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Full Length Research Paper

Arbuscular mycorrhizal fungi improve the growth of olive trees and their resistance to transplantation stress

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Two native Algerian mycorrhizal fungi (*Glomus mosseae* and *Glomus intraradices*) were tested for their effect on the growth of micropropagated olive tree (*Olea europaea* L.). The effect of inoculation of plantlets with *G. mosseae* was also compared with chemical fertilization using osmocote. Specific molecular techniques were then used to detect the presence of the two fungi. Highly significant increases in growth were evident for inoculated plants compared with uninoculated ones. For a slightly lower shoot growth, *G. mosseae* doubled the root growth of the inoculated plantlets, compared to that of the fertilized plants. This change in the root: shoot ratio permitted greater utilization of soil resources and strengthened the plant's capacity to resist transplantation shock and water stress. The abundance of the two fungi in the roots of wild olives just as in the inoculated olives is indicative of the predominance of *G. intraradices* when the natural microflora is present.

Key words: Arbuscular mycorrhizal fungi, olive tree, inoculation, chemical fertilizer, transplantation, growth.

INTRODUCTION

The olive tree is a particularly mycotrophic plant (Roldan-Fajardo and Barea 1986) and plays an important part in the economy of several countries in the Mediterranean region where there is predominant water stress. These regions have a long dry season where low water availability has a large impact, notably in the transport and the uptake of the solutes needed for vegetative growth (Monneveux and This, 1997).

Mycorrhizal fungi, which are active in the rhizosphere, take part in the cycles and transfer of the mineral elements in the soil and into the roots (George et al., 1992). Certain minerals such as phosphorus, iron, zinc and copper are of very limited mobility in the soil and are only found in extremely low concentrations in soil solution. Their use by plants may be increased by the presence of symbiotic microflora, notably mychorrhizal fungi, which assist their nutrition, growth (Gianinazzi et al., 1982; Smith and Read, 1997; Jeffries et al., 2003; Duponnois et al., 2005) and their tolerance to different types of biotic and abiotic stress (Rosendahl and Rosendahl, 1991; Caravaca et al., 2003a; Al-Karaki and McMichael, 2004; Selosse et al., 2004).

In vitro production of micro-plantlets of the olive is a developing biotechnology that is well-suited to new methods of plant production. The aim of the technique is to eliminate pathogenic microorganisms, but it also eliminates beneficial ones such as mycorrhizal fungi, which help plants cope with transplantation stress (Ruız-Lozano et al., 1996; Porras-Soriano et al., 2009) once they are placed into normal culture conditions. Routine inoculation of young plants in the nursery has been a suggested strategy (Plenchette, 2000).

The inoculation of *in vitro* plantlets with arbuscular mycorrhizal (AM) fungi to improve their growth has been studied for many different cultures (Lovato et al., 1996;

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Abbreviations: AM, *Arbuscular mycorrhizal*; **MD,** mycorrhizal dependence; **PCR,** polymerase chain reaction; **ITS,** internal transcribed spacer; **MF,** mycorrhizogenic fungi; **rDNA,** ribobosomal deoxyribonucleic acid.

Verma and Arya 1998; Caravaca et al., 2003a; Ouahmane et al., 2007). Results vary according to the mycorrhizal strain and plant cultivars (Azcon-Aguilar and Barea, 1997; Calvente et al., 2004; Soriano et al., 2006; Binet et al., 2007). This emphasizes the importance of testing the effectiveness of different mycorrhizal fungi to create the appropriate inoculum for production of top-quality olive plants.

To study the potential of the mycorrhizal fungi *Glomus mosseae* and *Glomus intraradices* to stimulate the growth of micropropagated olive plants and to compare their capacity to resist water stress after re-potting with that of plantlets receiving chemical fertilizer (osmocote), controlled experiments were carried out using strains of *G. mosseae* and *G. intraradices* isolated from Algerian olive plantations. Molecular tools were used in parallel to assist in the search for the appropriate inoculum capable of co-existence with the natural field community, by detecting the different taxons of native Glomeromycetes that colonize the root system of the wild olive *Olea oleaster* (Hoffm and Link) grown in nurseries. This latter approach is widely-used as root-stock in commercial olive growth in this country. A molecular detection was also carried out on *in vitro* plantlets of the Aglandau variety of the olive-tree with an aim of comparing the presence of *G. mosseae* and *G. intraradices* inoculated separately in controlled conditions and in absence of the native microflora.

The study objectives were to evaluate the mycorrhizogenic potential of *G. mosseae* and *G. intraradices* on the growth of micropropagated olive plants, compare the capacity to resist water stress after re-potting of *in vitro* cultivated olive plantlets that received osmocote with that of plantlets inoculated with *G. mosseae* and to detect mycorrhizogenic fungi by molecular methods for their study in the field.

