner, J.M. (1965). *Total nitrogen*. In: C.A. Black et al (ed). Methods of Soil Analysis. Part 2. Agronomy 9:1149-1178. American Society of Agronomy, Inc. Madison, Wisconsin, USA.

Chance, B., & Maehly, A.C. (1955). Assay of catalase and peroxidase. *Methods in Enzymology*, 2, 764-775.

Chapman, H.D. (1965). Cation exchange capacity. In: Black, C.A.,Evans, D.D.,Ensminger, L.E.,White, J.L., & Clark, F.E. (ed). Methods of soil analysis part 2. *Chemical and microbiological properties*. Monogr. 9. 2nd Edition. Agron. Monogr. 9. ASA and SSSA, Madison, WI.

Chapman, H.D., & Pratt, D.F. (1961). *Methods of analysis of soil, plant and water*. University California Division Agricultural Science.

Cottenie, A. (1980). *Soil and plant testing as a basis of fertilizer recommendation*. FAO Soils Bulletin, no. 38/2. Rome: FAO, Land and Water Development Division.

Daniels, B.A., & Trappe, J.M. (1980). Factors affecting spore germination of the vesicular-arbuscular mycorrhizal fungus, *Glomus epigaeus*. *Mycologia*, 72, 457-471.

Dhindsa, R.S., PlumbDhindsa, P., & Thorpe, T.A. (1981). Leaf senescence: correlated with increased levels of membrane permeability and lipid peroxidation and deceased levels of superoxide desmutase and catalase. *Journal of Experimental Botany*, 32, 93-101.

FAO. 2011. http://faostat.fao.org/site/339/default.aspx.

Gaber Gomaa, S., OsamaKansowa, A., & HossamSaad, E. (2010). Effects of various chemical agents for alleviation of drought stress in rice plants (*Oryza sativa* L.). *Notulae Botanicae Horti Agrobotanici Cluj*, 38(1), 139-148

GianinazziPearson, V., Branzanti, B., & Gianinazzi, S. (1989). In vitro enhancement of spore germination and early hyphal growth of a vesicular-arbuscular mycorrhizal fungus by host root exudates and plant flavonoids. *Symbiosis*, 7, 243-255.

Gupta, S., & Gupta, N.K. (2005). High temperature induced antioxidative defense mechanism in contrasting wheat seedlings. *Indian Journal of Plant Physiology*, 10, 73-75.

Hassan, S., & Mathesius, U. (2012). The role of flavonoids in root–rhizosphere signalling: opportunities and challenges for improving plant–microbe interactions. *Journal of Experimental Botany*, 63(9), 3429–3444.

Helal, R.M., & Samir, M.A.A. (2008). Comparative response of drought tolerant and drought sensitive maize genotypes to water stress. *Australian Journal of Crop Science*, 1(1), 31-36.

Kariman, K., Goltapeh, E.M., & Minassian, V. (2005). Arbuscular mycorrhizal fungi from Iran. *Journal of Agricultural Technology,* 1 (2), 301-313.

Klute, A. (1986). Water retention: Laboratory methods. p. 635–662. In: A. Klute (ed.) Methods of soil analysis. Part 1. Physical and mineralogical methods. 2nd ed. *Agron. Monogr.* 9. ASA and SSSA, Madison, WI.

Koo, R.C.J. (1980). Results of citrus fertigation studies. Proceedings of the Florida State. *Horticultural Society*, 93, 33-36.

Kormanik, P.P., & McGraw, A.C. (1982). Quantification of vesicular-arbuscular mycorrhizae in plant root. In: Methods and principles of mycorrhizal reseach,ed. By N .C. Schenk, *The American Phytopathological Society*. St.Paul.

Lambais, M.R., RiosRuiz, W.E., & Andrade, R.M. (2003). Antioxidant responses in bean (*Phaseolus vulgaris*) roots colonized by arbuscular mycorrhizal fungi. *New Phytologist*,160, 421-428.

Lindsay, W.L., & Norvell, W.A. (1978). Development of a DTPA soil test for zinc, iron, manganese, and copper. *Soil Science Society of American Journal,* 42, 241-428.

Marschner, H. (1986). *Mineral Nutrition of Higher Plants*. 2nd. edition Academic Press, London.

Mousavi S.R, Galavi, M., & Rezaei, M. (2012). The interaction of zinc with other elements in plants: a review. *International Journal of Agriculture and Crop Sciences*, 4 (24), 1881-1884.

