Effect of Endomycorrhizal Inoculum on the Growth and Protection of Olive Plants Against *Phytophthora palmivora*

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Abstract

This study demonstrated beneficial effects of the symbiotic relationship between mycorrhizal arbuscular fungi (AM) and olive trees. Young olive trees were treated with a composite mycorrhiza treatment with and without inoculation of the pathogenic *Phytophthora palmivora*. A non-inoculated control was also included. Mycorrhizal plants had greater morphology compared with non-mycorrhizal plants. Of particular interest was the fact that plants inoculated with the pathogen, in the presence of mycorrhizae, had much higher growth compared with those that were inoculated with the pathogen only, indicating disease resistance due to mycorrhizal colonization. A total of 36 mycorrhizal fungal species were isolated from the rhizosphere of mycorrhizal olive plants with a spore count of 121 spores/100 g soil, compared with 27 species and a spore density of 67 spores/100 g in the rhizosphere of plants inoculated with both *Phytophthora palmivora* and mycorrhizae. Species frequency also varied between the two treatments.

Introduction

The olive tree (*Olea europaea* L.) is a characteristic species of the Mediterranean landscape (Dahbia 2009) and plays a very important socio-economic and environmental role in several countries of this region (Abousalim et al. 2005). Olive trees are integral for the maintenance of ecological continuity, the reduction of greenhouse gas production, the fight against erosion, the valorization of agricultural land, and sustainability of tree populations in mountain areas (Angles 2016). In Morocco, the olive production area has increased from 773,000 ha in 2009 to more than 1,000,000 ha in 2016 (Sadiki 2016) with nearly 1,500,000 metric tons of olives generated per year (El Mouhtadi et al. 2014). In addition, olive production actively contributes to the income of 7 million families and the settlement of the rural population by creating more than 11 million working days (MAMVA 1996). Olive cultivation, however, is experiencing several problems such as pests and diseases (Chliyeh et al. 2014a, Zouiten et al. 2001) and environmental stresses under prolonged spring and summer drought (Khabou et al. 2009, Meddad 2010, Semane et al. 2017).

The rhizosphere of olive tree roots forms a large reservoir of biological diversity, including mycorrhizal fungi that establish a symbiotic relationship with olive roots (Kachkouch et al. 2014). These arbuscular mycorrhiza (AM) improve assimilation of mineral nutrients and benefit growth of the host plant in poor soils (Chliyeh et al. 2016, Plenchette 2005). For example, uptake of nutrients with low mobility and low concentration in the soil solution, such as phosphorus, iron, zinc, and copper, can be increased in the presence of symbiotic microflora, especially mycorrhizal fungi (Duponnois et al. 2005, Gianinazzi et al. 1982, Smith and Read 1997). Mycorrhizae also allow plants to better withstand environmental stresses such as salinity, drought, and even some pathogenic soil microorganisms (Caravaca et al. 2003, Dahbia 2009, Meddad 2010, Rosendahl and Rosendahl 1991, Schreiner et al. 1997, Selosse et al. 2004). These telluric pathogens include *Phytophthora palmivora*, a fungal agent responsible for root rot of olive trees, which was recently encountered in different Moroccan olive groves (Chliyeh et al. 2013, 2014b; Msairi et al. 2017).

The objective of our study was to evaluate the effects of a composite endomycorrhizal inoculum (originating from the rhizosphere of olive trees) on the growth of young olive trees and to determine if the inoculum protected against *Phytophthora palmivora*.

Materials and Methods

Mycorrhizal Inoculum

A composite endomycorrhizal inoculum was prepared from the roots of mycorrhizal olive plants. The inoculum contained multiple endomycorrhizal species, 22 of which were identified morphologically. All of the 22 identified species have been isolated previously from the rhizosphere of olive trees in different regions of Morocco (Chliyeh et al. 2016). Barley (*Hordeum vulgare* L.) was used as a host plant to multiply the composite mycorrhizal inoculum. Barley seeds were disinfected with 5 percent sodium hypochlorite for 2 minutes, then germinated in plastic pots filled with a mixture of sterile sand and endomycorrhizal inoculum. After 4 weeks of culture, the barley roots were excised, rinsed 3 times with distilled water, and cut into 1- to 2-mm long fragments. These root fragments were used as the endomycorrhizal inoculum.

Pathogen Inoculum

Fungal pathogen inoculum was produced using an isolate of *Phytophthora palmivora* obtained from the dried twigs of an olive tree growing in Morocco's Sidi Kacem region. The inoculum was cultivated for 14 days on oatmeal agar plates (60 g oatmeal, 12.5 g of agar, and 1 L of distilled water). The mycelium was then transferred to sterile Petri dishes containing 20 ml of sterile distilled water and incubated overnight at 28 °C in the light. Subsequently, the dishes were cooled for 5 min at -20 °C to induce zoospore release. The inoculum concentration was adjusted to 10^6 zoospores/ml.

