Arbuscular mycorrhizal fungi enhanced the growth, phosphorus uptake and *Pht* expression of olive (*Olea europaea* L.) plantlets

Tao Wu[1]*, Li Pan[1]*, Isaac Zipori[2], Jihua Mao[1], Rongbo Li[1], Yongpeng Li[1], Yongjie Li[1], Yuebo Jing[1] and Haiyun Chen[1]

[1] Yunnan Academy of Forestry and Grassland, Kunming, Yunnan, China
[2] Gilat Research Center, Agricultural Research Organization, M.P. Negev, Gilat, Israel

* These authors contributed equally to this work.

Corresponding Author:
Yuebo Jing,
Lan'an Road No.2, Kunming, Yunnan, 650201, China
1401764297@qq.com
Haiyun Chen,
kmchenhy@163.com

Abstract

Olive (*Olea europaea* L.) is a highly mycotrophic species that has been introduced and cultivated in China for half a century. The arbuscular mycorrhizal fungi (AMF) are extremely valuable as a kind of biofertilizer to promote the health and vigor of olive plants. However, it is still unclear how native AMF impact growth and mineral nutrients, especially phosphorus absorption in the area where olive tree was introduced in China. In the present study, through a pot experiment, the effects of native AMF on the growth, phosphorus uptake and expression levels of four phosphate transporter genes (*Pht*) of olive plantlets were characterized. We found that (1) typical AMF colonization was observed within the roots of inoculated olive plantlets, and the growth of plantlets was significantly promoted; (2) some indigenous consortia (AMF1 and AMF2) notably promoted the absorption of phosphorus, fertilizers significantly increased the foliar content of nitrogen, and both AMF inoculation and fertilization had no significant effect on the uptake of potassium; and (3) AMF inoculation enhanced the expression of phosphate transporter genes in inoculated olive roots. This work demonstrates the effectiveness of native AMF on the cultivation of robust olive plantlets and highlights the role of AMF in increasing phosphorus uptake. There is great potential in using native AMF consortia as inoculants for the production of healthy and robust olive plantlets.

Keywords

Olea europaea L.; native arbuscular mycorrhizal fungi; growth promotion; phosphorus uptake; phosphate transporter gene

40

Introduction

41 **Introduction**

42 Olive (*Olea europaea* L.), a multifunctional long-living tree crop, is relevant not only for table
43 olive and oil production but also for its impact on human nutrition and rural lifestyle (Fabbri et al.
44 2009). Olive has long been the symbol of the Mediterranean and is now spreading in many other
45 new areas (Gutierrez et al. 2009; Horden & Purcell 2012). A global production trend of olive has
46 been on the rise and the countries that were previously importers of the product, such as the US,
47 China, Chile and Australia are now producing. Olive trees were introduced to China in the 1960's,
48 and now grown in 14 provinces, mainly Gansu, Sichuan, and Yunnan, covering an area of 167000
49 ha (Wang et al. 2019). These areas are located in western China, and the soil is mostly acidic red
50 soil and yellow soil, phosphorus (P) is one of the major plant nutrients that is least available in the
51 soil. Aluminum and iron ions, which predominate in acidic soils, interact strongly with P and
52 render it unavailable to plants (Raghothama & Karthikeyan 2005).

53 Arbuscular mycorrhizal fungi (AMF) can form mutualistic symbioses with approximately 80%
54 of land plant species. Notably, AMF are known to improve phosphorus (P) nutrition in plants by
55 making them accessible to unavailable soil P sources (Allen 1996). They can acquire nutrients
56 from soil volumes that are inaccessible to roots, and provide the host plant with mineral nutrients
57 and water, in exchange for photosynthetic products (Berruti et al. 2016). Olive is a typical
58 mycotrophic species (Calvente et al. 2004; Hayman et al. 1976; Roldán-Fajardo & Barea 1986),
59 and many studies have reported that the early presence of AMF increases the growth of olive
60 rooted cuttings and micropropagated plants (Estaún et al. 2003; Martín et al. 2006) and enhances
61 olive plant growth (Aganchich et al. 2022), absorption of nutrients (Rodrigues et al. 2021),
62 physiological parameters (Tekaya et al. 2017), tolerance to stress caused by transplanting
63 (Bompadre et al. 2014; Dag et al. 2009), drought (Ouledali et al. 2018), salinity (Ben Hassena et
64 al. 2021) and disease (Boutaj et al. 2021). Thus, AMF show superior prospects as biofertilizers,
65 especially in tropical soils (Igiehon & Babalola 2017), which are usually dominated by iron and
66 aluminum oxides and maintain a lower available P than temperate soils (Tiessen 2005).