Nahar, K., & Gretzmacher, R. (2002). Effect of water stress on nutrient uptake, yield and quality of tomato (*Lycoperscon esculentum* Mill.) under subtropical conditions. *Die Bodenkultur*, 53 (1), 45-51.

Nakano, Y., & Asada, K. (1981). Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplast. *Plant Cell Physiology*, 22, 867-880.

Neill, S., Desikan, R., Clarke, S., Hurs, A.R., & Hancock, J.T. (2002). Hydrogen peroxide and nitric oxide as signaling molecules in plants. *Journal of Experimental Botany*, 53, 1237–1247.

Nelson, D.W., & Sommers, L.E. (1996). Total carbon, organic carbon, and organic matter. Methods of Soil Analysis: Part 3.Chemical Methods. Sparks, D. L. (Ed.). *Soil Science Society of America and American Society of Agronomy*. Madison WI.

Nonami, H., & Boyer, J.S. (1990). Wall extensibility and cell wall hydraulic conductivity decrease in enlarging stem tissues at low water potentials. *Plant Physiology*, 93, 1601–1609.

Olsen, S.R., Cole, C.V.,Watanabe, F.S., & Dean, L.A. (1954). *Estimation of available phosphorus in soils by extraction with sodium bicarbonate*. U.S. Dep. of Agric. Circ.

Rhoades, J.D. (1996). Salinity: Electrical conductivity and total dissolved solids, In: Methods of Soil Analysis, Part 3. Chemical Methods. Sparks, D. L. (Ed.). *Soil Science Society of America and American Society of Agronomy*. Madison WI.

Roldán, A., DíazVivancos, P., Hernández, J.A., Carrasco, L., & Caravaca, F. (2008). Superoxide dismutase and total peroxidase activities in relation to drought recovery performance of mycorrhizal shrub seedlings grown in an amended semiarid soil. *Journal of Plant Physiology*, 165, 715–722.

Saab, I.N., Sharp, R.E., Pritchard, J., & Voetberg, G.S. (1990). Increased endogenous abscisic acid maintains primary root growth and inhibits shoot growth of maize seedlings at low water potentials. *Journal of Plant Physiology*, 93, 1329-1336.

Safir, G.R., Boyer, J.S., & Gerdemann, J.W. (1972). Nutrient status and mycorrhizal enhancement of water transport in soybean. *Journal of Plant Physiology*, 49, 700–703.

Sairam, R.K., Srivastava, G.C., & Saxena, D.C. (2000). Increased antioxidant activity under elevated temperature: a mechanism of heat stress tolerance in wheat genotypes. Biologia *Plantarum*, 43, 245-251.

Sangtarash, M.H., Qaderi, M.M., Chinnappa, C.C., & Reid, D.M. (2009). Differential responses of two *Stellaria longipes* ecotypes to Ultraviolet-B radiation and drought stress Flora-Morphology, Distribution. *Flora Morphology Functional Ecology of Plants*, 204(8), 593-603.

Scandalios, J.G. (1993). Oxygen stress and superoxide dismutases. *Plant Physiology*, 101, 7–12.

Schnepf, A., Jones, D., & Roose, T.(2011). Modelling nutrient uptake by individual hyphae of arbuscular mycorrhizal fungi: temporal and spatial scales for an experimental design. *Bulletin of Mathematical Biology*, 73, 2175-2200.

Sepaskhah, A.R., & Yarami, N. (2009). Interaction effects of irrigation regime and salinity on flower yield and growth of saffron. *The Journal of Horticultural Science and Biotechnology*, 84(2), 216-222.

Singh, N.B., Singh, D., & Singh, A. (2009). Modification of physiological responses of water stressed *Zea mays* seedlings by leachate of *Nicotiana plumbaginifolia*. *General and Applied Plant Physiology*, 35(1,2), 51–63.

Syvertsen, J.P. (1985). Integration of water stress in fruit trees. *Horticultural Science*, 20, 1039-1043.

Thomas, G.W. (1996). Soil pH and soil acidity. In: J. M. Bigham (ed). Methods of soil analysis: Part 3. Chemical methods. Sparks, D.L. (Ed.). *Soil Science Society of America and American Society of Agronomy*. Madison WI.

Tommerup, I.C. (1984). Effect of soil water potential on spore germination by vesicular-arbuscular mycorrhizal fungi. *Transactions of the British Mycological Society*, 83(2), 193-202.

Wu, Q.R., & Xia, X. (2006). Arbuscular mycorrhizal fungi influence growth, osmotic adjustment and photosynthesis of citrus under well-watered and water stress conditions. *Journal of Plant Physiology*, 163, 417-425.

