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Arbuscular mycorrhizal fungi increased growth, nutrient uptake and tolerance to salinity in olive trees under nursery conditions

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Summary

Inoculating olive plantlets with the arbuscular mycorrhizal fungi (AMF) *Glomus mosseae*, *Glomus intraradices* or *Glomus claroideum* increased plant growth and the ability to acquire nitrogen, phosphorus, and potassium from non-saline as well as saline media. AMF-colonized plants also increased in survival rate after transplant. Osmotic stress caused by NaCl supply reduced stem diameter, number of shoots, shoot length and nutrients in olive plants, but AMF colonization alleviated all of these negative effects on growth. *G. mosseae* was the most efficient fungus in reducing the detrimental effects of salinity; it increased shoot growth by 163% and root growth by 295% in the non-saline medium, and by 239% (shoot) and by 468% (root) under the saline conditions. AMF colonization enhanced salt tolerance in terms of olive growth and nutrient acquisition. Mycorrhizal olive plants showed the lowest biomass reduction under salinity (34%), while growth was reduced by 78% in control plants. This *G. mosseae* effect seems to be due to increased K acquisition; K content was enhanced under salt conditions by 6.4-fold with *G. mosseae*, 3.4-fold with *G. intraradices*, and 3.7-fold with *G. claroideum*. Potassium, as the most prominent inorganic solute, plays a key role in the osmoregulation processes and the highest salinity tolerance of *G. mosseae*-colonized olive trees was concomitant with an enhanced K concentration in olive plants.

Abbreviations: AMF, arbuscular mycorrhizal fungi.

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Introduction

Olive (*Olea europaea*) trees are traditionally grown on infertile land because this species is adapted to poor soils and drought (Barranco et al., 1999). In Spain, olives are considered the country's most important crop and are widely grown. The area given over to olive trees is approximately 2,476,000 ha distributed in zones with different productions varying according to the ecological characteristics of each area.

The major factors affecting crop production in Mediterranean agro-ecosystems are water and nutrients (N, P, K). Water availability has been recognized as the most critical determinant of crop productivity in Mediterranean zones. Thus, it is necessary to improve the level of efficiency in the plant capture and/or use of nutrients (N, P and K) by this important crop. On the other hand, an excess of nutrients applied to crops to maximize olive productivity is also a powerful driving force of environmental damage. Mechanisms determining the balance between nutrients to maximize productivity and minimize pollution risks will be required in the future. Olive crops have received far less attention than many others prevailing crops in non-Mediterranean areas.

New olive orchards are usually planted with mist-propagated plants derived from hardwood cuttings. This propagation system is very efficient (Caballero-Mellado and del Río, 1997). Shortening the juvenile period of these plantlets is, however, very important from an economic standpoint. Soriano-Martín et al. (2006) reported that olive trees of the Cornicabra cultivar showed greater growth and a shorter juvenile period when colonized with arbuscular mycorrhizal fungi (AMF). In addition, growth during the nursery period must be encouraged. Tolerance or resistance to transplantation may also be improved by these fungi (Ruíz-Lozano et al., 1996). Olive trees have great adaptability to adverse soil conditions and are typically grown on marginal soils with low fertility.

Water deficit and osmotic imbalance are the most common stresses affecting crops in arid and semi-arid regions; this is the situation faced by olive trees growing across most of the Mediterranean basin. This is particularly true in Spain. Nutrient deficiencies and excess salinity can also inhibit plant growth. Plants are stressed in three ways by salinity: (1) low water potential in the root medium leads to water deficits in plants, (2) the toxic effects of ions, mainly Na and Cl, and (3) nutrient imbalance caused by depression in uptake and/or transport (Marschner, 1995). Consequently, crop productivity on saline soils is relatively low (Feng et al., 2002). Osmotic stress interferes with growth and has a negative effect on productivity and survival (Shen et al., 1999). Indeed, olive production is often limited by drought and salinity (Estaún et al., 2003; Ganz et al., 2002; Rinaldelli and Mancuso, 1998). When plants enter into a symbiotic relationship with AMF, however, this can lead to morphological and physiological changes that increase stress tolerance and improve the cellular conservation of water (Porcel et al., 2006). Water deficit reduces the absorption power of the roots, but colonization with AMF seems to compensate for this (Barea and Jeffries, 1995; Ruíz-Lozano et al., 1996).

