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Arbuscular mycorrhizal fungus *Rhizophagus irregularis* influences key physiological parameters of olive trees (*Olea europaea* L.) and mineral nutrient profile

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Abstract

In this study, we hypothesized that colonization of olive trees (*Olea europaea* L.) with the arbuscular mycorrhizal fungus *Rhizophagus irregularis* could modify the profiles of rhizosphere microbial communities with subsequent effects on nutrient uptake that directly affects olive tree physiology and performance. In this context, a greenhouse experiment was carried out in order to study the effects of mycorrhizal colonization by *R. irregularis* on photosynthesis, pigment content, carbohydrate profile, and nutrient uptake in olive tree. After six months of growth, photosynthetic rate in mycorrhizal (M) plants was significantly higher than that of nonmycorrhizal plants. A sugar content analysis showed enhanced concentrations of mannitol, fructose, sucrose, raffinose, and trehalose in M roots. We also observed a significant increase in P, K, Ca, Mg, Zn, Fe, and Mn contents in leaves of the M plants. These results are important, since nutrient deficiency often occurs in Mediterranean semiarid ecosystems, where olive trees occupy a major place.

Additional key words: arbuscular mycorrhizal symbiosis; carbohydrates; chlorophyll; gas exchange; lipids; mineral nutrition.

Introduction

Arbuscular mycorrhizal (AM) fungi are widespread in soils, and growth of mycorrhizal plants is often enhanced in comparison to nonmycorrhizal (NM) plants. This beneficial effect on plant growth has largely been attributed to high uptake of nutrients, such as P, Zn, Cu, and Fe (Porras-Soriano *et al.* 2009). Apart from the influence of AM fungi on nutrient uptake, other positive aspects of mycorrhization include an increase of plant tolerance to drought (Ruiz-Sánchez *et al.* 2010), salt stress (Porras-Soriano *et al.* 2009), as well as resistance to pathogens (Wehner *et al.* 2010), alleviation of oxidative stress, and enhancement of antioxidant responses (García-Sánchez *et al.* 2014).

AM fungi have been shown to interact with different groups of soil bacteria and to modify the rhizosphere microbial community (Wamberg *et al.* 2003, Mechri *et al.* 2014). Microbial communities can alter nutrient cycling in the rhizosphere, thus affecting nutrient availability to plants (Marschner *et al.* 2004, De Maria *et al.* 2011). It has been demonstrated that some bacteria are able to synthesise several plant growth regulators including indole-3-acetic acid and cytokinins, which can increase the root surface absorption area resulting in a better uptake of water and nutrients (Glick *et al.* 1998, Wu *et al.* 2005). Additionally, it has been reported that rhizosphere microorganisms can differently alter bioavailability of nutrients through the release of chelating substances, acidification of the microenvironment, and by changing the redox potential, modifying soil conditions which contribute to the mobilisation and uptake of nutrient in the tissues of terrestrial plants (Marschner *et al.* 2004). Recently we have reported that colonisation of olive trees with the AM fungi *R. irregularis* increased the number of actinomycetes and decreased the level of Gram-negative and Gram-positive bacteria in mycorrhizal rhizosphere soil

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Abbreviations: AM – arbuscular mycorrhizal; Car – carotenoids; Chl – chlorophyll; DM – dry mass; ICP-AES – inductively coupled plasma atomic emission spectroscopy; FAMEs – fatty acid methyl esters; FID – flame ionization detection; M – mycorrhizal; NM – nonmycorrhizal; Pi – inorganic phosphorus; XDH – xylitol hydrogenase; XK – xylulose kinase; XR – xylose reductase.
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(Mechri *et al.* 2014). A higher concentration of glucose and trehalose and a lower concentration of fructose, galactose, sucrose, raffinose, and mannitol were also detected in mycorrhizal rhizosphere soil (Mechri *et al.* 2014). In view of the above background, the following questions were addressed: how olive trees would respond to the specific microenvironment created after the colonisation of their roots by the AM fungi *R. irregularis*? Could AM fungi contribute to the improvement of olive tree performance?

