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RESEARCH ARTICLE

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Effectiveness of arbuscular mycorrhizal fungi in the protection of olive plants against oxidative stress induced by drought

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Abstract

Olive trees are often subjected to a long dry season with low water availability which induces oxidative stress. The present study was conducted to evaluate the effectiveness of native *Rhizophagus manihotis* (Rma) and non-native *Funneliformis mosseae* (Fmo) arbuscular mycorrhizal (AM) fungi (AMF) in enhancing olive protection against oxidative stress induced by water deficit. Olive plantlets, cv. Picholine marocaine, were inoculated (AM-plants) or not (NM-plants) with Rma or Fmo and subjected to well-watered (75% of field capacity) or water-stressed (25% of field capacity) conditions. After two months, obtained results showed that water stress significantly decreased growth and biomass production of NM-plants, but AMF alleviated the detrimental effects of water deficit on the growth of olive plants. Inoculation with Rma increased shoot height by 120%, root length by 56%, fresh weight by 170% (shoot) and 210% (root), and dry weight by 220% (shoot) and 220% (root) compared to NM-plants. AM colonization enhanced drought tolerance in terms of protection against oxidative stress. Mycorrhizal plants showed lower levels of hydrogen peroxide (H_2O_2), malondialdehyde (MDA) and electrolyte leakage (EL) than NM-plants. Rma colonization decreased two times H_2O_2, 2.6 times MDA and two times EL levels compared to NM-plants. This protective effect seems to be due to the enhanced activities of superoxide dismutase (534 U mg^{-1} protein), catalase (298 U mg^{-1} protein), guaiacol peroxidase (47 U mg^{-1} protein), and ascorbate peroxidase (305 U mg^{-1} protein) which were highest in Rma-plants. Moreover, Rma-plants showed the lowest oxidative damage to lipid and highest soluble protein content. Thus, the native AMF Rma ought to be considered as a biological tool for enhancing olive tolerance to drought.

Additional key words: mycorrhizal colonization; *Olea europaea*; water deficit; antioxidant activity; drought tolerance.

Introduction

Olive tree (*Olea europaea* L.) is a high economic value crop species with high hardiness and high adaptability to the semi-arid environment (Gimenez *et al.*, 1997). However, in Mediterranean regions, olive trees are often subjected to a long dry season with low water availability leading to water stress which affects almost all plant functions. Water deficit is often associated with increased level of reactive oxygen species (ROS), such as superoxide radical (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical (OH) and singlet oxygen (O^{1-}). Plants are endowed with a complex antioxidant system to cope with ROS accumulation (Smirnoff, 1993). However, under water stress ROS accumulation often exceeds the removal capacity of the antioxidant system leading to the oxidative stress which causes oxidative damage, in-

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Abbreviations used: AM (arbuscular mycorrhiza); AMF (arbuscular mycorrhizal fungi); APX (ascorbate peroxidase); CAT (catalase); DW (dry weight); EC (electrical conductivity); EL (electrolyte leakage); FC (field capacity); Fmo (*Funneliformis mosseae*); FW (fresh weight); G-POD (guaiacol peroxidase); MDA (malondialdehyde); NBT (nitroblue tetrazolium); NM (non-mycorrhizal); RDW (root dry weight); RFW (root fresh weight); RL (root length); Rma (*Rhizophagus manihotis*); ROS (reactive oxygen species); SDW (shoot dry weight); SFW (shoot fresh weight); SH (shoot height); SOD (superoxide dismutase); TBA (thiobarbiturique acid); TCA (trichloroacetic acid); WS (water stress); WW (well water).