Tolerance to osmotic stress in plants is a complex phenomenon and involves many changes at the biochemical and physiological levels (Ingram and Bartels, 1996). However, the mechanisms behind the modulation of tissue water conductivity and osmotic adjustment (which help maintain tissue water potential (Bohnert et al., 1994) appear to be affected by AMF colonization (Ruíz-Lozano and Azcón, 2000). Rabie and Almadini (2005) suggested that AM fungi protect leaf metabolism from Na^+ toxicity. Osmotic stress affects plants at different stages of growth, and the effect of salinity on plant metabolic activities may change according to the AMF used. Thus, the selection of an effective fungal strain could improve plant development under stress.

The effects of *Glomus* species on olive tree production have been studied in micropropagated plants (Calvente et al., 2004). Soriano-Martín et al. (2006) showed how colonization with AMF can shorten the length of the juvenile period of olive plantlets obtained by mist propagation. Although their results were limited, they appear to confirm that colonization with such fungi increases growth both before and after transplant.

Many hectares of land devoted to olive production in Spain suffer the problems of drought and salinity. Saline water is considered to be an alternative source of irrigation for agriculture in countries suffering from a shortage of fresh water, for example in the Mediterranean zone. Thus, the use of new biological methodologies is a necessary and practical way to improve agricultural plant tolerance under salinity. Studies have found that AM symbiosis can alleviate the stress of salinity on plant growth. Nevertheless, the effects of AM colonization on trees growth under saline conditions have not yet been reported.

In this study, we used as test plant *Olea europea* L. cultivar Cornicabra that is the second var at the level of cultivated area. This cultivar is notable for its high oleic acid composition (77%) and total

polyphenols and tocophenols (which reached 488 and 215 ppm, respectively) and their concentrations are higher than those determined in commercial olive oils such as Hojiblanca and Arbequina varieties (García et al., 2003). Its oil is popular and has a very high stability. Cornicabra oil, due to its organoleptic properties, is one of the most common olive oils sold (Aranda et al., 2004).

In Spain, new plantations are established with plants obtained via the mist propagation of hand wood olive cutting. This model of plant production has been widely adopted by nursery growers owing to the advantages it offers (Caballero-Mellado and del Río, 1997).

The present study therefore examined the effects of root colonization by drought-adapted *Glomus* species (isolated from the rhizosphere of olive trees) on olive trees in terms of post-transplant survival, biomass production (dry weight, leaf and root area, maximum length of stem, stem diameter and number of shoots), and nutrient acquisition under non-saline and saline soil conditions.

Materials and methods

The experiment had control and AM inoculation treatments with *Glomus mosseae*, *Glomus intraradices* or *Glomus claroideum*. Some treatments received a salt solution (6 g NaCl kg^{-1}) sequentially added to substrate and some treatments did not receive such solution. The treatments were replicated 50 times and a total pots were 400 placed in a randomized complete block design.

In this study, the substrate used in all assays was a mixture (1:1; v:v) of washed river sand and white Sphagnum peat. The sand had a particle size <0.3 mm, porosity 40%, pore index 0.67, specific mass 1.19 g/cm^3 at a moisture level of 25%, and specific mass 1.99 g/cm^3 when saturated. The peat, which had a maximum particle size of 20 mm (with a maximum of 40% of grains <1 mm in diameter) was mixed with 4 kg/m^3 calcium dolomite. This peat had a water retention capacity of 67% of its volume, porosity 95%, dry density 70 kg/m^3, organic matter content 96%, and ash content 4%. Analysis of a 1:5 (v:v) mixture of peat and water returned a pH of 5.9, and an electrical conductivity (EC) of 4.0 ds/m. The substrate was tyndallized in an autoclave for 1 h on two consecutive days before use.