Materials and methods

Experiment description and determination of degree of mycorrhization: Experimental design used in this work was described previously (Mechri *et al.* 2014). Briefly, spores of *Glomus intraradices* DAOM 197198, now *R. irregularis* DAOM 197198 (Krüger *et al.*2012), used in this study, were inoculated in a sample of olive plantlets (15 cm long and three pairs of leaves) produced *in vitro*. After two weeks of acclimation in a greenhouse, the olive plantlets were potted into individual 10-L pots of 20 cm in diameter (one plant per pot) filled with a sandy P-poor soil (pH: 7.68; sand: 91.6%; silt: 1.5%, clay: 6.9%, C_{tot}: 4.3 g kg^{-1}, Olsen-P: 8.38 mg kg^{-1}) collected directly from an olive tree field. At the time of re-potting, 1,000 spores of *R. irregularis* were deposited directly below the roots of each plantlet. The experiment was conducted in a fully randomized block design with two treatments and three replications. Treatments consisted of nonmycorrhizal plants (NM; 0 spores of *R. irregularis*) and mycorrhizal plants (M; 1,000 spores of *R. irregularis*). The experiment was carried out under controlled greenhouse conditions. The average air temperature in the greenhouse was 25–30°C. Plants were grown under natural light. Plants were watered every second day to maintain a soil water level corresponding to 65% of the field capacity. Six months after planting, plants were harvested and leaves and roots were collected from each treatment.

For the estimation of *R. irregularis* colonization, roots were stained with Trypan blue (Phillips and Hayman 1970) and the colonization of root pieces was analysed using a stereomicroscope (*Carl Zeiss, Jena GmbH*, Germany).

Photosynthetic performance and pigment content: Photosynthetic rates was measured six months after planting by using a *LI-6400* gas-exchange system (*Li-Cor*, *Lincoln*, NE, USA) on six replications per treatment. During photosynthetic measurements, the air mean temperature was 25°C, the CO_2 concentrations were 400 µmol mol^{-1}, and the photosynthetic photon flux density was maintained at 1,500 µmol m^{-2} s^{-1}. Chlorophyll (Chl) and carotenoids (Car) were extracted by grinding 0.5 g of fresh leaves in 80% acetone. The extract was filtered and centrifuged at 15,000 × *g* for 5 min. The supernatant was collected and read at 663 and 647 nm for Chl *a* and Chl *b*, respectively, and at 470 nm for Car content (*Perkin Elmer Lambda 25*, Boston, MA, USA). The concentrations of pigments were calculated according to the equations described by Camejo *et al.* (2005).

Nutrient concentrations in the leaves: The foliar nutrient concentration was determined on dried material of M and NM plants. Total N was determined in accordance with the Kjeldahl method. For the determination of other element concentrations, about 100 mg of dry sample of leaves were ashed in a muffle furnace at 700°C for 24 h, and mineralized with HNO_3. Foliar P, Ca, K, Mg, Zn, Mn, Fe, B, and Cu were analyzed by ICP-AES (*Thermo Jarrell Ash Corp., Franklin*, MA, USA).

Total lipids in the roots: Fatty acids of M and NM roots were analyzed by gas liquid chromatography after conversion to the corresponding methyl esters. The procedure used was described by Schutter and Dick (2000) and used mild alkaline hydrolysis [0.2 M KOH in methanol] to extract whole cell fatty acids and simultaneously convert fatty acids of root lipids to methyl esters. Briefly, 30 mg of M and NM roots plants were mixed with 15 ml of 0.2 M KOH in methanol, and the preparation was incubated for 1 h at 37°C, during which ester-linked fatty acids were released and methylated. Fatty acids methyl esters (FAMEs) were extracted into an hexane organic phase, and the sample was centrifuged at 480 × *g* for 10 min to separate the aqueous and hexane phases. The hexane layer was transferred to a clean tube, and the hexane was evaporated off, after which FAMEs were resuspended in 250 µl of hexane for analysis. Samples (1 µl) of the hexane phase were separated by gas chromatography (GC) on a *HP-5MS* capillary column (30 m × 0.25 mm) and quantified using a flame ionization detector (*6890*, *Agilent*, USA). The following temperature program was set: 60°C for 1 min, from 60 to 160°C at 10°C min^{-1}, from 160 to 270°C at 5°C min^{-1}, and finally remained at 270°C for 2 min in order to clean the column.