MATERIALS AND METHODS

Extraction of spores of arbuscular fungi and controlled inoculation

G. mosseae and *G. intraradices* spores used in these experiments were initially extracted by wet sieving (Gerdemann and Nicholson, 1963) using soil from Algerian olive groves situated in the commune of Bekouche Lakhdar, wilaya de Skikda (36° 71' N and 7° 29' E). *G. mosseae* (Nicol. and Gerd.) Gerdemann and Trappe and *G. intraradices* Schenck and Smith identified both by morphological and molecular criteria, were chosen according to their abundance in the olive tree rhizosphere and their abundance after trapping and monospecific multiplication on the leek (*Allium porrum* L.). They were inoculated in a sample of olive plantlets (variety Aglandau) produced by the experimental group of INRA *in vitro* (Dijon, France). After 2 weeks of acclimatization in a greenhouse, the plantlets were potted into individual 400 ml pots filled with a sterile mix of soil and gravel (3:1, v:v). The soil had been previously γ-irradiated radiation (10 kGy) and the gravel steam-disinfected in an autoclave (120°C for 2 h). The soil (pH 8.0; 20 ppm P_2O_5) was from the domaine of Epoisses (INRA Center at Dijon-Bretenieres). At the time of re-potting, each plant was inoculated with 100 spores of *G. mosseae* or *G. intraradices*; five duplicates were made for each treatment and uninoculated plants were kept as a control. The experiment was carried out in controlled greenhouse conditions (under normal daylight for around 12 h, average daily temperature 18 - 22°C, 60 - 70% relative humidity). The plants were watered daily with distilled water. After 9 months growth in individual pots, the height, number of inter-nodes and aerial and root biomass (fresh and dry; drying at 70°C for 1 week) were measured. The efficiency of the mycorrhizal inoculum was calculated as the percentage increase in height, number of inter-nodes, root fresh mass, aerial fresh mass, root dry mass and aerial dry mass produced by each AM fungus over the uninoculated control. The mycorrhizal dependence (MD) of the plants was found using the formula of Plenchette et al., (1983): 100 × [(dry mass of mycorrhizal plants -dry mass of control plants) ÷ dry mass of mycorrhizal plants].

Test of resistance to water stress

After acclimatization in the greenhouse and at the time of re-potting into the 400 ml pots, 10 olive plantlets (variety Aglandau) were inoculated with 25 carpophores of *G. mosseae* per plant. Ten other plants were treated with chemical fertilizer; for this, a granule of 2.5 g of osmocote was placed on the soil surface in each pot. The substrate was the same as that used in the previous experiment. Daily watering was made according to the needs in the field. The osmocote fertilizer was composed of 15% nitrogen, 10% anhydrous phosphate, 15% potassium oxide, 2% magnesium oxide, 4.5% sulphur, 0.02% boron, 0.05% copper, 0.15% iron, 0.075% manganese and 0.015% zinc. After 6 months growth, all plants were again re-potted, this time into 1 L pots with the same soil mixture and the osmocote granules were removed. From this point onwards watering was reduced. The soil water content was kept at 10% of the field capacity. A month after re-potting, the fresh mass of the aerial and root growth was measured and the ratio of root: shoot growth was recorded.

Staining and estimation of mycorrhizal colonization

Endomycorrhizal colonization was estimated on the set of inoculated plantlets used in the experiment. Five repetitions of 1 g of root samples taken from each plant were incubated in a 10% solution of KOH at 90°C for 1 h and then stained with Trypan Blue following the method of Phillips and Hayman (1970). AM colonization was estimated for 30 root fragments of 1 cm length mounted in a drop of glycerol and observed in a photonic microscope, with annotation according to the method of Trouvelot et al. (1986) using the MYCOCALC computer programme (www.dijon.inra.fr/mychintec/Mycocalc-prg/download.html).

Statistical analysis

Results are presented as the mean ± standard deviation. Comparisons of means between inoculated and uninoculated plants were made for all the parameters tested and were distinguished by the Dunnett test ($P < 0.05$). Comparisons between plants inoculated with *G. mosseae* and those inoculated with *G. intraradices* were made by the Turkey test ($P < 0.05$). Between group comparisons of those plants inoculated with *G. mosseae* and those fertilized with osmocote were made using Student's T test at a significance level of 5%.

Table 1. Effect of inoculation with *G. mosseae* and *G. intraradices* native to Algeria on the growth of olive plants.