Inoculation of Young Olive Trees

In May 2015, a total of 24 young olive trees (18-months old), grown at a nursery in the Meknes region, were excavated from their substrate. Roots were rinsed under running water to remove soil, after which trees were divided into groups of six and randomly assigned to four treatments (control, pathogen inoculum, mycorrhizal inoculum, or pathogen+-mycorrhizal inoculums). For the control treatment, seedlings were transplanted to pots filled with disinfected Mamora sand. For the pathogen inoculum treatment, trees were soaked for 6 hours in a spore suspension of *Phytophthora palmivora* (10^6 zoospores/ml) and subsequently planted in pots containing the disinfected Mamora sand. For the mycorrhizal inoculum treatment, trees were transplanted to pots containing the disinfected Mamora sand and 3 g of endomycorrhizal barley root fragments incorporated into the top of the pot. For the pathogen+mycorrhizal inoculum treatment, six plants were soaked for 6 hours in the spore suspension of *P. palmivora*, then transplanted to pots containing the disinfected Mamora sand and fragments of mycorrhizal barley roots. Pots for all treatments were 30 cm in diameter and 40 cm deep with a volume of 27 liters. After transplanting, all pots were transported to the university greenhouse and seedlings were watered regularly with distilled water.

After 6 months of culture, the olive plants from all four treatments were severed at the base of the stem. Roots were rinsed with tap water, then the root and aerial parts were dried on absorbent paper for 6 hours under ambient laboratory conditions. Morphology was assessed on each plant by measuring stem height, root biomass, and number of leaves, twigs, and buds.

Spore Extraction

After 6 months of greenhouse cultivation, olive plants were excavated from the pots and mycorrhizal spores were extracted from the growing medium of the two treatments that included mycorrhizal inoculum according to the wet sieving method described by Gerdemann and Nicolson (1963). A sample was collected from each of the 6 pots of each treatment inoculated with mycorrhizae to determine the mycorrhizal population associated with the olive tree in the presence and absence of the pathogen. In a 1 L beaker, 100 g of each composite soil sample was immersed in 0.5 L of tap water and stirred for one minute with a spatula. After 10 to 30 seconds of decantation, the supernatant was passed through four superposed sieves with a decreasing mesh size (500, 200, 80, and 50 microns). This operation was repeated twice. The contents recovered after passing through the different sieves were divided into two

tubes and centrifuged for 4 min at 9000 rpm. The supernatant was discarded and a viscosity gradient was created by adding 20 ml of a 40-percent sucrose solution to each centrifuge tube (Walker et al. 1982). The mixture was rapidly stirred and the tube was returned to the centrifuge for 1 min at 9000 rpm. In contrast to the first centrifugation step, the supernatant was poured into the sieve with a mesh size of 50 microns. The resulting substrate was rinsed with distilled water to remove sucrose, and then disinfected with an antibiotic solution (streptomycin). The spores were then recovered with a little distilled water in a flask.

Spore identification was performed according to the species descriptions provided by the International Cultural Collection of Mycorrhizal Arbuscular Vesicular Fungi (INVAM 2017) and by following both the classification of Redecker et al. (2013) and the criteria proposed by Schenck and Smith (1982), Schenck and Perez (1987), and Morton and Benny (1990).

Statistical Analyses

Seedling morphology data were analyzed using analysis of variance (ANOVA) for a completely randomized design. Significant differences among the four treatments were determined using the least significant difference test at the 5 percent threshold. Data were analyzed using Statistica software (StatSoft Inc.).

Results

Mycorrhizal inoculation had a positive effect on olive plant shoot morphology after 6 months of greenhouse cultivation (table 1). Plants inoculated with *Phytophthora palmivora* without the mycorrizal inoculum showed disease symptoms of dieback, leaf drop, decay, and root system degradation (figure 1). In contrast, plants inoculated with both *P. palmivora* and mycorrhizal fungi showed no signs of disease and had significantly greater height, number of branches, number of leaves, and root biomass compared with plants inoculated with *P. palmivora* only (table 1). AM fungal species isolated from mycorrhizal soils without the presence of *P. palmivora* showed an association of 36 species with a spore density of 121 spores/100 g of soil (table 2,

Figure 1. The effect of an endomycorrhizal inoculum on root and shoot development of olive plants; I: plant inoculated with *Phytophthora palmivora*, M: mycorrhizal plant, C: control plant. (Photo by S. Msairi 2015)

Table 1. The effect of a composite endomycorrhizal inoculum on the growth parameters of olive plants inoculated with *Phytophthora palmivora*. Within a column, means followed by the same letter are not significantly different at the 0.05 level.

Inoculation treatment	Number of branches	Number of leaves	Number of buds	Root mass (g)	Height (cm)
Phytophthora palmifora	9.0 c	101.0 d	13.3 a	13.1 c	53.0 b
Phytophthora palmifora + mycorrhizae	27.0 a	258.3 c	27.0 a	44.6 b	67.4 a
Mycorrhizae	18.0 b	381.3 a	15.3 a	96.9 a	74.1 a
Control (no inoculation)	29.8 a	338.6 b	11.8 a	75.1 ab	76.5 a

Table 2. The identification of the isolated mycorrhizal fungi from the rhizosphere of mycorrhizal olive trees. Photos of each are shown in figure 2.