Soluble carbohydrates in the leaves and roots: Soluble carbohydrates were extracted according to the method described by Bartolozzi *et al.* (1997). Briefly the soluble carbohydrates from composite leaves and roots samples were extracted twice in 80% ethanol at 70°C. Extracts were dried and converted into trimethylsilyl ethers with a silylation mixture made up of pyridine, hexamethyldisilazane, and trimethylchlorosilane. Soluble carbohydrates were analyzed using a *Hewlett-Packard 5890 series II* gas chromatograph equipped with a flame ionization detection (FID) system and a *HP-5MS* capillary column (30 m × 0.25 mm). Injector and detector temperatures were 280°C and 300°C, respectively. The following temperature program was set: 80°C for 1 min, from 80 to 170°C at 10°C min^{-1}, from 170 to 200°C at

15°C min^{-1}, from 200 to 315°C at 25°C min^{-1}, and finally 315°C for 8 min. Using this program, 23.6 min were required to elute the trimethylsilyl derivatives. Identification of individual carbohydrates was achieved by the use of the relative retention times, *i.e.*, in comparison to that of the trimethylsilyl derivates of standard carbohydrates.

Statistical analysis: The experiment was a completely randomized design with three replications. The significance of differences between mean values was determined by one-way analysis of variance (*ANOVA*). *Duncan*'s multiple range test was used to compare the means. The significance probability levels of the results are given at the $P<0.05$ level.

Results

Degree of mycorrhizal colonization: The roots of NM olive trees were observed after six months after planting, confirming the lower level of mycorrhizae (3.1%) as compared to that of the M plants (51.9%). NM olive trees had only 0.9% of arbuscule abundance as compared to that of M plants (29.5%).

Photosynthetic rate, pigment content and nutrient uptake: The rate of photosynthesis in M plants was significantly higher than that of NM plants. In this experiment, the percentage increase in the rate of photosynthesis of M compared with NM plants was 19% (Table 1). There was no significant difference in the Chl *a*, Chl *b*, and Car contents of the M plants comparing with NM plants (Table 1). In comparison with the NM plants, leaf nutrient analysis of the M plants showed significantly higher concentrations of P, K, Ca, Mg, Mn, Fe, and Zn. However, the foliar concentrations of N, Na, and Cu in the M plants were not significantly different from those in NM plants (Figs. 1, 2). The foliar B concentration decreased significantly in the M plants. The foliar B concentration in leaves of M olive trees was nearly 30% lower than that in NM trees (Fig. 2).

Soluble carbohydrates and total lipids: Analyses of leaf extracts from M and NM plants revealed that mannitol was the predominant sugar compound of the total amount of soluble carbohydrates (Table 2). The amounts of glucose, fructose, galactose, mannose, arabinose, rhamnose, xylose, inositol, mannitol sucrose, trehalose, and raffinose in the leaves of the M plants were not significantly different from that in the leaves of NM plants (Table 2).

Similarly, the most abundant sugar in M and NM roots was mannitol (Table 3). Soluble carbohydrates in roots showed a significant change under inoculation of olive trees with *R. irregularis*. The M roots contained significantly higher contents of fructose, mannitol, sucrose, trehalose, and raffinose, whereas the amount of mannose, arabinose, rhamnose, inositol, glucose, and galactose in the roots of the M plants were not significantly different from that in the roots of NM plants. A significant decrease of xylose was observed in M compared with NM roots (Table 3). The amount of total lipids in the roots of M plants was significantly higher than that of NM plants. In this experiment, the percentage increase of total lipid amount was 24% in the roots of M plants compared with NM plants.

Table 1. Photosynthetic rate, chlorophyll *a,* chlorophyll *b*, and carotenoid contents of mycorrhizal and nonmycorrhizal olive tree plants. The effect of *Rhizophagus irregularis* treatment was tested with one-way *ANOVA* (mean value ± SE, $n = 6$ for photosynthesis, $n = 3$ for chlorophylls, $n = 3$ for carotenoids), and mean values in individual line followed by *the same letter*(s) are not significantly different at $P<0.05$ (*Duncan*'s test).