The plants used were obtained by mist propagation according to the method of Porras et al. (1998). Propagation was performed in a tunnel with a controlled environment (perlite substrate, temperature 22 ± 2 °C, air temperature 20 ± 2 °C) using semi-woody olive (cv. Cornicabra) cuttings. Mist was produced by 0.8 mm mirror nozzles at a pressure of 0.25 MPa. The cuttings undergoing propagation were 15 cm long and had three pairs of leaves at the top end. The bottom basal end of each cutting was immersed in an ethanol solution containing 4 g of indol-3-butyric acid/L to favor root development.

The three species of AMF used were: *G. mosseae* (Nicol and Gerdem) (BEG 119), *G. intraradices* (Schench and Smith) (BEG 123) and *G. claroideum* (Schench and Smith) from the EEZ collection. These were isolated locally from dry infertile soil (Spain), propagated by the Dept. of Microbiology, Estación Experimental del Zaidín, Granada (Spanish Research Council-CSIC), and maintained in sterile substrate formed by a mixture of sand and vermiculite (1:1, v:v) using maize and alfalfa as host plants. Inoculum from each AM fungus possessed similar infective characteristics.

The experimental procedure followed was as follows: Semi-hardwood cuttings of cv. Cornicabra olive trees were planted in the propagation tunnel on July 15, 2004, where they remained for $2\frac{1}{2}$ months. Selected plants were then divided into four blocks and transplanted into 120 cm^3 pressed peat pots containing the prepared substrate. During this procedure, three blocks were inoculated with one of the three AMF species by placing 5 g of inoculum (mixed rhizosphere samples containing 75% of infected roots and approximately 40 spores/g of inoculum) directly in the substrate at the position of the roots. One block of control plants was left uninoculated. Each control pot received 5 ml of 5 g inoculum filtrate that was sieved through a 25 μm filter in an attempt to provide similar microbial populations (excluding AM fungi) in all treatments.

The cuttings were then left in the tunnel under the same conditions for another 2 months to harden. At the end of this period the number of plants lost was determined (first transplant). One hundred plants were then taken from each block and divided into two subgroups of 50. These plants, still in their pressed peat pots, were transplanted into 2.5 L polythene pots filled with substrate prepared as above, and either subjected or not subjected to NaCl application, resulting in the following treatments: Controls and AM-inoculated plants with either *G. mosseae*, *G. intraradices* or *G. claroideum*. These final blocks were assayed without salt or with 6 g NaCl kg^{-1}.

From the time of transplant, 50 mL of Hewitt's solution (Hewitt, 1952) was supplied every month to each pot.

After transplant NaCl was added to the pots in the form of 250 mL of a 60 g/L NaCl solution in distilled water. To avoid percolation losses and osmotic shock, this solution was sequentially added over 5 applications (alternative days supplying 50 mL per day) from the day of transplant. Each pot/plant received 15 g of NaCl.

These olive plants (one plant per pot) were then acclimatized to field conditions, and the substrate moisture level of the pots was maintained at 60% of the field capacity. Water was supplied by micro-sprays and the irrigation system was controlled automatically via a mercury tensiometer modified according to Porras-Piedra et al. (1991).

Olive plants were grown under field conditions. After 1 year of transplanting, the 40 plants in each treatment subgroup (one plant per pot) were examined and the following variables recorded: maximum length of the stem, number of shoots, and stem diameter 5 cm above the substrate. They were then uprooted and the following values recorded: percentage of AMF colonization, leaf area and root area (measured as an area parallel to the long axis of the plant), the dry weight of the aerial parts of the plant, the dry weight of the roots, and the leaf N, P and K concentrations.

The percentage colonization of the roots was determined using the root staining method of Phillips and Hayman (1970) and the colonized/non-colonized fields method of Mcgonigle et al. (1990).

Mycorrhizal dependence (based on dry matter yield) was calculated using the following equation:

$$\frac{\text{Dw of AM plants} - \text{Dw of non-AM plants}}{\text{Dw of AM plant}} \times 100$$

Nutrient utilization efficiency was defined as the amount of biomass produced per unit of nutrients in plant tissues. It was calculated by the ratio

$$\frac{\text{Shoot biomass}}{\text{Nutrients(\% or content)}}$$

The maximum height of each plant was measured using a tape (precision 0.25 mm). The diameter of the stem (5 cm above the substrate) was measured using a digital calliper (MIT) (precision 0.01 mm). The number of shoots was visually counted.