cluding peroxidation of lipid, oxidation of DNA and proteins, destruction of photosynthetic pigments, and inactivation of photosynthetic enzymes (Smirnoff, 1993). To mitigate the oxidative damage initiated by ROS, plants have developed a complex antioxidant defense system that includes enzymatic and non-enzymatic antioxidants. The induction of ROS scavenging enzymes, such as superoxide dismutase (SOD), catalase (CAT), guaiacol peroxidase (G-POD), and ascorbate peroxidase (APX) is the most common mechanism that protect plants against oxidative damage (Mittler, 2002). Under limiting conditions, mycorrhizal association is the main pathway through which most plants can obtain water and mineral nutrients (Moucheshi *et al.*, 2012). In this mutual symbiosis, plants exchange photosynthates, not only for mineral nutrients, but also for increased tolerance to drastic environmental conditions such as drought (Al-Karaki *et al.*, 2004; Faghire *et al.*, 2010) and soil salinity (Estrada *et al.*, 2013). These positive effects seem to be due to the enhanced antioxidant enzymes activities (Baslam *et al.*, 2010; Abbaspour *et al.*, 2012; Fouad *et al.,* 2013), and improved water relations and nutrient acquisition (Faghire *et al.*, 2010; Fouad *et al*., 2013) in mycorrhizal plants. However, the effectiveness of the mycorrhizal symbiosis varies according to mycorrhizal fungi strains and plant cultivars (Soriano-Martin *et al.*, 2006; Binet *et al.*, 2007). Though many investigations have reported the positive effects of arbuscular mycorrhizal fungi (AMF) on morphological and physiological variables related to olive tolerance to water deficit and salt stress (Soriano-Martín *et al*., 2006; Porras-Soriano *et al.*, 2009, Fouad *et al.*, 2012, 2013), little is known about their specific action on antioxidant mechanisms which regulate different biochemical changes *e.g.* protein, enzyme activities, carbohydrates, etc., related to the alleviation of oxidative damage induced by water stress. The present investigation was carried out to provide a more detailed insight into the relationship between the mycorrhizal status and changes of antioxidants enzyme activities as well as oxidative damage levels in relation to drought tolerance of olive plantlets. The potential of native (*Rhizophagus manihotis*) and non-native (*Funneliformis mosseae*) arbuscular mycorrhizal fungi to alleviate oxidative stress damage in olive plants (cv. Picholine marocaine) under drought conditions was investigated in order to reveal performance variability in drought resistance between these two mycorrhizal strains.

Material and methods

Mycorrhizal inoculum

Two species of arbuscular mycorrhizal fungi were used: reference strain *Funneliformis mosseae* (Nicol. and Gerd.) Gerd. and Trappe (BEG no. 12) from Experimental Station del Zaidín of Granada (CSIC) and indigenous strain *Rhizophagus manihotis* isolated from the rhizosphere of *Cupressus atlantica* Gaussen located in the N'Fis valley (Haut Atlas, Morocco) at the Idni Station (8° 17' 02" W, 31° 54' 34" N, 1700 m above sea level) and identified by Ouahmane *et al.* (2007).

Inoculation and plant culture

Semi-woody cuttings of the most cultivated olive variety in Morocco (cv. Picholine marocaine) were mist rooted using the method described by Porras-Piedra *et al.* (2005) in Babram Nursery Society, Marrakech (Morocco). Rooted cuttings were selected uniformly (with 10 cm long and three pairs of leaves) and transplanted into 1 L bags containing 1 kg of a mixture (v/v) of sand and gray loam soil from the Faculty of Sciences and Technologies (Marrakech, Morocco) previously sterilized for 3 h in oven at 180°C. Soil had a pH of 8.03; organic matter was 1.25%; nitrogen was 2.5 mg kg^{-1}; available phosphorus was 4.85 mg kg^{-1}; total phosphorus was 118 mg kg^{-1}; potassium was 142.89 mg kg^{-1} and electrical conductivity (EC) was 145 µs cm^{-1}.

Plantlets were divided into three groups. Two groups were inoculated with one of the two AMF species by placing inoculum directly at the position of the roots at the time of transplanting. The AMF inoculum consisted of rhizospheric soil containing spores and colonized root fragments of *Hordeum vulgare* L. in amounts of 15 g per pot, which were predetermined to have achieved high levels of root colonization. The block of non mycorrhizal plants (NM-plants) received the same amount of autoclaved inocula. The experiment was carried out under greenhouse with an approximate temperature of 30/20°C (day/night) and 16 h photoperiod (22 klux ≈ 400 µmol m^{-2} s^{-2}) at the Faculty of Sciences and Technologies (Marrakech, Morocco).

Water stress and experimental design

The factorial experiment consisted in six treatments crossing three mycorrhizal inoculations levels: non-inoculated (NM-plants), inoculated with *Funneliformis mosseae* (Fmo-plants), and inoculated with *Rhizophagus manihotis* (Rma-plants) with two soil water regimes: well water (WW) corresponding to 75% of field capacity and water stress (WS) corresponding to 25% of field capacity. Each treatment was replicated twelve times and was arranged in a complete randomized block design. In order to control the level of drought stress, the soil water content in each bag was measured daily and the amount of water lost was refilled to keep the target soil water content. Two months after the start of the soil-water treatments, 18 plants were chosen at random, 3 from each treatment, and harvested for determination of growth and biochemical parameters.