Parameter	Treatment	
	Mycorrhizal	Nonmycorrhizal
Photosynthetic rate [μmol m^{-2} s^{-1}]	27.5 ± 1.56^{A}	22.2 ± 0.4^{B}
Chlorophyll *a* [μg mg^{-1}]	0.89 ± 0.1^{A}	0.77 ± 0.04^{A}
Chlorophyll *b* [μg mg^{-1}]	0.39 ± 0.09^{A}	0.36 ± 0.03^{A}
Carotenoid [μg mg^{-1}]	0.23 ± 0.02^{A}	0.20 ± 0.01^{A}

Discussion

The present study showed a significant difference in the percentage of root colonization between inoculated and uninoculated olive trees. This finding is consistent with that of Meddad-Hamza *et al.* (2010) who found higher percentage of olive trees root colonization using *R. irregularis* (70%). Seifi *et al.* (2014) observed a significant difference in the percentage of root colonization between the cultivars. Koroneiki (66%) showed higher colonization as compared to Valanolia (60.4%), which corresponded well with most of the growing attributes showing higher growth in Koroneiki. In this experiment, noninoculated olive trees also showed some degree of colonization, which may be due to contamination with some local AM species existing in the greenhouse environment. Such root colonization in control plants was also observed in previous studies (Krishna *et al.* 2006, Eftekhari *et al.* 2012).

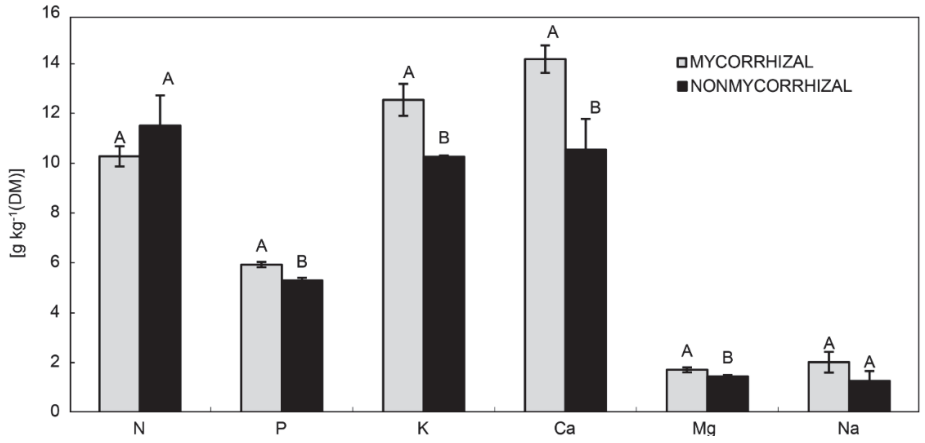

Fig. 1. The foliar nitrogen, phosphorus, potassium, calcium, magnesium, and sodium concentrations of mycorrhizal and nonmycorrhizal olive trees. Bars represent the mean of each treatment, and error bars indicate standard deviation (n = 3). Means with *different letters* are significantly different at P<0.05 (*Duncan*'s test).

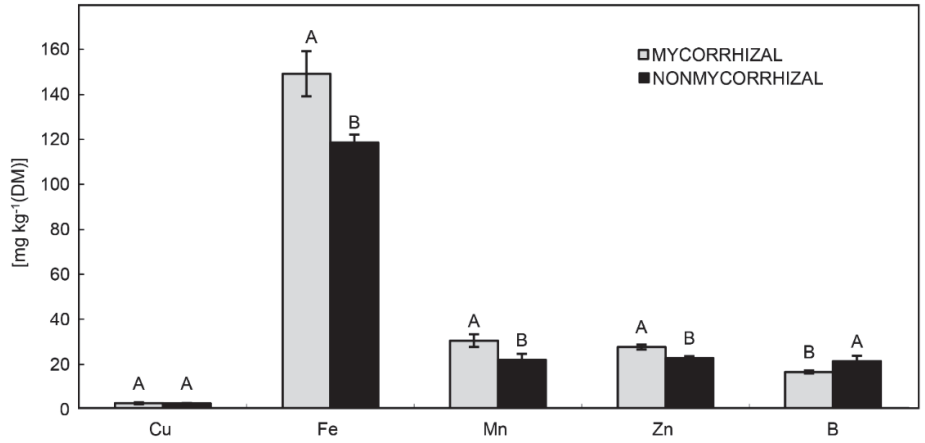

Fig. 2. The foliar copper, iron, manganese, zinc, and boron concentrations of mycorrhizal and nonmycorrhizal olive trees. Bars represent the mean of each treatment, and error bars indicate standard deviation (n = 3). Means with *different letters* are significantly different at P<0.05 (*Duncan*'s test).