The dry weight of the aerial part of the plant and the root system was determined by drying them separately in an oven (Memmert) at 75 °C until a stable weight was achieved (measured with a balance [GIBERTINI precision 0.01 g]).

The leaf and root areas were determined using an HP ScanJet 6300C scanner connected to a PC. The images acquired were digitized and stored as bitmap files (BMP) (resolution 640 × 480 pixels).

The leaf macronutrient concentrations were determined in a specialized laboratory, following the method of Lachica et al. (1973) to measure the N content using a flow injection atomizer. We measured P by spectrophotometric analysis using the vanadate–molybdate–yellow method (Olsen et al., 1965), and K by atomic absorption spectrometry.

The influence of the AM fungi on post-transplant plant loss, and on plant loss during acclimatization for the 3 months after adding the salt to the substrate was examined using the χ^2 test ($\alpha = 0.05$). To compare the influence of the fungi on the growth variable measured and the assimilation of nutrients, Fisher's multiple range test was used (with a 5% risk of considering any pair of means significant when in fact they were not).

Results

Survival rate

As shown in Table 1, colonization with any AMF assayed increased the chances of survival after the first transplant from the rooting bed to the pressed peat pots. Similarly, the highest percentage of AM-colonized plants survived after being transplanted (second transplant) into the 2.5 L pots with or without saline solution applied. In both transplants, AM-inoculated plants survived in the highest proportion relative to control non-inoculated olive plants.

Plant growth and root colonization

Compared to the non-inoculated plants, inoculations with any of the three *Glomus* species increased shoot and root dry weight under

Table 1. Effect of AM inoculation (with *G. mosseae*, *G. intraradices* or *G. claroideum*) on the percentage of olive plants survival after transplanting.

Treatments	Percentage of plants survival		
	First transplant	Second transplant	
	–	–	NaCl
Control	94.7b	88c	84b
G. mosseae	97.3a	100a	92a
G. intraradices	96.7a	94b	90a
G. claroideum	97.3a	94b	90a

Values in columns followed by different letters are significantly different ($p \leqslant 0.05$).

Table 2. Shoot and root dry weight (g), surface area (SA, cm^2) and shoot/root ratio of control and AMF-inoculated (with *G. mosseae*, *G. intraradices* or *G. claroideum*) olive plants growing under saline or non-saline conditions.

Treatments	Shoot		Root		Shoot/root ratio
	dw (g)	SA (cm^2)	dw (g)	SA (cm^2)	
No NaCl					
Control	3.00c	66.98c	0.75c	80.58d	4.00a
G. mosseae	7.89a	206.60a	2.96a	282.36a	2.66b
G. intraradices	4.94b	135.26b	2.05b	225.60b	2.41b
G. claroideum	4.97b	131.56b	1.99b	195.37c	2.50b
NaCl					
Control	1.89c	46.98c	0.44c	63.23c	4.00a
G. mosseae	6.41a	151.98a	2.50a	240.20a	2.66b
G. intraradices	3.95b	107.54b	1.46b	170.60b	2.41b
G. claroideum	4.05b	94.56b	1.62b	169.66b	2.50b

Values in columns followed by different letters are significantly different ($p \leqslant 0.05$).

Table 3. Number of shoots per plant, stem diameter and maximum length of stem (cm) of control and AMF-inoculated (with *G. mosseae*, *G. intraradices* or *G. claroideum*) olive plants growing under saline or non-saline conditions.