Photo number	Name	Number of spores	Shape	Color	Spore surface	Average size (µm)
1	*Acaulospora* sp.	3	globular	clear yellow	smooth	32
2	*Acaulospora* sp.	7	oval	dark yellow/ clear brown	smooth	31
3	*Acaulospora* sp.	2	globular	brown	irregular	28
4	*Acaulospora colossica*	19	oval	clear brown	irregular	23
5	*Acaulospora foveata*	15	globular	dark yellow	smooth	40
6	*Acaulospora gedanensis*	3	oval	dark yellow	irregular	45
7	*Acaulospora mellea*	14	globular	clear yellow	smooth	38
8	*Acaulospora morrowiae*	9	globular	yellow	irregular	25
9	*Acaulospora nicolsonii*	3	globular	yellow	irregular	22
10	*Acaulospora scrobiculata*	41	globular	clear yellow	smooth	38
11	*Claroideoglomus etunicatum*	24	oval	dark brown	granular	30
12	*Gigaspra* sp.	1	oval	dark yellow	smooth	50
13	*Gigaspora* sp.	1	globular	yellow	irregular	31
14	*Gigaspora margarita*	27	globular	dark yellow	granular	35
15	*Glomus ambisporum*	18	oval/ globular	dark brown	irregular	36
16	*Glomus aureum*	9	globular	clear brown	smooth	30
17	*Glomus clarum*	30	globular	dark yellow	granular	37
18	Glomus constrictum	4	globular	dark brown	irregular	25
19	*Glomus deserticola*	30	globular	brown	irregular	50
20	*Glomus glomerulatum*	6	globular	dark yellow	smooth	38
21	*Glomus heterosporum*	30	globular	dark brown	irregular	45
22	Glomus hyderabadensis	6	globular	brown	smooth	42
23	*Glomus intraradices*	39	oval/ globular	brown	irregular	30
24	*Glomus leptotichum*	4	oval	yellow	smooth	45
25	*Glomus macrocarpum*	17	globular	yellow/ brown	irregular	32
26	*Glomus margarita*	3	oval	clear brown	granular	34
27	*Glomus microcarpum*	5	globular	yellow	smooth	36
28	*Glomus mosseae*	18	globular	clear brown	granular	27
29	*Glomus versiforme*	66	globular	clear brown	irregular	55
30	*Glomus walker*	6	globular	clear brown	smooth	32
31	*Pacispora* sp.	3	globular	dark yellow	granular	34
32	*Pacispora* sp.	3	globular	yellow/green	granular	35
33	*Scutellospora* sp.	6	globular	dark brown	granular	28
34	*Scutellospora gilmorei*	6	globular	transparent/ light green	granular	24
35	*Scutellospora heterogamma*	2	oval	brown	granular	63
36	*Scutellospora savannicola*	4	oval	clear brown	irregular	29

figure 2). These species were distinguished on the basis of morphological criteria representing 6 genera with the dominant AM fungal species being *Glomus versiforme, G. intraradices, Acaulospora scrobiculata, G. clarum,* and *G. deserticola*. In contrast, species isolated from mycorrhizal soils in the presence of *P. palmivora* showed an association of 27 species with a spore density of 67 spores/100 g of soil with the dominant AM species being *Acaulospora scrobiculata, A. genensidas, Scutellospora nigra, Glomus radiates,* and *Gigaspra margarita* (table 3, figure 3).