67 The concent of P in plant tissue is 500-2000 times that of soil available phosphorus (Bieleski
68 1973; Raghothama & Karthikeyan 2005). The ability of AMF or bioinoculants to assimilate P is
69 one of the key factors for plants to effectively utilize soil P. The high expression of the plant's own
70 phosphorus transporter gene (*Pht*) is another important factor (Nussaume et al. 2011). This sharp
71 concentration gradient between plants and soil illustrates the critical role of *Pht* genes. A majority
72 of *Pht* genes exhibit strong expression in roots, a few *Pht* genes express in aerial organs such as
73 leaves or flowers (Nussaume et al. 2011). After the plant roots were induced by AMF, the
74 expression levels of *Pht* genes increased sharply (Nagy et al. 2005; Xu et al. 2007a; Yang et al.
75 2012a). However, the specific situations in olive are not clear.

76 In this study, we hypothesized that, after years of planting in China, (1) the olive trees harbored
77 some indigenous AMF; (2) these AMF can produce promising effects on the growth and
78 phosphorus (P) uptake of olive plants, and the expression of genes related to P uptake can also be
79 promoted with the root colonization of AMF. In this context, we collected rhizosphere soil of olive

80 trees from five growing sites in Yunnan as the source of indigenous AMF consortia and compared
81 them with commercial AMF inoculum and fertilizer to study their effects on olive plantlets.
82 Furthermore, the gene expression of P absorption via the formation of AM was also characterized.
83

Materials & Methods

Plant materials and cultivation conditions

86 Olive cultivar 'Koroneiki' was selected since it is one of the most common olive cultivars in
87 Yunnan province with promising performance (Li et al. 2020). The plantlets used in this study
88 were obtained by mist propagation. The semi-woody cuttings of approximately 10 cm long and
89 two pairs of leaves at the top end were selected for the study. The bottom basal end of each cutting
90 was immersed in an ethanol solution containing 5 g of indol-3-butyric acid/L to promote root
91 development. Propagation was performed in an arch tunnel with a controlled glass greenhouse
92 under an air temperature of 20 ± 2 ℃, a relative humidity of 55-70%, a range of irradiance of 400-
93 650 μmol photon·m^{-2}·s^{-1} (Martín et al. 2006). After three months, the rooting cuttings were
94 transferred to 7.5 cm × 11.5 cm plastic pots filled with growing medium.
95   The growing medium was a mixture of 50% peat moss and 50% vermiculite (*v/v*) with a pH of
96 6.85, organic matter of 251.83 g/kg, hydrolyzable nitrogen of 406.76 mg/kg, available phosphorus
97 of 43.12 mg/kg, available potassium of 469.38 mg/kg, total nitrogen, phosphorus and potassium
98 of 5.08 g/kg, 0.62 g/kg and 16.57 g/kg, respectively. The growing medium was autoclaved twice
99 for a period of 2 h at 121°C, with a 24 h interval between the two sterilizations.

AMF and fertilization treatments

101 The native soil inocula were collected from five orchards located in the representative olive
102 introduction and cultivation areas in Yunnan Province. The five growing sites sampled were coded
103 AMF1~AMF5. AMF1 was sampled from the orchard of Olive Cultivation Technology Extension
104 Base of Diqing Forest Institute of Yunnan Province. AMF2 from the orchard of Yunnan Lvyuan
105 Industrial Development Company Limited. AMF3 from the orchard of Chuxiong Xinyuan
106 Biotechnology Company Limited. AMF4 from the orchard of Lijiang Tianyuan Olive Technology
107 Development Company Limited. AMF5 from the orchard of Lijiang Sanquan Olive Industrial
108 Development Company Limited. For specific location information, please refer to table S1. The
109 spore numbers of AMF1 to AMF5 were 350, 121, 82, 198 and 116 in 20 grams of soil. The
110 dominant AMF species of AMF1, AMF2, AMF3 and AMF5 were *Funneliformis geosporum*, that
111 of AMF4 was *Septoglomus constrictum* (see the supplementary table S1), the detailed AMF
112 communities characterization was reported in our previous work (Jing et al. 2022). A commercial
113 AMF inoculant (coded AMF6) was assayed as a reference alongside the native soil inocula. AMF6
114 was produced in the form of granules, spores and roots, *viz. Rhizophagus intraradices*, *F. mosseae*,
115 *R. aggregatus* and *Claroideoglomus etunicatum* (without other additives). Three fertilizer
116 treatments were used here to compare their effects with AMF inocula, namely, Fert1 (3 gram/pot
117 of compound (15:15:15) fertilizer), Fert2 (3 gram/pot of earthworm manure, whose main
118 components include 56.2% organic matter, 1.78% total nitrogen, 1.67% total phosphorus, 1.02%