Wu, Q.S., & Zou, Y.N. (2009a). Mycorrhiza has a direct effect on reactive oxygen metabolism of drought-stressed citrus plant. *Soil and Environment*, 55 (10), 436–442.

Wu, Q.S., & Zou, Y.N. (2009b). Mycorrhizal influence on nutrient uptake of citrus exposed to drought stress. *The Philippine Agricultural Scientist*, 92(1), 33-38.

Wu, Q.S., Xia, R.X., & Zou,Y.N. (2006). Reactive oxygen metabolism in mycorrhizal and non-mycorrhizal citrus (*Poncirus trifoliata*) seedlings subjected to water stress. *Journal of Plant Physiology*, 163, 1101—1110.

Wu, Q.S., Zou, Y.N., Xia, R.X., & Wang, M.Y. (2007). Five *Glomus* species affect water relations of *Citrus tangerine* during drought stress. *Botanical Studies*, 48, 147-154.

Wutscher, H.K. (1989). Alternation of fruit tree nutrition through rootstocks. *Horticultural Science*, 24, 578- 584.

Zarei, M., König, S., Hempel, S., Nekouei, M.K., Savaghebi, G., & Buscot, F. (2008). Community structure of arbuscular mycorrhizal fungi associated to *Veronica rechingeri* at the Anguran zinc and lead mining region. *Environmental Pollution*, 156(3), 1277-1283.

تحقیقات کشاورزی ایران (۱۳۹۵) ۳۵(۲) ۱۹-۲۶

اثر قارچهای میکوریز آربوسکولار و تنش آبی بر فعالیت برخی آنزیم های آنتی اکسیدان و جذب عناصر غذای دو پایه مرکبات

مهدی زارعی* ، زهراپیمانه، عبدالمجید رونقی

بخش علوم خاک، دانشکده کشاورزی، دانشگاه شیراز، شیراز، ج. ا. ایران.

*نویسنده مسئول

اطلاعات مقاله

تاریخچه مقاله:
تاریخ دریافت: ۱۳۹۳/۴/۲۹
تاریخ پذیرش: ۱۳۹۳/۹/۱۸
تاریخ دسترسی: ۱۳۹۵/۴/۸

واژه‌های کلیدی:
مرکبات
جذب عناصر غذایی
آنزیم‌های آنتی اکسیدان
تنش آبی
گلوموس موسه

چکیده- تنش خشکی از مهمترین فاکتورهای کاهش تولید در کشاورزی است زیرا بر تمام فعالیت‌های گیاه اثر دارد. در این پژوهش گلخانه‌ای اثر تنش خشکی و قارچ گلوموس موسه بر روی رشد، جذب عناصر غذایی و آنزیم‌های آنتی اکسیدان دو پایه مرکبات نارنج و رافلمون) در خاک استریل بررسی شد. در این مطالعه از سه فاکتور در قالب طرح کاملاً تصادفی با سه تکرار استفاده شد. فاکتورها شامل سه سطح تنش خشکی (دور های آبیاری ۲، ۴، ۶ و ۸ روز)، میکوریز در ۲ سطح (تلقیح قارچ گلوموس موسه و شاهد) و دوپایه مرکبات (نارنج و رافلمون) بودند. دانهال‌های میکوریزی دو پایه مرکبات به خوبی توسط قارچ *گلوموس موسه* کلنیزه شده بودند. با افزایش سطح تنش خشکی درصد کلنیزاسیون ریشه، عملکرد ماده خشک اندام هوایی، جذب نیتروژن، فسفر، منگنز، مس و آهن در اندام هوایی دو پایه مرکبات به طور معنی‌داری کاهش ولی جذب روی اندام هوایی و فعالیت آنزیم‌های آنتی اکسیدان سوپراکسید دسموتاز، کاتالاز، گلوتاتیون پراکسیداز و آسکوربیک پراکسیداز) در برگ در هر دو پایه مرکبات افزایش یافت. با تلقیح دو پایه مرکبات با قارچ در مقایسه با گیاهان بدون قارچ عملکرد ماده خشک اندام هوایی، جذب نیتروژن، فسفر و آنزیم‌های آنتی اکسیدان افزایش یافت. ممکن است قارچ های میکوریز آربوسکولار با افزایش جذب عناصر غذایی در اندام هوایی و فعالیت آنزیم‌های آنتی اکسیدان برگ مرکبات اثرات تنش خشکی را تعدیل نماید.