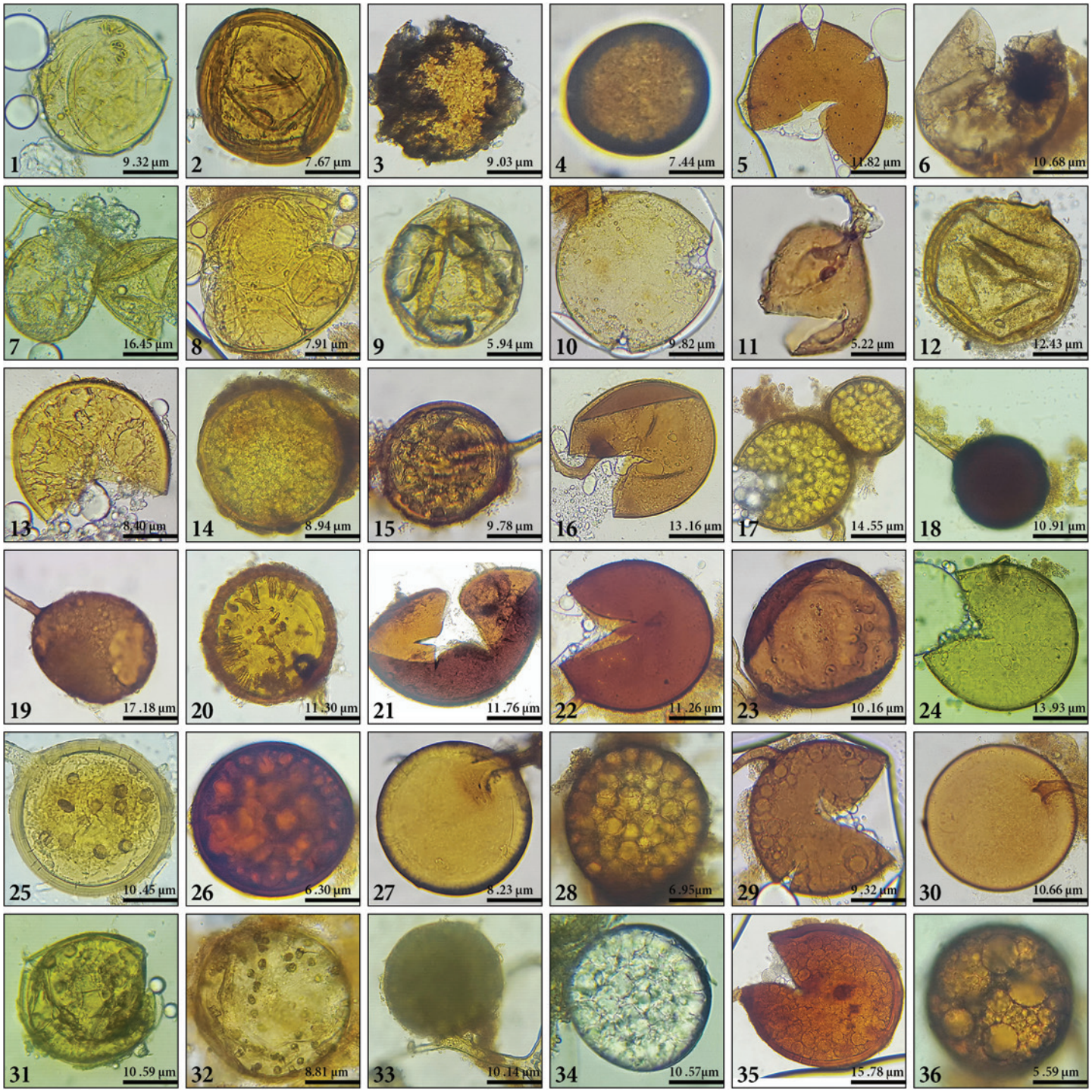

Figure 2. Species of endomycorrhizal fungi that were isolated from the rhizosphere of mycorrhizal olive plants; numbers correspond to table 2. (Photos by S. Msairi 2015)

Table 3. Identification of mycorrhizal fungi isolated from the rhizosphere of mycorrhizal olive plants and inoculated with *Phytophthora palmivora*. Photos of each are shown in figure 3.

Photo number	Name	Number of spores	Shape	Color	Spore surface	Average size (µm)
1	*Acaulospora* sp.	4	globular	dark yellow	irregular	22
2	*Acaulospora adenticulate*	4	globular	yellow	granular	7
3	*Acaulospora colombiana*	2	oval/ undetermined	clear brown	granular	47
4	*Acaulospora foveata*	9	globular	brown	irregular	37
5	*Acaulospora genensidas*	22	globular	yellow	granular	25
6	*Acaulospora lacunose*	14	oval	brown	granular	30
7	*Acaulospora laevis*	6	globular	dark yellow	granular	44
8	*Acaulospora nicolsonii*	9	oval	clear brown	irregular	27
9	*Acaulospora scrobiculata*	40	globular	brown	irregular	29
10	*Ambispora leptoticha*	2	oval	yellow	smooth	30
11	*Gigaspora* sp.	6	globular	green	granular	45
12	*Gigaspora* sp.	3	globular	yellow	smooth	31
13	*Gigaspora* sp.	11	globular	yellow	granular	33
14	*Gigaspora margarita*	17	globular	brown	smooth/ granular	40
15	*Glomus* sp.	5	globular	dark yellow/ clear brown	granular	21
16	*Glomus* sp.	7	globular	brown	smooth	35
17	*Glomus* sp.	3	oval	transparent clear brown	smooth	25
18	*Glomus aureum*	9	globular	yellow	smooth	26
19	*Glomus clarum*	10	globular	brown	granular	31
20	*Glomus fecundisporum*	15	globular	brown	granular	30
21	*Glomus macrocarpum*	10	globular	yellow	smooth	48
22	*Glomus mosseae*	8	oval	brown	granular	35
23	*Glomus radiatus*	19	globular	yellow/ brown	smooth	32
24	*Glomus rubiforme*	4	oval/ undetermined	transparent clear brown	smooth	43
25	*Pacispora scintillans*	3	globular	yellow	smooth	48
26	*Scutellospora* sp.	5	globular	brown	granular	29
27	*Scutellospora nigra*	21	globular	dark brown	smooth	30