Determination of plant biomass, growth and AMF colonization

Shoot height (SH), root length (RL), shoot (SFW) and root (RFW) fresh weights were measured in all the harvested plants. Shoots and roots were dried separately at 80°C for 48 h to record shoot (SDW) and root (RDW) dry weights. Some of the fresh roots were carefully washed and cut into 1 cm root pieces. Root pieces were then cleaned with 10% (w/v) KOH and stained with 0.05% (w/v) trypan blue in lactophenol for mycorrhizal status evaluation (Phillips & Hayman, 1970). The intensity of root colonization was determined by optical microscopic observations of 20 root fragments. Each root fragment observed was assigned a class note between 0 and 5, corresponding to the estimation of the level of mycorrhizal colonization. Zero was the class note corresponding to 0% of root colonization, 1 lower than 1%, 2 between 1% and 10%, 3 between 10% and 50%, 4 between 50% and 90% and 5 more than 90%. The intensity of root colonization (M%) was calculated using the following formula (Trouvelot *et al.*, 1986): $M\% = [(95 \times n5) + (70 \times n4) + (30 \times n3) + + (5 \times n2) + n1] / N$, where N is the total number of fragments observed, and n5, n4, n3, n2 and n1 are the number of fragments rated respectively 5, 4, 3, 2 and 1.

Electrolyte leakage

This technique is based on the increase of cellular membrane permeability and concomitantly greater electrolyte diffusion out of cells when leaf tissue is injured by a stress situation (Selvakumar & Thamizhiniyan, 2011). After harvest, the uppermost fully expanded leaves of three plants per treatment were immediately cut into discs of 0.8 cm diameter. The discs were washed briefly three times in deionized water to remove solutes released during cutting of the discs. Ten discs of each plant were then placed in a vial filled with 15 mL of deionized water and placed in a water bath at a constant temperature of 32°C. After 2 h the initial EC of the medium (EC1) was measured using an electrical conductivity meter. The samples were autoclaved afterwards at 121°C for 20 min to kill the tissues completely and release all electrolytes. The samples were then cooled to 25°C and the final EC (EC2) was measured. The electrolyte leakage (EL) was estimated using the following formula (Dionisio-Sese & Tobita, 1998): $EL = EC1/EC2 \times 100$.

Malondialdehyde (MDA) concentration

Leaves samples (0.5 g) were homogenized in 5 mL of 5% (w/v) trichloroacetic acid (TCA) and centrifuged at 3,000 × g for 10 min. The extracts were used for malondialdehyde assay according to thiobarbituric acid (TBA) reaction as described by Dhindsa *et al.* (1981). One milliliter aliquot of supernatant was mixed with 4 mL of 20% TCA containing 0.5% TBA. The mixture was heated at 100°C for 30 min, quickly cooled, and then centrifuged at 10,000 × g for 10 min. The absorbance (A) of the supernatant was read at 532 nm (A_{532}). The unspecific turbidity was corrected by A_{600} subtracting from A_{532}. The concentration of MDA was calculated using an extinction coefficient of 155 mM^{-1} cm^{-1}.

Hydrogen peroxide (H_2O_2) content

Hydrogen peroxide content in leaves was determined by Patterson's method (Patterson *et al.*, 1984), with slight modifications as described previously by Aroca *et al.* (2003). Five-hundred milligrams of fresh leaves were homogenized in a cold mortar with 5 mL 5% (w/v) TCA containing 0.1 g of activated charcoal and 1% (w/v) polyvinylpolypyrrolidone (PVPP). The homogenate was centrifuged at 18,000 × g for 10 min. The supernatant was filtered through a millipore filter (220 μm). A volume of 1.2 mL of 100 mM potassium phosphate buffer (pH 8.4) and 0.6 mL of the colori-

metric reagent were added to 130 µL of the supernatant. The colorimetric reagent was freshly made by mixing 1:1 (v/v) 0.6 mM potassium titanium oxalate and 0.6 mM 4-2 (2-pyridy-lazo) resorcinol (disodium salt). The samples were incubated at 45°C for 1 h and the absorbance at 508 nm was recorded. The blanks were made by replacing leaf extract by 5% TCA.