Inoculation	Parameters measured [z]							
	H (cm)	NInt	RFM (g)	AFM (g)	RDM (g)	ADM (g)	M (%)	MD (%)
GM	51.87 ± 5.32*a*	18.25 ± 4.03 *a*	6.66 ± 1.25 *a*	9.44 ± 1.74 *a*	2.78 ± 0.70 *a*	4.89 ± 1.31 *a*	51.06 ± 7.4	73.9
GI	46.9 ± 6.99 *a*	20.4 ± 3.51 *a*	4.09 ± 1.10 *a*	7.75 ± 1.33 *a*	1.80 ± .0.54 *a*	3.37 ± 0.55 *a*	69.96 ± 4.5	67.3
UI	9.25 ± 1.76	5.25 ± 0.96	1.42 ± 0.34	1.96 ± 0.26	0.44 ± 0.12	1.12 ± 0.15	/	/

GM: *G. mosseae*; GI: *G. intraradices*; UI: uninoculated; H: Height; NInt: number of internodes; RFM: root fresh mass; AFM: aerial fresh mass; RDM: root dry mass; ADM: aerial dry mass; M: intensity of colonization by arbuscular mycorrhizal fungi; MD: mycorrhizal dependency.
[z]Mean ± standard error, n = 5; *a* indicates results that are not significantly different (P < 0.05).

Molecular detection of arbuscular fungi

With the aim of using molecular tools to detect inoculated fungi and those existing naturally in the soil, two groups of plants were created. One group was comprised of olive plants (Aglandau variety) obtained from *in vitro* culture growth on sterile soil for 9 months after inoculation with *G. mosseae* or *G. intraradices* spores (100/plant). Another group was comprised of oleaster plants, *Olea oleaster* (Hoffm. and Link) grown in polyethylene bags containing 5 kg of natural soil from the Belkhir nursery (Algeria). Root samples were taken from all plants in each group and were mixed to make one single sample for each experimental group. From this sample, five sub-samples of 100 mg each of root material were used for the extraction of ribosomal DNA (rDNA) and approximately 1 g was used to estimate the level of mycorrhizal colonization (M%).

Extraction and purification of rDNA

One hundred milligrams (100 mg) of each root sample was crushed in 1.5 ml Eppendorf tubes using a micro-pestle. The DNA was extracted and purified using a Macherey-Nagel NucleoSpin kit, following the protocol furnished by the supplier and collected in 50 µl of ultra-pure water.

Polymerase chain reaction (PCR) amplification of rDNA

Double amplification (nested PCR) allowed amplification of a targeted part of the large subunit covering domains D1 and D2 of the 5' region and the internal transcribed spacer (ITS) region of the rDNA root samples. Nested PCR was carried out using the universal primer ITS3 (White et al.,1990) and the primer specific to fungi, FLR2 (Trouvelot et al., 1999) at the first PCR and the specific primers for the different genera and species of fungi AM at the second PCR, which were FLR3/FLR4 (Gollotte et al., 2004) for the detection of the set of mycorrhizogenic fungi (MF), GloITS/LR3rev for the detection of the *Glomus* genus, 8.24/FLR3 (Farmer et al., 2007) for the detection of *G. intraradices*, 5.25/FLR4 (van Tuinen et al., 1998) for the detection of *G. mosseae*, 23.5/FLR4 mixed primers for the detection of *Scutellospora*/*Gigaspora* and Acaul/FLR4 for the detection of *Acaulospora*. Five repeats were made. For a reaction volume of 20 µl, the PCR product contained 2 µl of 10× buffer (100 mM Tris-acetate pH 9, 500 mM KCl, 1.5 mM $MgCl_2$, 0.1% Triton X-100 in phosphate buffer saline (PBS), 2 mg/ml bovine serum albumin, 2 µl of 1 mM dNTP, 0.1 µl of each primer (100 mM), 0.05 µl of Taq polymerase (15 U/µl, Qbiogene) and 14.85 µl of ultra-pure water. Root DNA extracts were undiluted of diluted (1:10, 1:100, 1:1000, 1:10 000) and 1 µl of each suspension was added to 19 µl of PCR mixture. To check on the effectiveness of PCR, a control using water was used for the first and second PCR runs. The amplification was carried out in a Biometra thermocycler programmed in 15 cycles. The PCR programme consisted of an initial denaturation for 5 min at 95°C, then each cycle included denaturation of the double stranded DNA (94°C for 1 min), hybridization (58°C for 1 min) and polymerization (72°C for 1.5 min) for cycles 1 - 14. The last cycle was followed by a final elongation at 72°C for 10 min. The amplified product was used in 1 µl volumes for a second amplification. The second PCR programme involved 30 cycles with the same steps as before. PCR amplimers were separated by electrophoresis in a 1.4% agarose gel using 7 µl mixed with one drop of the appropriate buffer and placed into the wells of the gel. Three microliters (3 µl) of 1 kb ladder marker were coincidentally used. After migration at 100 V for 25 min, the gel was stained with ethidium bromide and observed under ultraviolet transilluminance. The DNA was checked by observation of fluorescent bands.