119 total potassium, pH 7.66) and Fert3 (Fert1 + Fert2). Plantlets with neither inoculation nor fertilizer
120 application were used as the control (CK). Six repetitions were performed for each treatment.
121 Inoculation was carried out at the time of transferring the rooted cuttings into the containers. Three
122 grams of inoculum was deposited directly below the roots of the rooted cuttings.
123   To expose the cuttings in every treatment to an equal nutritional level, for the AMF1
124 treatment, 3 grams of sterilized rhizosphere soil from AMF2 to AMF5 were added to the pot, and
125 for AMF2, 3 grams of sterilized rhizosphere soil from AMF1, AMF3 to AMF5 were added to the
126 pot, and so on. For the AMF6, Fert1, Fert2, Fert3 and CK treatments, the rhizosphere sterilized
127 soil of AMF1 to AMF5 was added to the pot, at the rate of 3 g each.
128   The plantlets were grown in the greenhouse of the Yunnan Academy of Forestry and Grassland
129 and watered manually with tap water The frequency of watering depended on the environmental
130 conditions prevailing inside the greenhouse, generally once a week. After six months of growth,
131 the shoots and roots of the olive plantlets were harvested separately.

Detection of AMF colonization

133 The roots were first washed with tap water, and randomly collected root segments were then
134 cleared with 10% (*w/v*) KOH at 90°C in a water bath for approximately 60 min. After cooling to
135 room temperature, root samples were thoroughly washed with tap water, stained with blue ink
136 (Hero® 203, Shanghai China), mounted on microscope slides and then examined under a
137 compound light microscope (Olympus-BX53, Olympus Corporation, Tokyo, Japan) for the
138 presence of AM fungal structures. The percentage of root length occupied by hyphae, arbuscules
139 and vesicles was quantified on each sample by a modified line intersection method (McGonigle et
140 al. 1990). At least 200 intersections per root sample were examined. Four replicates were
141 performed for each treatment and control.

Plantlet growth measurements

143 Growth parameters were measured for all plantlets, including height, root collar diameter, and
144 above- and belowground biomass. The dry weights of the shoots and roots were recorded after
145 oven-drying at 70°C until they reached a constant mass. The foliar nutrient concentration was
146 determined on dried material in the tested plantlets. Dried leaves were milled and passed through
147 a 0.25 mm sieve, and a sample of 0.5 g of leaf powder was taken for digestion with H_2SO_4 and
148 H_2O_2 (*v/v*) 1:4. Nitrogen, P and K contents were determined by the Kjeldahl Method (FOSS
149 KJELTEC 8400, Denmark), ultraviolet–visible spectrophotometry (HITACHI U-5100, Japan) and
150 atomic absorption spectrophotometry (HITACHI Polarized Zeeman Atomic Absorption
151 Spectrophotometer ZA3000, Japan), respectively. In addition, the ratio of root:shoot growth was
152 calculated to reflect the robustness of the plantlets and the efficiency of AMF inoculation (Tobar
153 et al. 1994). Six replicates were performed for each treatment and control.

qRT–PCR of phosphate transporter genes

155 The wild olive assembly and annotated genome (Unver et al. 2017) were used as queries for
156 phosphate transporter protein genes (*Pht*). First, we use "phosphate transporter" as the keyword to

157 search target genes in the wild olive genome of *Olea europaea* var. *sylvestris* (RefSeq assembly
158 accession: GCF_002742605.1), then obtained the name and location information of target genes,
159 found the corresponding protein sequence. Secondly, we got 30 gene members of the three
160 phosphate transporter gene families based on the verifying of sequence length and function
161 annotation information. Thirdly, we randomly selected several genes from each gene family as
162 representatives, synthesized primers and amplified them. Finally, four olive *Pht* genes with good
163 effect were selected to analyze their gene expression level in this study. According to the
164 annotation and retrieval results of the wild olive genome, they are named *OePht1;11*, *OePht1;2*,
165 *OePht1;4* and *OePho3* respectively. The four olive *Pht* studied here refer to table S2.