Enzyme extraction and assay

For enzyme extracts and assays, fresh leaves (0.5 g) were frozen in liquid nitrogen and then ground at –4°C in 5 mL solution containing 50 mM K-phosphate buffer (pH 7.0), 1% (w/v) PVPP, and 0.1 mM EDTA. The homogenate was centrifuged at 15,000 × g, –4°C for 30 min, and the supernatants were kept at –20°C for subsequent enzymatic assays (Arafat & He, 2011).

Total superoxide dismutase (SOD) activity was measured according to Beyer & Fridovich (1987) based on the ability of SOD to inhibit the reduction of nitro-blue tetrazolium (NBT) by superoxide radicals generated photochemically. One unit of SOD was defined as the amount of enzyme required to inhibit the reduction rate of NBT by 50% at 25°C. Catalase (CAT) activity was measured by the disappearance of H_2O_2 (Aebi, 1984). The reaction mixture (3 mL), which contained 10.6 mM H_2O_2, was initiated by adding 25 µL of the extract and monitoring the change in absorbance at 240 nm and 25°C for 3 min. Ascorbate peroxidase (APX) activity was measured in an 1 mL reaction volume containing 50 mM K-phosphate buffer (pH 7.0), 0.1 mM H_2O_2, and 0.5 mM ascorbate. Adding the H_2O_2 started the reaction and the decrease in absorbance at 290 nm was recorded for 1 min to determine the oxidation rate of ascorbate (Amako *et al.*, 1994). Guaiacol peroxidase (G-POD) activity was measured by following the change of absorption at 470 nm due to guaiacol oxidation. The activity was assayed for 1 min in a reaction solution (3 mL final volume) composed of 100 mM potassium phosphate buffer (pH 7.0), 20 mM guaiacol, 10 mM H_2O_2 and 0.15 mL enzyme extract (Polle *et al.*, 1994). Soluble proteins were found out by Bradford (1976) procedure using bovine serum albumin as the standard.

Statistical analysis

Data were analyzed with two-way ANOVA (IBM SPSS 20.0). Water status and AMF were used as first and second factor, respectively. Significant differences and interaction between factors were calculated at 5%. When F and p values were significant, means comparison was made using Tukey's (honest significant difference HSD) post hoc test $p < 0.05$.

Results

Root colonization

Root mycorrhizal colonization was observed in all inoculated olive plants regardless of fungi strain and water regime. The highest colonization was observed with *Rhizophagus manihotis* irrespective of water regime (Table 1). Water deficit significantly reduced the intensity of colonization; but the reduction rate was lower in Rma-plants (14%) than in Fmo-plants (20%).

Growth and biomass production

Compared to the NM-plants, inoculation with any of the two AMF species increased growth (SH and RL) and biomass production (SFW, RFW, SDW and RDW) both under WW and WS conditions (Table 1). Drought stress significantly decreased growth and biomass production in NM-plants; but AMF inoculation reduced the detrimental effect of drought on olive plant growth. Drought reduced by 27% SH, by 39% RL, by 43% SFW; by 51% RFW, by 39% SDW and by 40% RDW in NM-plants compared to respective rates of 13%, 27%, 25%, 36%, 18% and 13% in Fmo-plants and 7%, 16%, 15%, 15%, 8% and 8% in Rma-plants.

Hydrogen peroxide and malondialdehyde contents

As shown in Table 1, H_2O_2 and MDA levels were increased by water stress both in AM-plants and NM-plants, but this increment was significantly highest in NM-plants. Accumulation of H_2O_2 due to water stress was 5 times lower in Rma-plants and 4 times lower in Fmo-plants than in NM-plants. While MDA accumulation was 29 times lower in Rma-plants and 13 times lower in Fmo-plants than in NM-plants. Moreover, MDA concentration in water stressed Rma-plants was similar to that observed in well watered NM-plants (Table 1).