Table 2. Sugar contents in source leaves of mycorrhizal and nonmycorrhizal olive tree plants. The effect of *Rhizophagus irregularis* treatment was tested with one-way *ANOVA* (mean value ± SE, n = 3), and mean values in individual line followed by *the same letter* are not significantly different at P<0.05 (*Duncan*'s test).

Carbohydrates [μg mg^{-1}]	Treatment	
	Mycorrhizal	Nonmycorrhizal
Arabinose	0.34 ± 0.1^{A}	0.27 ± 0.04^{A}
Rhamnose	0.54 ± 22^{A}	0.25 ± 0.04^{A}
Xylose	0.89 ± 0.22^{A}	0.88 ± 0.12^{A}
Fructose	2.58 ± 0.39^{A}	4.27 ± 2.12^{A}
Galactose	6.98 ± 1.38^{A}	9.21 ± 1.07^{A}
Glucose	8.93 ± 1.09^{A}	11.1 ± 2.6^{A}
Mannitol	34.8 ± 7.04^{A}	38.2 ± 3.23^{A}
Myo-inositol	3.07 ± 0.67^{A}	3.51 ± 0.47^{A}
Sucrose	4.17 ± 1.4^{A}	4.67 ± 0.95^{A}
Trehalose	0.40 ± 0.07^{A}	0.42 ± 0.09^{A}
Raffinose	2.53 ± 1.05^{A}	2.43 ± 0.55^{A}

***R. irregularis* increased the rate of photosynthesis and modified carbohydrate profiles in olive trees**: The present study showed that the rate of photosynthesis in the M plants was significantly higher than that of NM plants. In the present study, Chl was not significantly different in the M plants compared with NM plants. There are numerous reports describing AM-induced enhancement of the photosynthetic rate (Harris *et al.* 1985, Boldt *et al.* 2011). It has been hypothesized that the increased sink strength of mycorrhizal roots leads to faster removal of sugars from leaves which would enable higher photosynthetic rates (Wright *et al.* 1998, Kaschuk *et al.* 2009).

In this experiment, the percentage increase in the rate of photosynthesis of M compared with NM plants was 19%. This is in good agreement with previous studies which have shown that the additional amount of CO_2 assimilated by M compared with NM roots ranged from 4 to 20% of the total net CO_2 fixed by the plants (Harris *et al.* 1985, Wright *et al.* 1998). This suggest that the additional assimilate gain was used to support the growth and maintenance of the fungi.

Table 3. Sugar contents in roots of mycorrhizal and nonmycorrhizal olive tree plants. The effect of *Rhizophagus irregularis* treatment was tested with one-way *ANOVA* (mean value ± SE, $n = 3$), and mean values in individual line followed by *the same letter* are not significantly different at $P<0.05$ (*Duncan*'s test).

Carbohydrates [μg mg^{-1}]	Treatment	
	Mycorrhizal	Nonmycorrhizal
Arabinose	0.03 ± 0.02^{A}	0.03 ± 0.01^{A}
Rhamnose	0.06 ± 0.01^{A}	0.06 ± 0.01^{A}
Xylose	0.10 ± 0.01^{B}	0.15 ± 0.02^{A}
Fructose	0.67 ± 0.08^{A}	0.45 ± 0.02^{B}
Galactose	0.46 ± 0.06^{A}	0.42 ± 0.11^{A}
Glucose	2.39 ± 0.73^{A}	1.98 ± 0.43^{A}
Mannitol	14.2 ± 1.27^{A}	6.85 ± 0.31^{B}
Myo-inositol	0.99 ± 0.31^{A}	0.94 ± 0.11^{A}
Sucrose	4.03 ± 0.27^{A}	2.74 ± 0.45^{B}
Trehalose	0.68 ± 0.16^{A}	0.32 ± 0.05^{B}
Raffinose	0.60 ± 0.09^{A}	0.43 ± 0.04^{B}