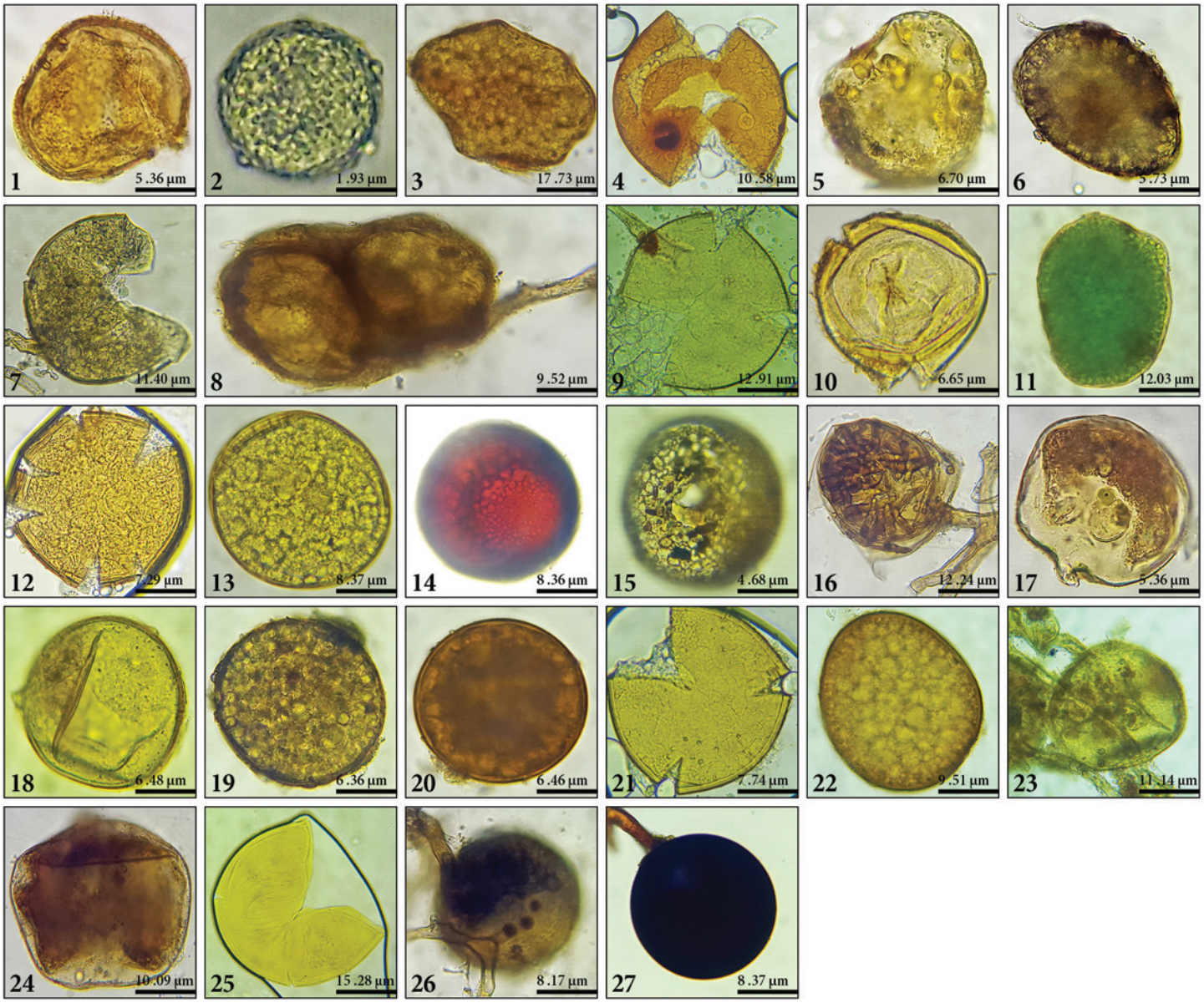

Figure 3. Species of endomycorrhizal fungi were isolated from the rhizosphere of olive plants that were inoculated with *Phytophthora palmivora* in the presence of mycorrhizae; numbers correspond to table 3. (Photos by S. Msairi 2015)

Discussion

Mycorrhizae inoculation resulted in good root colonization and improved shoot and root growth of young olive trees compared with non-mycorrhizal plants (control). These results are similar to research reported by Chliyeh et al. (2014) in which inoculation of olive plants with a composite endomycorrhizal inoculum showed good establishment of mycorrhizal symbiosis and improved growth compared with non-inoculated controls. Other studies have also noted that olive trees growing on a substrate containing endomycorrhizal fungi had improved growth compared with controls (Meddad et al. 2010, Semane et al. 2017). According to these authors, all studied growth parameters of mycorrhizal plants, including number of leaves, number of buds, height, root biomass, and shoot biomass, were higher than those of non-mycorrhizal control plants. Favorable effects of mycorrhizae have also been reported in other plant species such as the argan tree (*Argania spinosa*) (Sellal et al. 2017), rice (*Oryza sativa* L.) (Bernaola and Stout 2019), leek (*Allium porrum* L.) (Hibilik et al. 2018, Tran et al. 2019), sorghum (*Sorghum bicolor* [L.] Moench), carrot (*Daucus carota* L. var. *sativus* Hoffm.) (Kim et al. 2017), and common reed (*Phragmites australis* [Cav.] Trin. ex Steud.) (Liang et al. 2018).

In a study on sweet cherry (*Prunus avium* L.), plants inoculated with *Glomus intraradices* and *G. caledonium* showed a positive effect on foliage formation, dry weight, and stem diameter in mycorrhizal plants compared with control plants (Cordier 1996). The main effect of *G. intraradices* was on plant dry weight, while that of *G. caledonium* was on plant stem diameter. The increased growth of olive trees grown in mycorrhizal soil is likely due to increased access to soil water and nutrients. Mycorrhizal fungi can be considered biofertilizers, bio-regulators, and bio-protectants (Gianinazzi et al. 2010).

AM fungi in our study also showed a positive effect against disease caused by *Phytophthora palmivora*. Other studies have shown a positive effect of mycorrhiza on the growth and protection of plant species against certain root pathogens, including *Phytophthora* (Bärtschi et al.1981, Cordier et al. 1998, Duponnois et al. 1993, Duponnois and Cadet 1994, Guillemin et al. 1994). Similar to our study, the pathogen *P. cinnamomi* did not cause negative effects on fresh biomass development of sweet cherry roots pre-colonized by the AM fungi *Glomus mosseae*, whereas a decrease in root growth was observed in non-mycorrhizal plants (Cordier 1996).