Treatments	No NaCl			NaCl		
	No shoots	Stem diameter (mm)	Maximum length of stem (cm)	No shoots	Stem diameter (mm)	Maximum length of stem (cm)
Control	13.8c	3.79c	16.7c	12.4c	3.44c	15.2c
G. mosseae	54.4a	6.10a	42.5a	47.6a	5.24a	37.8a
G. intraradices	36.2b	4.93b	34.8b	24.1b	4.43b	27.2b
G. claroideum	36.7b	4.91b	32.8b	25.1b	4.52b	26.2b

Values in columns followed by different letters are significantly different ($p \leqslant 0.05$).

both non-saline and saline conditions (Table 2). The magnitude of the growth response to AMF colonization was more effective in improving root development than shoot development, and particularly under the salt-stress conditions (Table 2). The increase achieved in olive tree biomass by the colonization with the most effective fungus, *G. mosseae*, ranged from 163% (shoot) to 295% (root) in the non-saline substrate, and from 239% (shoot) to 468% (root), respectively, under the saline conditions. The other two AM fungi assayed also increased root and shoot biomass, but to a lesser extent (Table 2).

The shoot dry weight of the non-colonized plants was 37% lower as affected by the salinity, while in the AM-colonized plants, this negative effect of NaCl supply was only about 20% irrespective of the colonizing fungus (Table 2). Similarly, the reduction in olive root dry weight in the saline substrate compared to the non-saline substrate was more than two-fold greater in the non-inoculated than in the inoculated (particularly by *G. mosseae*) olive plants (Table 2).

The most effective AM fungus in increasing olive growth and salt tolerance (in terms of growth) was *G. mosseae*. The shoot/root ratio was lower in the AM-colonized plants (Table 2).

Shoot and root surface areas were increased by all three fungal species (Table 2).

The shoot and root weights of *G. mosseae*-colonized olive trees under saline conditions were 114% (shoot) and 233% (root) greater than in control plants growing under non-salinity (Table 2). AM colonization also led to enhanced shoot number, stem diameter and shoot length in olive plants (Table 3). Inoculation with *G. mosseae* increased these growth values more than with *G. intraradices* or *G. claroideum*. The main effect of AM colonization, however, was the increased number of shoots produced (Table 3). In fact, *G. mosseae* inoculation increased the no. of shoots by 294% and by 61% the stem diameter. All of the positive effects from AMF colonization on olive tree biomass production in the juvenile period were particularly important for olive performance under the saline as well as non-saline conditions, and these results showed that olive trees

were highly dependent on AM colonization to reach the optimal growth (Tables 2 and 3). These results indicate the high mycorrhizal dependence of olive plants, particularly under the salt-stress conditions. The plant growth dependence on mycorrhizal symbiosis was greatest when olive plants were AM-colonized by *G. mosseae*. Nevertheless, irrespective of the colonizing fungus, the AM dependence, to reach optimum growth, increased to the highest extent under salinity (Table 4).

Mycorrhizal root colonization

Small differences were observed among the three fungal species in terms of their ability to colonize the olive roots (expressed as % in Table 4). Moreover, AM fungal infectivity was not reduced by the presence of NaCl in the medium. Nevertheless, differences associated with the AM fungus involved were observed with respect to the total amount of AM roots colonized (Table 4).

Table 4. AM colonization [% and total root length (cm)] and mycorrhizal dependence (MD) (based on dry matter) of AM-colonized olive plants growing under saline or non-saline conditions.

Fungal species	(%)		Total length		MD	
	–	NaCl	–	NaCl	–	NaCl
Control	0	0	0	0	0	0
G. mosseae	94.4a	94.0a	266.5a	225.8a	62.0a	70.5a
G. intraradices	92.6b	91.4b	208.9b	155.9b	39.3b	52.1b
G. claroideum	92.4b	91.0b	180.5b	154.4b	39.6b	53.3b

Values in columns followed by different letters are significantly different ($p \leqslant 0.05$).

Table 5. Shoot N, P and K concentration and content (A) and nutrients utilization efficiency (B) of control and AMF-inoculated (with *G. mosseae*, *G. intraradices* or *G. claroideum*) olive plants growing under saline or non-saline conditions.