In this study, we demonstrated that pools of mannitol, glucose, fructose, sucrose, and raffinose in the leaves of M and NM olive trees were similar. However, the pools of mannitol, fructose, sucrose, and raffinose were changed in M compared with NM roots. Roots of the M plants contained a significantly higher cncentrations of fructose, mannitol, sucrose, trehalose, and raffinose. We suggest that the pattern of carbon allocation within the M plants was altered so that an increased proportion of assimilated carbon was partitioned to the roots of M plants, which was needed for energy metabolism, maintenance, and growth of AM fungi. It is assumed that the increased sink strength of mycorrhizal roots leads to enhanced translocation of sugars from source leaves which would enable higher photosynthetic rates (Kaschuk *et al.* 2009). Consequently the increase of mannitol, sucrose, and raffinose in the M plants may be explained by this mechanism. It is known that mannitol, sucrose, and raffinose are the major phloem-translocated carbohydrates in *Olea europaea* (Flora and Madore 1993, Conde *et al.* 2007, Rejšková *et al.* 2007).

The comparison of M with NM roots revealed that the fructose content was higher in the M plants, while glucose content was not affected. We suggested that a significant proportion of sugars was used by the mycorrhizal fungus, because only the concentration of fructose increased, while the concentration of glucose, which is mainly transferred towards the fungus, was nearly constant. These results are in agreement with previous studies of Wright *et al.* (1998) and Boldt *et al.* (2011), who also suggested that glucose may be the main form of carbohydrates utilized by AM fungi (Shachar-Hill *et al.* 1995, Bago *et al.* 2000).

The fungus-specific sugar trehalose increased by 52.7% in M roots compared with the NM plants. In M roots, the glucose is incorporated by the AM fungus into trehalose and glycogen (Shachar-Hill *et al.*1995, Bago *et al.*2000), consequently the amount of observed trehalose may represent a considerable allocation of the plant's carbon to the fungus.

The decrease of xylose in M compared with NM roots may be explained by the need of AM fungi to acquire more carbon to support their increased metabolism. Indications of the use of xylose as a carbon source in the AM symbiosis are found in the work of Schliemann *et al.* (2008) who described an increased content of xylitol in *M. truncatula* mycorrhizal roots. Using ^{14}C-labeled sugars, Helber *et al.* (2011) showed that the symbiotic partner of plants *R. irregularis* DAOM 197198 expresses a sugar transporter (MST2) which is able to transport not only glucose but also xylose, mannose, and fructose with decreasing affinity in that order. The authors studied the expression of genome of *R. irregularis* DAOM 197198 for genes related to xylose catabolism during different stages of the fungal life cycle and identified two xylose reductases (XR1 and XR2), one xylitol dehydrogenase (XDH) and one xylulose kinase (XK). Our data could explain the unexpected results that artificially induced invertase activity in roots does not alter the AM fungal colonization status (Schaarschmidt *et al.* 2007), suggesting that glucose is not the only carbon sources and/or that enough carbohydrates are available.

Analysis of lipids revealed that total lipid content of M roots was significantly higher than that of NM roots. We suggest that these data indicate that sugars were used for lipid synthesis and for the production of the large extrametrical mycelium. In our study, the mycelium length in the soil as estimated using the phospholipid fatty acid 16:1ω5 was significantly higher in rhizosphere soil with mycorrhizal treatment compared to rhizosphere soil with nonmycorrhizal treatment (*see* Mechri *et al.* 2014). It has been shown that the mycelium length in the soil correlated most closely with the content of phospholipid fatty acid 16:1ω5 in the soil (Olsson *et al.* 1997). Previous experiments have shown a higher content of lipids in M roots (Wright *et al.* 1998, Bago *et al.* 2002). Lipids play a key role in the transport of assimilates from the plant–fungus interface (the arbuscule, in this case) to the extra-radical mycelium, where they are broken down and used as an energy source (Pfeffer *et al.* 1999, Bago *et al.* 2002).

***R. irregularis* increased the contents of P, K, Ca, Mg, Zn, Fe and Mn in M olive trees**: The present study showed that N uptake was not significantly different in the M plants compared with NM plants. There is evidence that AM fungi play a role in the uptake of nitrate and ammonium, which are assimilated and transported within the mycelium as arginine (Olsson *et al.* 2005), but compared with ectomycorrhizas, rates of N uptake by AM hyphae are too small to contribute substantially to plant N nutrition (Smith and Read 2008).