Studies have been conducted to better understand the bioprotection mechanisms of mycorrhizae. In tomato, AM bioprotection against *Phytophthora parasitica* is related to a reduction of pathogen propagation in the mycorrhizal plants' root systems, and to a resistance of cells containing arbuscules (Cordier et al. 1998). This resistance may be related to the activation of defense responses in host tissues (Benhamou et al. 1994, Gianinazzi 1991), or to the expression of certain defense-related genes in the cells containing the arbuscules (Blee and Anderson 1996; Gianinazzi-Pearson et al. 1992, 1996; Harrison and Dixon 1994; Lambais and Mehdy 1995).

Dalpé et al. (2005), identified five interacting mechanisms of mycorrhizae as biocontrol agents. Some mechanisms are directly related to the plant, either through growth stimulation by increasing nutrient supply and better plant health, or morphological transformation at the root level, or by induction or suppression of defense mechanisms, especially those involving multiple enzymes. Other mechanisms act on the parasite through direct competition with mycorrhizal fungi related to the availability of nutrients, sites of infection, and soil structure and quality, through a modification of the microflora and an increase in the rate of the organic matter (content).

Conclusion

The olive tree is a highly mycotrophic species and forms a positive mycorrhizal association with several AM species. These mycorrhizal fungi not only stimulate shoot and root growth but can have a remarkable protective effect against root rot caused by *Phytophthora palmivora*. This protective activity can be exploited to attenuate the progressive extension of *P. palmivora*, which could constitute a real danger for the olive tree and for the crops in the vicinity. The introduction of mycorrhizae as a biological control agent in agricultural practices can contribute to the development of a sustainable agriculture by reducing the application of chemical pesticides.

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REFERENCES

Abousalim, A.; Brhadda, N.; Walali Loudiyi, D.E.M. 2005. Essais de prolifération et d'enracinement de matériel issu de rajeunissement par bouturage d'oliviers adultes (*Olea europaea* L.) et de germination in vitro : effets de cytokinine et d'auxines. Biotechnology Agronomy. Society Environment. 9(4): 237–240.

Bärtschi, H.; Gianinazzi-Pearson, V.; Vegh, I. 1981. Vesicular-arbuscular mycorrhiza formation and root rot disease (*Phytophthora cinnamomi*) development in *Chamaecyparis lawsoniana*. Journal of Phytopathology. 102: 213–218.

Benhamou, N.; Fortin, J.A.; Hamel, C.; St Arnaud, M.; Shatilla, A. 1994. Resistance responses of mycorrhizal Ri T-DNA-transformed carrot roots to infection by *Fusarium oxysporum* f sp. *chrysanthemi*. Phytopathology. 84: 958–996.

Bernaola, L.; Stout, M.J. 2019. Effects of arbuscular mycorrhizal fungi on rice-herbivore interactions are soil-dependent. Scientific Reports. 9(1): 1–12.

Blee, K.A.; Anderson, A.J. 1996. Defense-related transcript accumulation in *Phaseolus vulgaris* L. colonized by the arbuscular mycorrhizal fungus *Glomus intraradices* Schenck and Smith. Plant Physiology. 110(2): 675–688.

Caravaca, F.; Barea, J.M.; Palenzuela, J.; Figueroa, D.; Alguacil, M.M.; Roldán, A. 2003. Establishment of shrub species in a degraded semiarid site after inoculation with native or allochthonous arbuscular mycorrhizal fungi. Applied Soil Ecology. 22: 103–111.

Chliyeh, M.; Ouazzani Touhami, A.; Filali-Maltouf, A.; El Modafar, C.; Moukhli, A.; Oukabli, A.; Benkirane, R.; Douira, A. 2013. *Phytophthora palmivora*: A new pathogen of olive trees in Morocco. Atlas Journal of Biology. 2(2): 130–135.

Chliyeh, M.; Touati, J.; Selmaoui, K.; Ouazzani Touhami, A.; Filali-Maltouf, A.; El Modafar, C.; Moukhli, A.; Benkirane, R.; Douira, A. 2014a. Bibliographic inventory of the olive tree (*Olea europaea* L.) fungal diseases in the world. International Journal of Pure and Applied Bioscience. 2(3): 46–79.

Chliyeh, M.; Rhimini, Y.; Selmaoui, K.; Ouazzani Touhami, A.; Filali-Maltouf, A.; El Modafar, C.; Moukhli, A.; Oukabli, A.; Benkirane, R.; Douira, A. 2014b. Comparative study of pathogenicity tests for *Verticillium dahliae* and *Phytophthora palmivora* causing wilt and decline of olive tree (*Olea europaea* L.) International Journal of Pure and Applied Bioscience. 2(2): 28–38.

Chliyeh, M.; Kachkouch, W.; Talbi, Z.; Ouazzani Touhami, A.; Filali-Maltouf, A.; El Modafar, C.; Moukhli, A.; Benkirane, R.; Douira, A. 2016. Evolution of a composite endomycorrhizal inoculums in function of time in the level of the olive plants rhizosphere. International Journal of Advances in Pharmacy, Biology and Chemistry. 5(1):1–14.

Cordier, C.; Trouvelot, A.; Gianinazzi, S.; Gianinazzi-Pearson, V. 1996. Arbuscular mycorrhiza technology applied to micropropagated *Prunus avium* and to protection against *Phytophthora cinnamomi*. Agronomie, EDP Sciences. 16(10): 679–688.