RESULTS

Effectiveness of *G. mosseae* and *G. intraradices* on olive plant

The data in Table 1 demonstrate the strong dependence of the olive *(Olea europea* L.) on mycorrhizae and the positive effect that these had on the species. After 9 months growth, all olive plants inoculated with *G. mosseae* or *G. intraradices* showed greater development than uninoculated plants. The former displayed greater growth in height, number of inter-nodes and fresh and dry weights for all aerial parts than the uninoculated plants. *G. mosseae*, while having a significantly lower level of colonization (51.06%) than *G. intraradices* (69.96%), had a marked effect on growth of the olive plant. The values for gain in total fresh weight were 376% for *G. mosseae* and 226% for *G. intraradices* (Table 2). But presently, despite the strong mycorrhizal dependence for *G. mosseae* (Table 1), the one-sided analysis of variance did not show any significant difference for all the measured parameters in the two groups of plants inoculated with these fungi. Both proved to be beneficial for growth. It should also be noted that the positive effect of these two species of fungi could arise from ecological compatibility. Fungi collected in the rhizosphere of olive groves seem to

Table 2. Efficiency of inoculation by *G. mosseae* and *G. intraradices*.

Inoculation	H (%)	Nint (%)	TFM (%)	RFM (%)	AFM (%)	RDM (%)	ADM (%)
GM	460	247	376	369	381	531	336
GI	407	288	226	188	295	309	200

GM: *G. mosseae*; GI: *G. intraradices*; UI: un-inoculated; H: Height; NInt: number of internodes; TFM: total fresh mass; RFM: root fresh mass; AFM: aerial fresh mass; RDM: root dry mass; ADM: aerial dry mass.

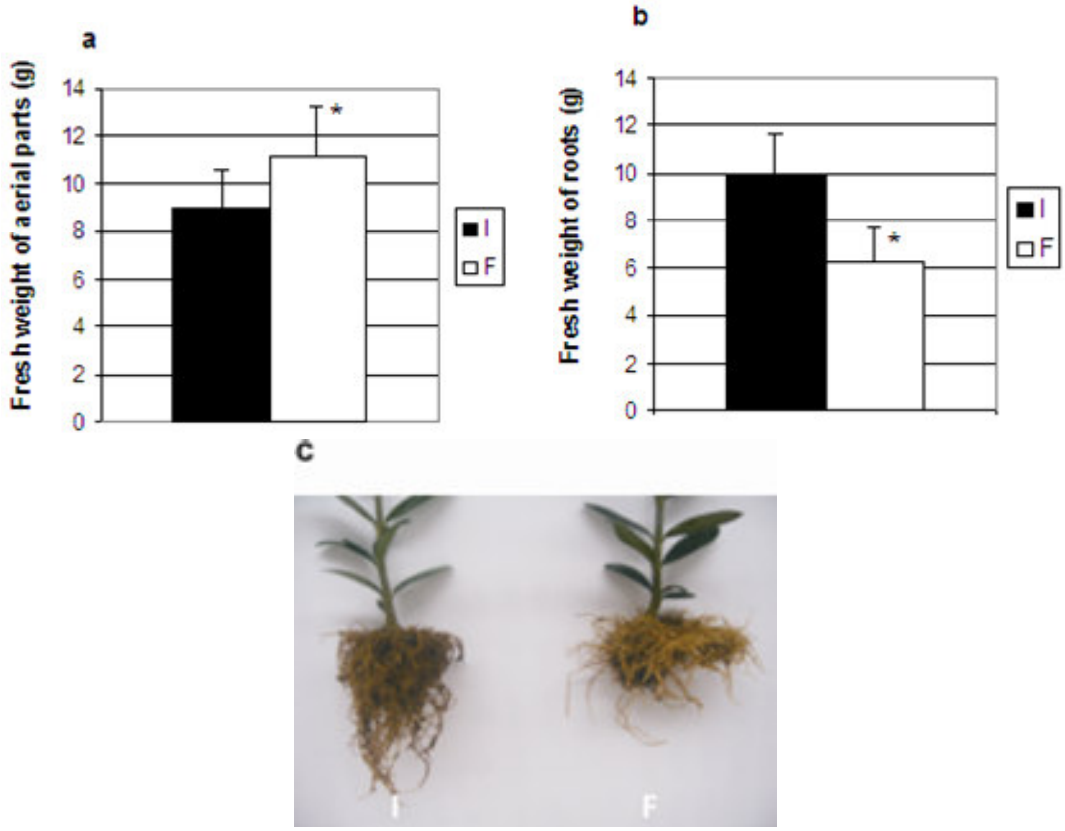

Figure 1. Fresh weight of shoot (a) and roots (b) and morphology of the root system (c) of plants inoculated with *G. mosseae* (I) and those fertilized with osmocote (F). *: I vs. F ($P < 0.05$).

act beneficially on the genotypes of the trees.