Treatments	No NaCl			NaCl		
	N	P	K	N	P	K
A						
Concentration (%)						
Control	0.77a	0.10a	0.40a	0.84a	0.08a	0.32a
G. mosseae	1.05b	0.14b	0.61c	0.90b	0.13b	0.60c
G. intraradices	0.97b	0.14b	0.54b	0.87b	0.127b	0.52b
G. claroideum	0.90b	0.14b	0.55b	0.97b	0.120b	0.55b
Content (mg)						
Control	23.0a	3.00a	12.00a	15.87a	1.51a	6.05a
G. mosseae	82.84c	17.70c	48.13c	57.65c	8.33c	38.46c
G. intraradices	47.91b	6.91b	26.67b	34.60b	5.01b	20.54b
G. claroideum	44.73b	6.96b	27.33b	39.28b	4.86b	22.22b
B						
Concentration (%)						
Control	3.89a	21.4a	7.5a	2.25a	23.6a	5.9a
G. mosseae	7.50c	56.3c	12.9b	7.12c	49.3c	10.7c
G. intraradices	5.10b	35.5b	8.31a	4.50b	31.1b	7.60b
G. claroideum	5.52b	35.5b	9.03a	4.17b	33.7b	7.36b
Content (mg)						
Control	0.13b	1.00c	0.25b	0.11b	1.25c	0.31c
G. mosseae	0.09a	0.44a	0.16a	0.11b	0.77a	0.17a
G. intraradices	0.10a	0.71b	0.18a	0.11b	0.79a	0.19a
G. claroideum	0.11a	0.71b	0.18a	0.10b	0.83b	0.18a

Values in columns followed by different letters are significantly different ($p \leqslant 0.05$).

Table 6. Percentage of shoot and root biomass (g) and N, P, K (mg) reduction by NaCl in control and AM-inoculated (with *G. mosseae*, *G. intraradices* or *G. claroideum*) olive plants.

Treatments	Shoot	Root	N	P	K
Control	37.0a	41.7a	31.0a	50.0a	50.0a
G. mosseae	19.0b	15.5c	30.4a	52.9a	20.1c
G. intraradices	20.0b	29.8b	27.8a	27.5b	28.0b
G. claroideum	18.5b	18.6c	19.0b	19.0c	19.0c

Values in columns followed by different letters are significantly different ($p \leqslant 0.05$).

Plant nutrition

The olive shoot N, P and K contents (Table 5) showed trends similar to those for growth in terms of variance as a function of the treatment applied. Colonization by *G. mosseae* increased the content of these nutrients more than the other two fungi did. The greatest differences among AM-inoculated plants were found in K concentrations or contents. Table 5 shows the nutrient utilization efficiency, and these results indicate the high effectiveness of all AM fungi on the use of these macronutrients by olive plants, particularly when colonized by *G. mosseae*. Curiously, this fungal effect was more obvious under saline-stress conditions, where nutrient acquisition by olive plants is more limited. Moreover, the detrimental effects of salt on nutrient acquisition was reduced by AM colonization (Table 6). The presence of salt reduced the contents of N, P and K by 31%, 50% and 50%, respectively, in the non-AM-colonized control plants, but these values were reduced to 28% (N), 27.5% (P) and 28% (K), respectively, in plants colonized with *G. intraradices* (Table 6). However, the most active fungus in increasing NaCl tolerance was *G. mosseae*, and it increased the N, P and K contents by 363%, 552% and 636%, respectively, over those values recorded in non-colonized olive plants under saline conditions. Curiously, the effect of *G. mosseae* on the percentage of N and P reduction by salt application was similar to that observed in control plants, while the K reduction was 2.5-fold lower in *G. mosseae* colonized than in control plants under saline conditions (Table 6).

Discussion

The finding that AMF-colonized olive plants grew more under saline conditions than did the non-colonized plants under non-saline conditions is very important information for Mediterranean agronomy. These results evidence the high mycorrhizal dependence of olive trees to reach optimum development, particularly under stress conditions. To decrease the unfavorable effects of salinity on olive growth, the use of AM inoculation ought to be considered as biological method to alleviate soil salinity, as it has been previously reported to do in other herbaceous crops (Giri and Mukerji, 2004; Rabie and Almadini, 2005).