Cordier, C.; Pozo, M.J.; Barea, J.M.; Gianinazzi, S.; Gianinazzi-Pearson, V. 1998. Cell defense responses associated with localized and systemic resistance to *Phytophthora parasitica* induced in tomato by an arbuscular mycorrhizal fungus. Molecular Plant-Microbe Interactions. 11(10): 1017–1028.

Dahbia, S. 2009. Etude des endomycorhizes de la variété Sigoise d'olivier (*Olea europaea* L.) et essai de leur application à des boutures semi – ligneuses. Oran University. 91 p. Master's thesis.

Dalpé, Y. 2005. Mycorrhizae: apotential tool for plant protection but not a panacea. Phytoprotection Quebec. 86(1): 53–59.

Duponnois, R.; Garbaye, J.; Bouchard, D.; Churin, J.L. 1993. The fungus-specificity of mycorrhization helper bacteria (MHBs) used as an alternative to soil fumigation for ectomycorrhizal inoculation of bare-root Douglas-fir planting stocks with *Laccaria laccata*. Plants and Soil. 157: 257–262.

Duponnois, R.; Cadet, P. 1994. Interactions of *Meloidogyne javanica* and *Glomus* sp. on growth and N2 fixation of Acacia seyal. Afro Asian Journal of Nematology. 4: 228–233.

Duponnois, R.; Colombet, A.; Hien, V.; Thioulouse, J. 2005. The mycorrhizal fungus *Glomus intraradices* and rock phosphate amendment influence plant growth and microbial activity in the rhizosphere of Acacia holosericea. Soil Biology and Biochemistry. 37: 1460–1468.

El Mouhtadi, I.; Agouzzal, M.; Guy, F. L'olivier au Maroc. OCL 2014, 21(2) D203 3 p.

Gerdemann, J.W.; Nicolso, T.H. 1963. Spores of mycorrhizal *Endogone* species extracted from soil by wet sieving and decanting. Transactions of the British Mycological Society. 46(2): 235–244.

Gianinazzi, S. 1991. Vesicular-arbuscular (endo-) mycorrhizas: cellular, biochemical and genetic aspects. Agriculture, Ecosystems and Environment. 35: 105–119.

Gianinazzi, S.; Golotte, A.; Binet, M.N.; Van Tuinen, D.; Redecker, D.; Wipf, D. 2010. Agroecology: the key role of arbuscular mycorrhizas in ecosystem services. Mycorrhiza. 20: 519–530.

Gianinazzi-Pearson, V.1982. Importance des mycorhizes dans la nutrition et la physiologie des plantes. Ed. INRA, Les mycorhizes en agriculture: partie intégrante de la plante, biologie et perspectives d'utilisation, Paris. 51–59.

Gianinazzi-Pearson, V.; Dumas-Gaudot, E.; Gollotte, A.; Tahiri-Alaoui, A.; Gianinazzi, S. 1996. Cellular and molecular defence-related root responses to invasion by arbuscular mycorrhizal fungi. New Phytologist. 133(1): 45–57.

Gianinazzi-Pearson, V.; Tahiri-Alaoui, A.; Antoniw, J.F.; Gianinazzi, S.; Dumas-Gaudot, E. 1992. Weak expression of the pathogenesis related PR-b1 gene and localisation of related protein during symbiotic endomycorrhizal interactions in tobacco roots. Endocytobiosis and Cell Research. 8: 177–185.

Guillemin, J.P.; Gianinazzi, S.; Gianinazzi-Pearson, V.; Marchal, J. 1994. Contribution of arbuscular mycorrhizas to biological protection of micropropagated pineapple (*Ananas comosus* (L.) Merr.) against *Phytophthora cinnamomi* Rands. Agricultural and Food Science. 3(3): 241–251.

Harrison, M.J.; Dixon, R.A. 1994. Spatial patterns of expression of flavonoid/isoflavonoid pathway genes during interactions between roots of *Medicago truncatula* and the mycorrhizal fungus *Glomus versiforme*. Plant Journal. 6(1): 9–20.

Hibilik, N.; Selmaoui, K.; Msairi, S.; Touati, J.; Chliyeh, M.; Mouria, A.; Artib, M.; Touhami, A.; Benkirane, R.; Douira, A. 2018. Study of arbuscular mycorrhizal fungi diversity and its effect on growth and development of leek plants (*Allium porrum* L.). Annual Research and Review in Biology. 24(2): 1–14.

INVAM, 2017. Arbuscular mycorrhizal fungi. Morgantown, WV : West Virginia University, Davis College of Agriculture, Natural Resources and Design, International Cultural Collection of Mycorrhizal Arbuscular Vesicular Fungi. http://fungi.invam.wvu.edu/.

Kachkouch, W.; Touati, J.; Ouazzani Touhami, A.; Filali-Maltouf, A.; El Modafar, C.; Moukhli, A.; Oukabli, A.; Benkirane, R.; Douira, A. 2014. Diversity of arbuscular mycorrhizal fungi in the rhizosphere of *Olea europaea* in three regions of Morocco (Tafilalt, Zagora and Taounate). International Journal of Pure and Applied Bioscience. 2(5):178–195.

Khabou, W.; Ben Amar, F.; Rekik, H.; Bekhir, M.; Touir, A. 2009. Performance evaluation of olive trees irrigated by treated wastewater. Desalination Journal. 246(1–3): 329–336.