166   To understand the homology of the four olive *Pht* genes with other known crop ones, a
167 phylogenetic tree was constructed using their DNA sequences together with 11 known *Pht* genes
168 ones in other crops, *MtPHT2;1*, *MtPT1* and *MtPT4* from alfalfa [*Medicago truncatula*] (Harrison
169 et al. 2002; Versaw & Harrison 2002), *LePT3* and *LePT4* from tomato [*Lycopersicon esculentum*]
170 (Xu et al. 2007a), *StPT1*, *StPT3* and *StPT4* from potato [*Solanum tuberosum*] (Nagy et al. 2005),
171 *OsPT11* and *OsPT13* from rice [*Oryza sativa*] (Paszkowski et al. 2002; Yang et al. 2012a) and
172 *ZmPT6* from maize [*Zea mays*] (Wright et al. 2005). All 15 nucleotide sequences representing 6
173 species were aligned in MAFFT program, then the phylogenetic tree was constructed with
174 MrBayes under the General Time Reversible (GTR) nucleotide substitution model and 10,000
175 burn-in length in Geneious Prime 2020.0.5 software.

176   Total RNA of olive roots was isolated using an RNAprep Pure Plant Kit (Tiangen, China).
177 DNase was used to eliminate the potential trace of genomic DNA in RNA samples. Then, 1.0%
178 agarose gel and a NanoDrop ND-2000 spectrophotometer (Thermo Fisher, USA) were used to
179 evaluate and quantify RNA, respectively. RNA samples were reverse-transcribed into cDNA with
180 the FastKing RT Kit (Tiangen, China), and synthesized cDNAs were used as templates for qRT–
181 PCR with the SuperReal PreMix Plus Kit (Tiangen, China). *O. europaea* translation elongation
182 factor-1 alpha (*OeEF1α*) served as the internal control for the qRT–PCR (Ray & Johnson 2014).
183 All the primer sequences are listed in Table 1.

184   A 20 µL reaction solution containing 1 µL cDNA (20 ng), 1 µL of each primer (10 µM), 10 µL
185 SYBR Green I Master mix reagent (Tiangen) and 7 µL ddH$_2$O was amplified with a LightCycler96
186 (Roche, Switzerland). The qRT–PCR program was as follows: 95°C for 15 min, followed by 45
187 cycles at 95°C for 10 s, 55°C for 10 s and 72°C for 10 s. The qRT–PCR was performed with three
188 biological replicates, and the data are shown as the mean ± SD. The relative transcript level was
189 calculated using the $2^{-\Delta\Delta Ct}$ method (Ray & Johnson 2014). Three biological replicates were
190 performed for each treatment and control.

191 **Statistical analysis**

192 Values of mycorrhizal parameters were summarized for each native and commercial AMF using
193 some descriptive statistics *viz*. mean, standard error (SE). Data related to olive plantlet growth
194 parameters per treatment were visualized using boxplots.   Differences in AMF colonization
195 intensities, plant growth parameters, the leaf concentrations of N, P and K between treatments, the
196 expression level of *Pht* genes, were analyzed using one-way analysis of variance (ANOVA),

197 comparisons among mean values in each treatment were made using the Tukey's test (HSD) ($p <$
198 0.05). The significance level adopted for all statistical tests was of 5% and the SPSS Statistics 24
199 (SPSS Inc., Chicago, IL, USA) was utilized for the statistical analysis. Boxplot diagrams were
200 drawn using the R package (R Core Team, 2019). All values were reported as means and standard
201 errors.