Table 1. Mycorrhizal colonization (M), shoot height (SH), root length (RL), shoot (SFW) and root (RFW) fresh weights, shoot (SDW) and root (RDW) dry weights, and hydrogen peroxide (H_2O_2), malondialdehyde (MDA) and protein contents, oxidative damage (OD), electrolyte leakage (EL), superoxide dismutase (SOD), guaiacol peroxidase (G-POD), catalase (CAT) and ascorbate peroxidase (APX) activities in leaves of olive plants non-inoculated (NM) or inoculated with arbuscular mycorrhizal fungi (AMF) *Funneliformis mosseae* (Fmo) or *Rhizophagus manihotis* (Rma) and subjected to well water (WW) or water stress (WS) conditions

	Water status						Water status (d.f. 1,12)	AMF status (d.f. 2,12)	Water status × AMF status (d.f. 2,12)
	WW			WS					
	NM	Fmo	Rma	NM	Fmo	Rma			
M (%)	0.0±0.00	57.23±1.23 b	63.83±0.58 a	0.0±0.00	45.50±1.00 d	54.87±0.25 c	440.23***	12790.46***	115.95***
SH (cm)	25.80±1.12 c	38.50±1.21 b	45.33±0.94 a	18.90±1.12 d	33.57±1.21 c	42.11±0.94 b	93.73***	581.44***	4.18*
RL (cm)	10.30±0.10 bc	10.40±0.36 b	11.77±0.06 a	6.30±0.10 e	7.63±0.15 d	9.83±0.11 c	1195.10***	314.02***	51.21***
SFW (g)	11.58±0.07 d	17.79±0.23 b	21.17±0.30 a	6.62±0.03 e	13.33±0.10 c	18.05±0.19 b	2365.40***	5071.92***	40.89***
RFW (g)	5.03±0.03 d	9.35±0.44 a	9.10±0.41 a	2.45±0.04 e	5.99±0.15 c	7.69±0.36 b	312.22***	435.44***	16.66***
SDW (g)	3.08±0.07 d	5.35±0.06 b	6.49±0.26 a	1.88±0.03 e	4.37±0.07 c	5.94±0.13 b	219.46***	1286.67***	11.22**
RDW (g)	1.49±0.01 e	2.61±0.09 c	3.14±0.06 a	0.89±0.01 f	2.26±0.05 d	2.88±0.06 b	250.73***	1780.81***	16.62***
H_2O_2 (µmol g^{-1} FW)	49.12±0.17 d	46.02±0.17 e	43.95±0.23 f	99.02±0.46 a	58.28±0.80 b	54.34±0.06 c	10443.18***	8054.02***	7562.07***
MDA (nmol g^{-1} FW)	68.37±0.04 cd	67.36±1.21 e	66.13±0.97 e	179.04±0.63 a	75.78±1.08 b	69.98±0.61 c	16363.69***	6906.80***	4633.74***
OD (nmol MDA mg^{-1} protein)	5.82±0.05 cd	5.14±0.10 cd	4.31±0.06 d	40.75±2.52 a	8.26±0.12 b	5.61±0.05 d	729.45***	578.16***	505.25***
EL (%)	18.20±0.42 d	16.36±0.69 d	17.29±0.58 d	46.75±1.02 a	28.25±0.99 b	23.73±0.58 c	1961.38***	445.91***	355.33***
Protein (mg g^{-1} FW)	11.75±0.11 d	13.11±0.10 b	15.34±0.03 a	4.41±0.30 f	9.17±0.07 e	12.48±0.05 c	5028.09***	2563.39***	411.13***
SOD (U mg^{-1} protein)	129.56±1.22 f	183.69±0.62 e	192.56±0.62 d	265.98±6.84 c	433.80±2.33 b	533.68±2.76 a	25577.48***	4116.95***	1524.29***
G-POD (U mg^{-1} protein)	9.59±0.10 f	11.70±0.16 e	13.96±0.19 d	23.29±0.34 c	32.30±0.50 b	46.73±0.63 a	16123.65***	2106.72***	1001.72***
CAT (U mg^{-1} protein)	174.63±1.38 f	208.91±0.74 d	216.61±1.35 c	190.83±1.66 e	266.45±2.82 b	298.12±1.30 a	4329.74***	3224.71***	588.28***
APX (U mg^{-1} protein)	66.10±0.62 f	71.37±1.05 e	76.54±0.51 d	189.68±1.43 c	237.44±2.36 b	305.30±2.01 a	60036.67***	2684.78***	1875.92***

Values are means of three replicates ± standard error. Within each variable means followed by different letters are significantly different at * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ as determined by the Tukey's HSD post hoc test.

Oxidative damage to lipid and electrolyte leakage

When plants were subjected to WW conditions, oxidative damage to lipid and electrolyte leakage of leaves was not significantly affected by mycorrhizal inoculation (Table 1). However, drought stress caused a strong increase of electrolyte leakage (157%) and of oxidative damage to lipid (600%) in NM-plants. In AM-plants, electrolyte leakage increase was less pronounced (37% in Rma-plants and 73% in Fmo-plants), while of oxidative damage to lipid due to water stress low in Fmo-plants and not significant in Rma-plants (Table 1).