Effect of arbuscular mycorrhization on resistance to water stress after re-potting

Comparison of plants inoculated with *G. mosseae* with those given chemical fertilizer (osmocote) allowed the positive effects of mycorrhization to be assessed in several aspects. One aspect concerned the effect on root development. For significantly lower development of aerial parts (Figure 1a), the root system of mycorrhizal plants was more developed (nearly double) compared to plants receiving osmocote (Figure 1b) and presented a different morphology (Figure 1c). Another aspect concerned the effect on tolerance to the stress of re-potting. A week after re-potting, observations made on plants inoculated with *G. mosseae* and those with chemical fertilizer showed a clear difference in reaction to repotting shock. Plants receiving fertilizer seemed to have experienced a shock that led to loss of weight, withering of aerial parts and a loss of leaves, while inoculated plants maintained their turgidity and vigour (Figure 2). These results could be explained by the presence of the fungus, which had a positive effect on the root to shoot ratio (Figure 3a). The effect was significantly higher (almost double) for mycorrhizal plants. A more highly developed and dense root system allowed the plant greater exchange between the roots and the aerial parts and more rapid water uptake (Figure 3b). The consequence of this was greater

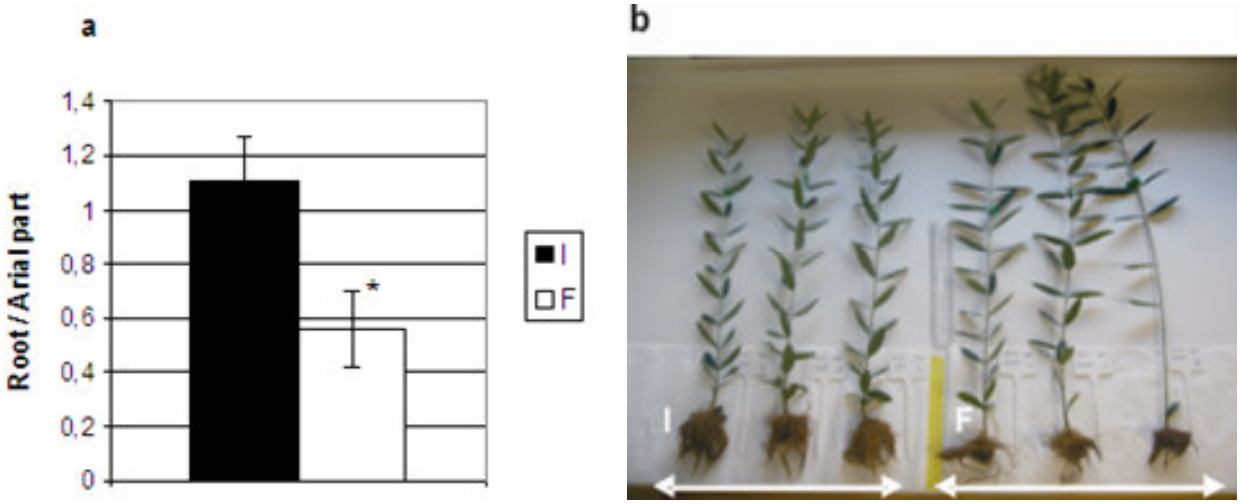

Figure 3. Relationship between root and aerial growth in plants inoculated with *G. mosseae* (I) and fertilized plants (F). * I vs. F ($P < 0.05$).

Figure 2. Signs of shock in a chemically-fertilized plant (F) compared with a plant inoculated with *G. mosseae* (I).

resistance both to the stress of re-planting and to water stress.

Molecular detection of mycorrhizal fungi in the wild olive and in the Aglandau variety of cultivated olive

The use of molecular probes for the detection of the set of mycorrhizogenic fungi on the roots of the wild olive revealed fluorescent bands at a dilution of 1:100 for the five biological repeats (Figure 4a). At the same time, M% was estimated at 19.72. The use of specific molecular probes for the *Glomus* genus revealed that the roots of the wild olive were colonized by this genus; a positive signal was obtained for the five repetitions carried out (Figure 4b). Also, *G. intraradices* was more abundant than *G. mosseae*, producing a positive signal at a 1:100 dilution for the five repetitions, while with *G. mosseae*, the fluorescent bands were apparent in only the initial sample dilution and then in only two repetitions (Figures 4c and d). A positive signal was also observed with the use of mixed probes specific to *Scutellospora/Gigaspora* until dilution 1/100 for two repetitions only. At the three other repetitions, there is no signal after dilution 1:10 (Figure 4e).