202

203 Results

204 Root colonization by AMF

205 No mycorrhizal colonization was detected, as expected, in the roots of plantlets under fertilizer
206 application (treatment Fert1, Fert2 and Fert3) and noninoculated ones (treatment CK). Except for
207 AMF4, all other treatments showed similar or higher root AMF colonization percentages compared
208 with the commercial AMF inoculum (AMF6). The percentage of AMF hypha colonization in roots
209 of olive plantlets ranged from 40.20% in AMF4 to 93.78% in AMF3, 3.32% (AMF4) to 42.46%
210 (AMF5) for the arbuscule colonization, 2.07% (AMF4) to 49.56% (AMF5) for the abundance of
211 vesicles (Table 2). Some typical structures of AMF colonizing the roots of olive plantlets are
212 presented in Figure 1.

213 Effect of AMF inoculation on the growth of olive plantlets

214 Six months after inoculation, all AMF inoculation treatments had a positive influence on plant
215 growth in terms of height, diameter, biomass and leaf area (Fig. 2). Compared to the noninoculated
216 CK, the commercial AMF inoculum increased the shoot and root fresh weights of plantlets by
217 66.27% and 91.90%, respectively. However, the native AMF inocula achieved higher effects (Fig.
218 2).
219   AMF3 showed the highest effect on aboveground growth, and the shoot dry weights of
220 inoculated plantlets were 2.16 times, 1.41 times, 2.02 times, 1.38 times and 1.24 times those of the
221 CK, Fert1, Fert2, Fert3 and AMF6 treatments, respectively. Whereas AMF1 showed the highest
222 effect on enhancing the belowground growth of the plantlets, the plantlets inoculated with AMF1
223 had root dry weights 2.84 times, 2.17 times, 2.71 times, 2.19 times and 1.35 times those of
224 treatment CK, Fert1, Fert2, Fert3 and AMF6 treatments, respectively. The root to shoot ratios of
225 olive plantlets inoculated with AMF1, AMF2, AMF3, AMF4, AMF5, AMF6, Fert1, Fert2, Fert3
226 and CK were 0.64, 0.52, 0.33, 0.48, 0.49, 0.44, 0.34, 0.35, 0.37 and 0.39, respectively.

227 Effect of AMF inoculation on the N, P, and K contents in olive plantlets

228 The leaf nitrogen (N) contents of all the AMF inoculated plantlets were lower than the CK, except
229 AMF2, which had a similar value to the control. Fertilization significantly increased the foliar N
230 content compared with AMF and CK (Fig. 3a). The foliar P contents of olive plantlets inoculated
231 with AMF1 and AMF2 were significantly higher than those of all other treatments (Fig. 3b). AMF
232 inoculation marginally enhanced the potassium (K) uptake of the plantlets, and the leaf K contents
233 of plantlets treated with AMF1 to AMF6 were 8.34%, 15.54%, 9.33%, 12.07%, 9.20% and 16.29%
234 higher than those of the control; however, the differences were not significant (Fig. 3c).

235 **Effect of AMF inoculation on expression levels of phosphate transporter genes**

236 A phylogenetic tree was constructed using a multiple DNA sequence alignment of four olive *Pht*
237 genes and ones of other plants (Fig. 4). The four olive *Pht* genes were clustered into three main
238 groups, *OePht1;11* and *OePht1;2* were clustered together with *PT4* genes of tomato (*LePT4*),
239 potato (*StPT4*) and alfalfa (*MtPT4*); *OePho3* and *OePht1;4* were individually clustered into a
240 separate group.

241 We characterized the expression level of the four *Pht* genes in olive roots inoculated with AMF
242 or fertilized with compound fertilizer and earthworm manure (Fig. 5). One member, *Pht1;11*,
243 exhibited strong expression in olive roots inoculated with AMF, especially in the plants treated
244 with AMF6, which of expression level jumped as much as 3400-fold compared to the
245 noninoculated control. *Pht1;4* was only expressed at high levels in the AMF1 treatment. The low
246 levels of *Pht1;2* transcripts in both the AMF and fertilizer treatments were completely different
247 from those in the control. The expression level of the *Pho3* gene responded positively to
248 inoculation and fertilization, but fertilization seemed more effective.