Protein content

Arbuscular mycorrhizal plants, especially Rma-plants showed higher content of soluble proteins under WW and WS conditions compared to NM-plants (Table 1). A significant reduction in protein content was observed as a consequence of drought stress, but the reduction rate was more relevant in NM-plants (62%) than in Fmo-plants (30%) and Rma-plants (19%).

Antioxidant enzymes (SOD, G-POD, CAT and APX) activities

Arbuscular mycorrhizal fungi colonization significantly increased the activity of antioxidant enzymes SOD, G-POD, CAT, and APX both in WW and WS plants (Table 1). The highest enzyme activities were observed in Rma-plants regardless the water status. Water stress caused a great increase of the activity of the four enzymes in AM-plant than in NM-plants. The highest increase of enzymes, APX (299%), SOD (177%), G-POD (235%), and CAT (37%), activities were observed in Rma-plants.

Discussion

In the present study, drought stress reduced root colonization of olive plants by arbuscular mycorrhizal fungi (AMF). Previous investigations have shown that drought can reduce AMF colonization by inhibiting spore germination and reducing growth and spread of hyphae after initial infection had occurred (Wu & Xia, 2006; Abbaspour *et al.*, 2012). Indeed, most of the energy used for the development and branching of the hyphae is obtained from photosynthesis. Decreases of plant photosynthesis induced by water stress could affect the status of root carbohydrates, and consequently the rate of AM colonization (Meddich *et al.*, 2000). In the current study, the degree of root colonization varied among fungal species, the native AMF (Rma) had higher root colonization than non-native AMF (Fmo), and it showed better symbiotic efficiency. However, according to most of the previous reports, there is no threshold value of root colonization for symbiotic efficiency and this depends on the plant and the fungal species involved (Meddich *et al*., 2000; Baslam *et al.*, 2010; Estrada *et al.*, 2013).

AM symbiosis enhances plant growth and biomass production of olive plants under water stress. Rma-plants showed enhanced shoot height, root length, shoots and root fresh weights, and shoots and root dry weights. Such growth response to mycorrhizal colonization was previously observed in other olive variety (Rougemont, 2007) and many other plant species such as *Citrus tangerine* (Wu & Xia, 2006), *Poncirus trifoliate* (Wu *et al*., 2008), date palm (Baslam *et al*., 2010; Faghire *et al.*, 2010), pistachio (Abbaspour *et al.*, 2012) and carob (Essahibi *et al.,* 2013). The positive effect on plant growth has been attributed to the improvement of water and nutrients uptake due to the greater absorption of the surface area provided by extensive fungal hyphae and the increased root length and density (Bethlenfalvay *et al.*, 1988; Faber *et al.*, 1991; Fouad *et al.*, 2012, 2013; Essahibi *et al.,* 2013). In previous study we showed enhanced water uptake and nutrients (potassium and phosphorus) accumulation in mycorrhizal olive plants compared to NM-plants both under well watered and water deficit conditions (Fouad *et al.*, 2013).

One of the earliest responses of plants to drought is the accumulation of reactive oxygen species (ROS). ROS are responsible of many of the degenerative reactions such as lipids peroxidation and proteins and nucleic acids oxidization (Ruiz-Lozano, 2003). It is well known that plant tolerance to water stress is associated with reduction of oxidative damage through induction of antioxidant enzymes (Wu *et al.*, 2006). According to several investigations, AM symbiosis enhances the activity of antioxidant enzymes, which helps plants to cope with ROS generated by water deficit (Wu *et al.*, 2006; Abbaspour *et al.*, 2012). In this study, H_2O_2 was highly accumulated in water stressed NM-plants compared to their relative AM-plants. The low level of H_2O_2 in AM-plants was correlated with tolerance to water stress (Porcel & Ruiz-Lozano, 2004; Wu *et al*., 2006; Baslam *et al*., 2010; Abbaspour *et al.*, 2012). Moreover, drought stressed AM-plants showed a higher content of soluble proteins in the leaves than drought stressed NM-plants. Similar results have been pointed out by previous reports indicating that AMF might alleviate or decrease RNA disassembly and might enhance the ability of the non-enzymatic antioxidant defense system by means of soluble proteins (Manoharan *et al*., 2010; Abbaspour *et al*., 2012).