The results obtained from this study indicated that leaf P content of the M plants was higher than that of NM plants. Several studies have demonstrated that plants colonized by mycorrhizal fungi are much more efficient in taking up soil P than noninoculated plants (Black *et al.*

2000, Bücking and Shachar-Hill 2005, Abdel-Fattah *et al.* 2014). To our knowledge, one of the reasons for the increase of leaf P in the M plants may be due to the increase in the number of actinomycetes in mycorrhizal rhizosphere soil (*see* Mechri *et al.* 2014). It has been reported that actinomycetes, by excretion of chelating substances, such as siderophores, which form stable complexes with phosphorus adsorbents, increase phosphate solubilisation (Welch *et al.* 2002, Hamdali *et al.* 2008a). Hamdali *et al.* (2008b) revealed that the presence of the actinomycete strains in the soil had the greatest stimulatory effect on P content of wheat tissues and plant growth in comparison with the control.

A possible and another explanation for the increase of leaf P content of M plants could be related to the high trehalose content in M roots. In this experiment, the percentage increase of the content of trehalose was 52.7% in M compared with NM plants. The higher fungal carbohydrate trehalose content in M roots enhances the remobilization of polyphosphates. The remobilization of polyphosphates increases the intracellular inorganic phosphorus (P_i) concentration in the hyphae (Bücking and Heyser 2003), and thereby promotes P_i efflux through the fungal plasma membrane into the interfacial apoplast. Consequently, the increase of phosphorus in the M plants compared with NM plants may be explained by this mechanism.

As our findings supported, it has been reported that mycorrhizal colonization can enhance Ca (Liu *et al.* 2002), Mg (Jentschke *et al.* 2001, Liu *et al.* 2002), and K absorption by plants (Giri *et al.* 2007, Gholamhoseini *et al.* 2013). To our knowledge, the increased K, Ca, and Mg in the M plants compared with NM plants may be explained by the direct enhanced uptake, by enlarging the absorption area of root systems with extraradicular hyphae, thus shortening the distance that nutrients must travel within the soil before they reach the roots. In our study, the hyphal length in the soil, as estimated using the phospholipid lipid fatty acid 16:1ω5, was significantly higher in rhizosphere soil with mycorrhizal treatment compared to rhizosphere soil with nonmycorrhizal treatment (*see* Mechri *et al.* 2014). Olsson *et al.* (1997) reported that the content of this phospholipid was an excellent marker for estimating fungal hyphal length. Rhodes and Gerdemann (1978) found that the external hyphae of AM fungi absorbed and transported ^{45}Ca to the host plants. George *et al.* (1992) observed K depletion in a hyphal compartment colonized by *Glomus mosseae* concurrently with K accumulation in the host plant. Jentschke *et al.* (2001) indicated that the increased Mg and K concentrations in M plants can be a consequence of the increased P availability from mycorrhizal fungal activity. The authors demonstrated that the translocation to mycorrhizal plant of K and Mg was strongly reduced, when hyphal P-fluxes were ceased.

In the present study, a significant difference in Zn content was observed between M and NM olive tree plants. AM fungi inoculation increased Zn concentration by 16% in leaf tissue comparing with the nonmycorrhizal control. Marschner and Dell (1994) estimated that 25% of the Zn uptake by plants can be supplied by AM fungi. Thus, it implies that colonisation of olive trees with *R. irregularis* may improve Zn uptake from a soil characterized by high pH which is generally associated with Zn deficiency (pH of the soil used in this study was 7.68). Rillig *et al.* (2001) reported that glomalin (a glycoprotein is produced in copious amounts by external hyphae of all members of AM genera) leads to the formation of a sticky string-bag of hyphae that acts as an adsorptive site for metallic cations which may result in enhanced availability of Zn. Other studies mentioned that the AM fungi *R. irregularis* improved organic status, dehydrogenase and phosphatase activities, and modified the Zn fractionation pattern of soils that collectively contributed for the availability of Zn and may assist in alleviating Zn deficiency in crop plants (Wamberg *et al.* 2003, Subramanian *et al.* 2009). However, the mechanisms that underlie the induction of mobilisation and uptake of Zn in plants inoculated with AM fungi have not yet been elucidated.