Natural/human-induced soil salinization is one of the main factors reducing the soil fertility of vast areas of the Mediterranean basin. As was observed in olive plants, salt reduces plant development and production in areas suffering from water limitation and osmotic stress, as do areas in the Mediterranean regions. Tolerance to salinity by olive trees, one of Spain's most important crops, is of great importance since salt concentration negatively correlates with yield (Ruíz-Lozano and Azcón, 1995). Thus, salinity tolerance by plants is a major concern in many countries. Given that AMF colonization appears to increase salt tolerance, the encouragement of symbiotic associations between these fungi and olive trees may be recommended in the future as a biotechnological practice in oliviculture.

In the present study, *G. mosseae* was the most efficient fungus in terms of olive tree performance, and particularly in the protection offered against the detrimental effects of salinity, although all of the fungi used offered important protection. Previous studies have also reported that the successful development of olive plants is highly dependent on an appropriate selection of AMF fungus (Soriano-Martín et al., 2006). Differences among AMFs with respect to the plant protection offered against salinity have also been reported by Cantrell and Linderman (2001) and Al-Karaki (2006). In previous studies, the mechanisms by which two AM fungi alleviated salt stress on lettuce plants appeared to be based on physiological processes (Ruíz-Lozano et al., 1996).

The presence of salt did not have a negative affect on AMF colonization (expressed as a percentage), although the total length of AMF-colonized roots was highly reduced as a consequence of reduced root length by NaCl supply.

Mycorrhizal pre-inoculation of olive cultivar Arbequina plants growing in different potting mixes in two different nursery experiments improved plant growth and crop yield up to 3 years after the inoculation (Estaún et al., 2003). In this cultivar (Arbequina), *G. intrardices* was more efficient at promoting plant growth than both *G. mosseae* and the native endophytes present in the orchard soil. The relevance of mycorrhizal fungal origin and host

plant genotype to induce growth and nutrient uptake has been recognized previously (Monzón and Azcón, 1996). These results indicate that specific compatibility relationships exist among symbionts, and underscore the importance of host-endophyte selection to maximize growth and nutrition of olive trees. AM symbiotic efficiency attributed to olive plants is dependent on plant cultivar and AM fungal species. The selection of the most suitable AM fungus for a specific plant variant is of practical interest for improving the effectiveness under particular environmental conditions.

With respect to the total length of AM colonization, a specific AM fungus-olive plant interaction that resulted in an increased growth response was observed. AM fungi differ in their ability to enhance nutrient uptake even when the extent of AM colonization is similar (Azcón et al., 1991; Ruíz-Lozano and Azcón, 2000). Specific mechanisms conferring functional differences could be expected from changes in fungal characteristics such as length of external mycelium, hyphae distribution, and/or nutrients translocation. In agreement with these ideas, Jakobsen et al. (1992) proposed a consistent relationship between the total length of root colonized by each fungus and the ability to alleviate the problems of nutrient limitation caused by the saline conditions. Feng et al. (2002) related the mycorrhizal tolerance to salt stress to higher accumulation of soluble sugars in plant roots.

Both the growth and nutrient content of the AMF-colonized olive plants were increased. Nevertheless, the notably high K intake of the AM-colonized plants might indicate that salt tolerance is afforded through such accumulation. When soil water is limited, as under saline conditions, plants suffer a loss of turgor and wilting – typical symptoms of K deficiency. As a solute in the vacuole, K plays an important role in the control of water relations, helping to maintain a high tissue water level, even under osmotically impaired conditions. Thus, the greatest K accumulation in the AM-colonized olive tissues could make a major contribution towards maintaining the osmotic potential of their cells and tissues. Colonization by *G. mosseae* increased the K content in olive plants 4-fold compared to non-colonized plants, while *G. intraradices* and *G. claroideum* increased this value by 2.2- and 2.3-fold, respectively. Indeed, in the olive plants grown on the non-saline substrate, potassium content was greater than in those grown on the saline substrate. The enhancement of K uptake by mycorrhizal olive plants under salinity can change the detrimental effect of Na on plant growth. Moreover, AM symbiosis is able to reduce the excess acquisition and/or translocation of Na and Cl to shoot tissue (Giri and Mukerji, 2004), but shoot Cl or Na uptake was not determined in the present study. It is well documented that potassium, as the most prominent inorganic solute, plays a key role in osmoregulation processes, in stomatal regulation and photosynthesis. The increase of these plant physiological activities may help explain the important growth effects obtained in *G. mosseae*-colonized olive plants under salinity conditions (Tian et al., 2004).