Kim, S.J.; Eo, J.K.; Lee, E.H.; Park, H.; Eom, A.H. 2017. Effects of arbuscular mycorrhizal fungi and soil conditions on crop plant growth. Mycobiology. 45(1): 20–24.

Lambais, M.R.; Mehdy, M.C. 1995. Differential expression of defense-related genes in arbuscular mycorrhiza. Canadian Journal of Botany. 73(1): 533–540.

Liang, J.F.; An J.; Gao, J.Q.;, Zhang, X.Y.; Yu, F.H. 2018. Effects of arbuscular mycorrhizal fungi and soil nutrient addition on the growth of *Phragmites australis* under different drying-rewetting cycles. PLoS ONE . 13(1): e0191999.

MAMVA. 1996. Ministère de l'Agriculture, de l'Equipement et de l'Environnement Plan d'action oléicole. Division de la production végétale, 45-50.

Meddad-Hamza, A.; Beddiar, A.; Gollotte, A.; Lemoine, M.C.; Kuszala, C.; Gianinazzi, S. 2010. Arbuscular mycorrhizal fungi improve the growth of olive trees and their resistance to transplantation stress. African Journal of Biotech. 9(8): 1159-1167.

Morton, J.B.; Benny, G.L. 1990. Revised classification of arbuscular mycorrhizal fungi (*Zygomycetes*): a new order, *Glomales*, two new suborders, *Glomineae* and *Gigasporineae*, and two new families, *Acaulospor aceae* and *Gigaspora ceae*, with an emendation of *Glomaceae*. Mycotaxon. 37: 471–491.

Msairi, S.; Chliyeh, M.; Selmaoui, K.; Mouria, A.; Ouazzani Touhami, A.; Benkirane, R.; Douira, A. 2016. New appearance of *Phytophthora palmivora* as a pathogen of the olive trees in Sidi Kacem Region (Morocco). Annual Research and Review in Biology. 11(5): 1–7.

Plenchette, C. 2005. Mycorhizes et nutrition phosphatée des plantes. Journées Techniques Fruits et Légumes et Viticulture Biologiques, 6-7 décembre, Beaune. 103–109.

Redecker, D.; Schüssler, A.; Stockinger, H.; Stürmer, S.L.; Morton, J.B.; Walker, C. 2013. An evidence-based consensus for the classification of arbuscular mycorrhizal fungi (*Glomeromycota*). Mycorrhiza. 23(7): 515–531.

Rosendahl, C.N.; Rosendahl, S. 1991. Influence of vesicular arbuscular mycorrhizal fungi (*Glomus* spp.) on the response of cucumber (*Cucumis sativus* L.) to salt stress. Environmental and Experimental Botany. 31(3): 313–318.

Sadiki M. Le secrétaire général du ministère de l'Agriculture et de la Pêche maritime. https://lnt.ma/agriculture-culture-de-lolivier-nette-progression-maroc/ (June 2019).

Schenck, N.C.; Smith, G.S. 1982. Additional new and unreported species of mycorrhizal fungi (*Endogonaceae*) from Florida. Mycologia. 77(4): 566–574.

Schenck N.C.; Perez, Y. 1987. Manual for identification of VA mycorrhizal fungi. Gainesville, FL: University of Florida.

Schreiner, R.P.; Mihara, K.L.; McDaniel, H.; Bethlenfalvay, G.J. 1997. Mycorrhizal fungi influence plant and soil functions and interaction. Plant and Soil. 188(2): 199–209.

Sellal, Z.; Ouazzani Touhami, A.; Mouden, N.; Ouarraqi, E.L.M.; Selmaoui, K.; Dahmani, J.; Benkirane, R.; El Modafar, Ch.; Douira, A. 2017. Effect of an endomycorrhizal inoculum on the growth of argan tree. International Journal of Environment Agriculture and Biotechnolgy. 2(2): 0928–0939.

Selosse, M.A.; Faccio, G.; Scappaticci, G.; Bonfante, P. 2004. Chlorophyllous and achlorophyllous specimens of Epipactis microphylla (*Neottieae, Orchidaceae*) are associated with ectomycorrhizal septomycetes, including truffles. Microbial Ecology. 47(4): 416–426.

Semane, F.; Chliyeh, M.; Talbi, Z.; Touati, J.; Selmaoui, K.; Ouazzani Touhami, A.; Filali-Maltouf, A.; El Modafar, Ch.; Moukhli, A.; Benkirane, R.; Douira, A. 2017. Effects of a composite endomycorrhizal inoculum on olive cuttings under the greenhouse conditions. International Journal of Environment, Agriculture and Biotechnology. 2 (3): 1070–1083.

Smith, S.E.; Read, D.J. 1997. Mycorrhizal symbiosis. San Diego: Academic Press.. 607 p.

Tran, B.T.T.; Watts-Williams, S.J.; Cavagnaro, T.R. 2019. Impact of an arbuscular mycorrhizal fungus on the growth and nutrition of fifteen crops and pasture plant species. Functional Plant Biology. 46(8): 732–742.

Walker, H.G.; Kohler, G.O.; Garrett, W.N. 1982. Comparative feeding value of alfalfa press cake residues after mechanical extraction of protein. Applied Animal Science. 55(3): 498–504.

Zouiten, N.; El Hadrami, I. 2001. Le psylle de l'olivier : état des connaissances et perspectives de lutte. Cahiers d'études et de recherches francophones/Agricultures. 10(4): 225–232.