No band was detected for the genus *Acaulospora* (Figure 4f). In contrast, using the same approach with DNA extracted from the roots of micropropagated Aglandau and inoculated in pots with spores of *G. mosseae*, a positive signal was obtained up to a dilution of 1:10 000 (Figure 5a). The use of a specific probe for *G. intraradices* confirmed our first interpretation. A more intense signal was obtained at the 1:10 000 dilution in Aglandau inoculated into pots with spores of *G. intraradices* (Figure 5b). The degree of mycorrhizal colonization by the latter was 69.96%. The observations were consistent with the suggestion that *G. intraradices* appears more colonizer than G. *mosseae*.

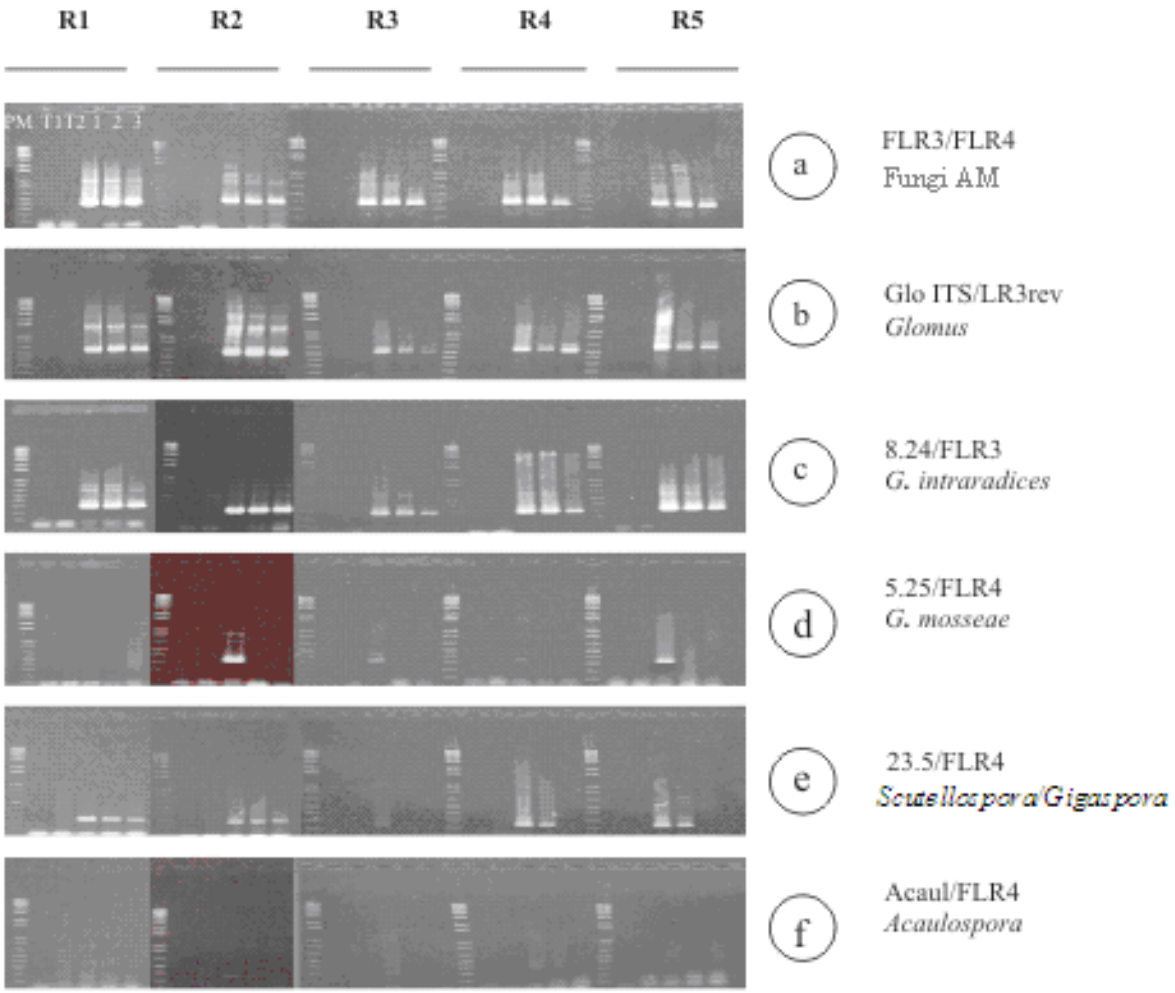

Figure 4. PCR detection of native AM fungi in oleasters. The detection was carried out using the FLR3/FLR4 primers for the entire set of mycorrhizogenic fungi, MF, GloITS/LR3rev (Genus *Glomus)*, 8.24/FLR3 *(G. intraradices)*, 5.25/FLR4 *(G. mosseae*), 23.5/FLR4 *(Scutellospora*/*Gigaspora*) and Acaul/FLR4 *(Acaulospora*). PM: molecular weight marker 1kb ladder; T1: water control for first PCR; T2: water control for second PCR. For each repetition (R1-R5), there is T1, T2 and three preparations (undiluted, 1:10, 1:100).

DISCUSSION

The inoculation with *G. mosseae* or *G. intraradices* showed a better development of the inoculated plantlets compared to the controls. These results agree with those of Caravaca et al. (2003b) who tested effects of the two fungi on the olive and those of Porras-Soriano et al. (2009) who studied the efficiency of the same fungal species on the Cornicabra variety of olive. However, the high level of AM colonization (> 50%) for these two strains of *Glomus* could bear no actual direct linkage to the efficiency of the fungi (Guissou et al., 1998; Requena et al., 2001; Calvente et al., 2004).

Several studies on other varieties of olive tree (Binet et al., 2007; Porras-Soriano et al., 2009) demonstrated the superiority of *G. mosseae.* This shows the importance of controlled mycorrhization in the micropropagation of olives after their transfer from *in vitro* conditions to growth in the soil.