In our investigation, the *R. irregularis* significantly increased foliar Mn concentration from 21.93 mg kg^{-1}(DM) in NM plants to 30.46 mg kg^{-1}(DM) in M plants, thus revealing the contribution of *R. irregularis* on improving Mn uptake by plants. Enhanced Mn concentrations have been reported for a range of AM fungal colonised plants (Al-Karaki and Clark 1999, Taylor and Harrier 2001). We did not measure the population density of Mn reducers in the rhizosphere, but it has been shown that AM colonization increased the population density of Mn reducers in the rhizosphere, thus increasing Mn availability to the plants and plant Mn uptake (Marschner and Timonen 2006).

Colonisation of olive trees with *R. irregularis* also increased Fe concentration in plants, suggesting that *R. irregularis* had a positive effect on the Fe nutrition of olive trees. This may reflect not only increased mobilisation from soil, but also enhanced transport to olive tree plants. It is well known that many rhizobacteria and fungi release iron chelators which can contribute to increased Fe availability to plants (Khan *et al.* 2006, Lemanceau *et al.* 2009). Santiago *et al.* (2013) reported that the changes in microbial communities rather than the increase of microbial biomass in soil can contribute to enhanced Fe accumulation in plants. Within our experimental conditions, we have reported that root colonization with the AM fungi *R. irregularis* induced significant changes in the microbial community structure of olive tree rhizosphere compared with nonmycorrhizal plants (Mechri *et al.* 2014). These change in microbial community observed in rhizosphere soil of the M plants appeared effective in improving the content of foliar Fe.

Did the mycorrhizal 'sink' for assimilates stimulate boron remobilization process? Boron mobility is species-dependent and is restricted to plant species that

translocate photosynthates as polyols (Liakopoulos *et al.* 2009). These carbohydrates readily form stable diesters with boric acid (Hu *et al.* 1997), thereby facilitating its translocation from source leaves to growing tissues. The lower content of boron in the M plants compared with NM plants may occur due to the formation and transport of complexes between boron and mannitol. The results presented here indicated that the rate of mannitol export from leaves of the M plants, due to AM-enhanced 'sink' demand for assimilates, was enhanced, as suggested by the elevated mannitol content in M roots. Furthermore, if more mannitol was continuously created in source tissues of the M plants compared with control, as suggested from the elevated photosynthetic rate, and loaded into the phloem, then the amounts of mannitol in the phloem of M plants should be increasing. This could increase the rate of mannitol–borate complex formation and facilitated the phloem mobility of boron. Hu *et al.* (1997) found that leaf phloem mannitol could be considered as a principal factor determining boron remobilisation since it forms complexes of boric acid and renders it mobile in the phloem. Consequently the decrease of boron in the M plants compared with NM plants may be explained by this mechanism. Liakopoulos *et al.* (2009) demonstrated that a quantitative relationship exists between mannitol translocation and boron mobility in olive plants. Drossopoulos *et al.* (1988) have reported that mannitol can be allocated to bark tissue of *Olea europaea* during autumn. Such circulation could promote a recycling of nutrients such as boron. The historical evidence for a role of AM in sugar transport and a possible effect of AM fungi on boron mobility described herein are intriguing and deserving a further study.

Conclusion: The results of this study demonstrated that inoculation of olive tree roots with *R. irregularis* induced several changes in physiological parameters that influence olive tree performance, particularly photosynthetic rate, nutrient uptake, and carbohydrate contents in leaves and roots. The increased sink strength of mycorrhizal roots led to faster removal of sugars from leaves which induced higher photosynthetic rate and increased concentrations of some sugars (fructose, mannitol, sucrose, trehalose, and raffinose) in the M plants compared with NM plants. The enhancement of root carbohydrate contents in turn led to the production of a large extrametrical mycelium which may contribute to the improvement of the nutrient status of olive trees.

Hence, our results clearly illustrated that *R. irregularis* is a good support for *Olea europaea* plants. The application of these microorganisms could be critical in the cultivation of *O. europaea* under arid and semiarid conditions, where water and the availability of nutrients in soil are the most important factors in determining plant growth and yield.

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