In the current study, water stress induced a lower electrolyte leakage and lower MDA content in AM-plants than in NM-plants. These results are consistent with previous reports indicating that AMF colonization decreased MDA level and improved membrane integrity (Wu *et al.*, 2006; Beltrano & Ronco, 2008). Electrolyte leakage reflects the cell membrane permeability and MDA reflects an end product of lipid peroxidation. The low value of electrolyte leakage together with the low MDA content provide evidence of lower cell membrane damage. Data from this study showed that plants colonized by Fmo had lower oxidative damage to lipid than NM-plants, while plants inoculated by Rma did not show oxidative damage to lipids. This provides evidence that native AMF was more effective in preventing cell membrane damage and enhancing olive tolerance to drought stress. Similar results showing high efficiency of native AMF have been reported in maize under salt stress (Estrada *et al*., 2013). Oxidative damage decrease in AM-plants has been observed under different stress conditions, and was correlated with an enhanced drought tolerance of AM-plants (Porcel & Ruiz-Lozano, 2004; Wu *et al*., 2006; Baslam *et al*., 2010). Thus reduced oxidative damage to lipids in AM-plants seems to be a consistent effect of AM symbiosis under several stress conditions.

Although AM symbiosis affected reactive oxygen metabolism and antioxidant production, the exact mechanisms involved are still unclear. In general, the low oxidative damage found in AM-plants provides

evidence that they suffered less drought stress due to: (1) a primary drought avoidance effect through increased water uptake by fungal hyphae network which kept plants protected against the generation of ROS, or (2) an increased activity of antioxidant defense system, especially enzymes such as SOD, G-POD, and CAT involved in the elimination of ROS. In our results, activities of antioxidant enzymes, such as SOD, CAT, G-POD and APX were higher in AM-plants than in NM-plants under water stress condition showing a consistent effect of AMF on oxidative stress alleviation. It is well known that SOD converts O_2^- into H_2O_2, which is then eliminated by G-POD and CAT (Reddy *et al*., 2004). The higher antioxidant enzymes activities associated with lower accumulation of H_2O_2 and less lipid peroxidation would explain the lower oxidative stress in AM-plants, giving proof that AMF contributed to protect their host against oxidative damage, in turn enhancing drought tolerance.

The effects of AM symbiosis on the antioxidant systems observed in this study were more prominent in native AMF Rma. This suggests that under water stress, olive antioxidant system could not be enough to cope with the oxidative stress induced by water deficit; AMF, particularly native one, are important to improve the capacity of olive to grow under such constraint. Our observations are in agreement with Bartels (2001) who proposed both the prevention of oxidative stress and the elimination of ROS as the most effective approaches used by plants to gain tolerance against several abiotic stresses, including water stress and salinity. In addition, several authors have correlated the growth performance of AMF plants under water stress with protection against oxidative stress (Lu *et al*., 1999; Alguacil *et al*., 2003).

In the present study AMF, particularly the native Rma, was the most efficient in terms of olive plant growth performance and protection against the detrimental effects of drought, although the non-native fungus Fmo offered important protection compared to non-inoculated plants. Results showing different response of plants towards mycorrhizal strains were previously reported (Estaun *et al*., 2003; Estrada *et al*., 2013). According to Estaun *et al*. (2003), Rma was more efficient at promoting growth of olive plant (cv. Arbequina) than Fmo and the native endophytes present in the orchard soil. While native AMF were more efficient in enhancing maize salt tolerance by alleviating the salt induced oxidative stress and membrane damage (Estrada *et al*., 2013). Thus specific compatibility relationships may exist among symbionts, and underscore the importance of host-AMF selection to maximize growth of olive performance (Soriano-Martin *et al*., 2006).

We conclude that arbuscular mycorrhizal fungi, particularly the native ones, improved olive plant performance under drought stress condition. The AMF must have contributed to this effect through: (1) the increased exchange surface between soil and the roots of host plants improving thereby water absorption and nutrients uptake, and (2) the protection of host plants against oxidative stress by increasing antioxidant enzyme activities responsible for the elimination of ROS, as evidenced by the lower accumulation of H_2O_2. Thus, the inoculation with native AMF *Rhizophagus manihotis* ought to be considered as a biological method to enhance olive tolerance to drought stress.

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