In higher plants, K^+ affects photosynthesis at various levels. The role of K^+ in CO_2 fixation has been demonstrated, and an increase in the leaf potassium content is accompanied by increased rates of photosynthesis, photorespiration and RuBP carboxylase activity, and a concomitant decrease in dark respiration. Enhanced respiration rates are a common feature of potassium deficiency (Bottrill et al., 1970).

Potassium is one of the most important inorganic solutes, and has an important role in processes such as water balance, cell extension, and solute transport in the xylem. Cell extension is the consequence of K^+ accumulation. In the plant cells, potassium is required not only for stabilizing pH^+ in the cytoplasm, but also for increasing the osmotic potential in the vacuole. Results from this study show the highest salt tolerance of mycorrhizal plants having the greatest K shoot concentration. This may be related to the stomatal regulation by K, which is a major mechanism controlling the water regime in the plant. In addition, potassium as osmotic solute is able to maintain a high tissue water level even under conditions of osmotic deficiency. These mycorrhizal effects in olive trees are particularly important, because no information has been available. Rabie and Almadini (2005) observed an enhancement of K uptake by AM plants under salinity, as also observed in this study. Nevertheless, Poss et al. (1985) reported that K uptake was affected little by AM colonization in plants grown under saline conditions.

When *G. intraradices* was the colonizing fungus, K acquisition by olive plants in the saline medium ranged from 3.4-fold that of non-colonized plants, and reached 6.4-fold in plants colonized by *G. mosseae*. The effect of NaCl salinity on olive biomass reduction (or protein synthesis reduction) might be due to the sensitivity of control olive plants to Cl^- toxicity and Na^+/K^+ imbalance. But in the most salt tolerant olive plants such as those that were AM-colonized, particularly by *G. mosseae*, the replacement of K^+ by Na^+, which allowed osmotic adjustment in leaves, may be the responsible factor. The higher K^+/Na^+ ratio is one of the determinants of plant salt tolerance (Naidoo and Naidoo, 2001; Thomas et al., 2003).

Marschner (1995) suggested that improved salt tolerance depends on selective ion uptake, allowing osmotic adjustments to be made and balanced nutrition to be achieved. Changes in the internal transport or storage of Na^+ or Cl^- might also explain the increased salt tolerance in AM-colonized plants (Al-Karaki, 2000; Baker et al., 1995).

The increased nutrient uptake observed may be explained by the fact that the extraradicular mycelia of AMF often extend some 7–10 cm or more into the soil beyond the rhizosphere, where they can absorb water and nutrients under different conditions of osmotic potential (Ruíz-Lozano and Azcón, 1995).

Salt tolerance as observed in AM-colonized olive plants may be produced by various integrated strategies with morphological, physiological and biochemical features. Levels of chloride and sodium in particular in the shoot normally increase with the external NaCl supply, but potassium concomitantly declines due to cation competition. Such induced K deficiency is an unlikely cause of growth depression and impaired osmoregulation. But in olive plants in this study, the mycorrhizal effect in enhancing K acquisition compensated for such a detrimental salt effect.

In addition, the importance of AM symbiosis in improving olive root development, particularly in nutrient-depleted soils under osmotic stress is of great interest.

In conclusion, the present results show that, especially under saline conditions, AMF colonization enhances plant uptake of essential nutrients, leading to a greater root surface area and biomass. AMF can alleviate and compensate for the growth limitations imposed by saline conditions; they therefore play an essential role in olive tree growth and biomass production. The encouragement of symbiotic association between AMFs and olive trees is therefore of great interest in olive production, especially in areas where soils are saline, as in many arid and semi-arid regions. The management of this symbiosis is a biotechnological procedure of major interest in the commercial production of olive trees.

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