Much research is currently aimed at improving the quality of vegetative production while at the same time preserving the environment. Inoculation with effective mycorrhizogenic fungi could represent a worthwhile alternative to treatment with chemical fertilizer. Underscoring this potential, we presently observed that inoculation of olive plantlets with the two strains of native Algerian mycorrhizal fungi produced increases in production of total vegetative matter by as much as 376% for plants inoculated with *G. mosseae* and 226% for those inoculated with *G. intraradices*. Although these values were superior to *G. mosseae* and the latter fungus was more advantageous in promoting growth of the different

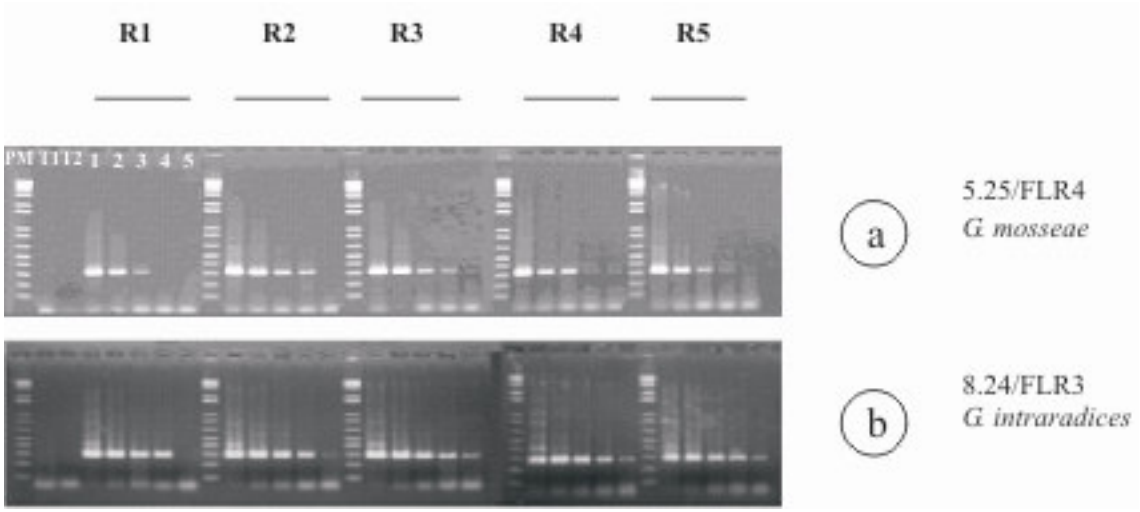

Figure 5. PCR detection of *G. mosseae* and *G. intraradices* inoculated into the cultivated olive (variety Aglandau) following 6 months of growth. The detection was carried out using the specific probes 8.24/FLR3 (*G. intraradices*) and 5.25/FLR4 (*G. mosseae*). PM: molecular weight marker 1kb ladder; T1: water control for first PCR; T2: water control for second PCR. For each repetition (R1-R5), there is T1, T2 and three preparations (undiluted, 1:10, 1:100, 1:1000, 1:10 000).

parameters measured when compared with *G. intraradices*, the differences were not significant. As far as root growth was concerned, no significant differences were currently evident between plants inoculated with *G. intraradices* and uninoculated control plants, whereas plants inoculated with *G. mosseae* displayed highly significant differences from the controls. Nevertheless, the high degree of colonization by *G. intraradices* (M% approximately 70%) certainly reflects its major hyphal network in the rhizosphere, which ensures better transport of nutrients to the host plant and causes the classic stimulation of aerial growth. It appears that the growth of aerial parts of the plant is linked to a correlation between the size of the root system and the degree of mycorrhizal colonization.

Further comparison of the results obtained by two culture techniques aimed at improving plant growth, mycorrhization and fertilization, indicated the clear superiority of mycorrhization. In fact, although the development of aerial parts of those plants inoculated with *G. mosseae* was significantly weaker than that of plants receiving osmocote, the root system showed the opposite effect. Inoculated plants showed a greater root to shoot ratio than did plants that had received the osmocote. Similar observations have been reported in other models (Tisserant et al., 1991; Berta et al., 1995; Dalpe, 2005; Porras-Soriano et al., 2009).

Tobar et al. (1994) have conclusively demonstrated that the root to shoot ratio reflects the degree of efficiency of AM fungi. Mycorrhization allows the plant to have a high root: shoot ratio, causing better hydro-mineral nutriation and thereby reinforcing the capacity to resist stress, especially the stress of transplantation (Caravaca et al., 2003b; Marschner, 1995). On the other hand, in the case of chemical fertilization, fertilizer placed near the roots makes the minerals immediately available for the plant, which reduces the need for extensive root development, resulting in a low root to shoot ratio. When the latter plants are transplanted into natural soil conditions without fertilizers, the low root to shoot ratio causes an abrupt fall in the uptake of soil solution by the plant and/or in the growth rate. This is what generally happens when young plants are moved from the nursery into the field. As Smith and Read (1997) suggest, mycorrhizal symbiosis may improve the quality of the root system, increasing the survival rate of young plants moved into the field. The suggestion was confirmed by Guissou et al. (2001) for fruit trees; mycorrhization does not improve the stress tolerance in this case, but stimulates mineral uptake and growth.

Finally, molecular biology studies of rDNA confirmed the presence of mycorrhizal fungi in the roots of olive trees, this being true for both wild olives and for the cultivated olive. The genus *Glomus* appears to be dominant over the other genera, *Scutellospora*/*Gigaspora* and *Acaulospora*, in the roots of the oleasters. *G. intraradices* is the dominant species and is the most commonly detected. *G. mosseae* varies from rare to completely absent. It is only present in trace amounts with the set of microflora in the roots of the oleasters. However, in monoculture, it is a very good root colonizer. The same result was found with *G. intraradices*; it was more frequent in monoculture than when in the natural soil microflora. These observations have been confirmed for sweet potato (Farmer et

al., 2007). Molecular detection of indigenous mycorrhizal populations in the roots of the olive tree in natural conditions is needed to link the success and the resistance that a given AM fungus can produce following its introduction. It is essential that the best inoculum be chosen to optimize the chances of root colonization and preserve the full mycorrhizogenic potential of the selected fungus.

Conclusion

This study affirms the importance of introducing mycorrhizal fungi onto olive plants produced *in vitro* and of developing the biotechnology to improve resistance to water stress after re-potting. The use of mycorrhization seems to be an extremely valid alternative to the use of chemical treatments. Trial inoculation of two mycorrhizal fungi (*G. mosseae* and *G. intraradices*) produced significantly increased growth over that of uninoculated controls. The success of the mycorrhization process appears not to depend so much on the degree of mycorrhization as on the efficiency of the fungus. This can be seen with *G. mosseae*, which, despite its lower level of colonization than *G. intraradices*, is just as good at improving growth of the olive plant.

Further, the use of mycorrhization and chemical fertilization demonstrates a clear superiority for mycorrhization, which allows the plants to obtain better nutrition from the soil, notably better water uptake and increases the capacity of plants to resist water stress after transplantation.

PCR examination of root colonization of the wild olive by the Glomeromycetes has shown the genus *Glomus* to be dominant in the roots of the oleasters. *G. intraradices* is the dominant species and is more frequently detected, with *G. mosseae* being less prominent when the microflora is complete. Both fungi colonize olive roots better when in monoculture.

Our results indicate the feasibility of *G. mosseae* and *G. intraradices* use in the production of olive plants, since both fungi are efficient at improving plant growth. However, being that *G. intraradices* appears to be more efficient and better at colonizing the roots of oleasters in the field, it would be interesting in future work to co-inoculate the two fungi into the olive and look at possible synergic effects on plant growth.

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