249

250 ## Discussion

251 Our results showed that the presence of AMF native to the olive growing sites of Yunnan Province
252 significantly promoted the growth, biomass and P uptake of olive plantlets. Several earlier studies
253 have shown that AMF can promote olive plantlet growth and nutrient uptake (Calvente et al. 2004;
254 M'barki et al. 2018; Porras-Soriano et al. 2009). In addition, we noted a distinct mycorrhizal
255 compatibility among the native soil inoculum indicated by differences in root colonization
256 intensity and the effectiveness on plantlets (Table 2, Fig. 2). In fact, different growth responses of
257 olive plants have been demonstrated following inoculation with different AMF strains (Calvente
258 et al. 2004; Castillo et al. 2006; Meddad-Hamza et al. 2010). Moreover, the effects of colonization
259 by AMF on olive plant growth also varied with the plant cultivars. Dag et al. found that the
260 response intensity in terms of height and biomass production of 12 commercial olive cultivars,
261 inoculated with *G. mosseae* and *G. intraradices* was highly cultivar specific (Dag et al. 2009).
262 Other researchers (Martín et al. 2006; Mohamed Oussouf et al. 2014) reported that specific
263 compatibility relationships may exist among symbionts, and underscore the importance of host-
264 AMF selection to maximize olive performance.

265 In this study, we found that the effects of native AMF were generally higher than those of the
266 commercial AMF inoculum (Fig. 2). Many studies have pointed out the higher efficiency of native
267 AMF compared to nonnative, introduced AMF (Affokpon et al. 2011; Briccoli Bati et al. 2015;
268 Estrada et al. 2013; Labidi et al. 2015). Our results are consistent with those found by Chenchouni
269 et al. (2020), demonstrating that the effects of local AMF strains on increasing different growth
270 parameters of olive plantlets were better than those of commercial AMF species (Chenchouni et
271 al. 2020). Several studies have highlighted that different isolates within the same species, rather
272 than different species, can cause large variations in plant response (Angelard et al. 2010; Gai et al.
273 2006; Munkvold et al. 2004). A study conducted earlier by Calvente et al. in 2004, showed that
274 the *G. intraradices* strain, isolated from the olive rhizosphere, was more effective than the exotic

275 *G. intraradices* from the culture collection (Calvente et al. 2004). More importantly, exploring and
276 exploiting native AMF can avoid any potential problems related to the application of nonnative
277 AMF inoculum in terms of biodiversity losses and homogenization as a result of anthropogenic
278 translocation of biota between biogeographic regions (Pellegrino et al. 2012; Schwartz et al. 2006).
279   There is evidence that AMF play a role in the uptake of nitrate and ammonium which are
280 assimilated and transported within the mycelium as arginine (Olsson et al. 2005), but compared
281 with ectomycorrhizas, rates of N uptake by the hyphae of AMF are too small to contribute
282 substantially to the N nutrition of plants (Smith & Read 2008). Accordingly, N uptake was not
283 significantly different in AMF inoculated plants compared to noninoculated plants in this study
284 (Fig. 3). The lower N concentration of inoculated olive plantlets can be explained by a 'dilution'
285 effect commonly observed in plants growing well in infertile conditions (Steenbjerg & Jakobsen
286 1963), as Dela Cruz et al. (1988) reported previously in *Albizia falcataria* seedlings (Dela Cruz et
287 al. 1988). In the present study, the N concentrations of olive plantlets inoculated with AMF1 to
288 AMF6 were 0.8346%, 0.9927%, 0.7827%, 0.7528%, 0.7730% and 0.6939% respectively, whereas
289 the noninoculated poorly growing olive plantlets (CK) showed the highest N concentration
290 (1.0047%) among all treatments (Fig. 2, 3).
291   Mycorrhizal symbiosis was found to be important for root system development, which is critical
292 for better mineral nutrition and stress resistance of seedlings. Tobar et al. (1994) conclusively
293 demonstrated that the root-to-shoot ratio reflects the degree of efficiency of AM fungi (Tobar et
294 al. 1994). In the present study, inoculation with AMF3 significantly enhanced the aerial parts of
295 olive plantlets, resulting in a low ratio of roots to shoots, whereas the plantlets treated with the
296 other four inocula achieved root to shoot ratios higher than those of the control. In addition, the
297 average root to shoot ratio of the plantlets treated with fertilizer was the lowest among all the
298 treatments (Fig. 2). Under chemical fertilization, minerals are immediately available for the plant,
299 which reduces the need and triggering for extensive root development, resulting in a low ratio of
300 root to shoot. Later, when the young plants are transplanted in the field without fertilizers, an
301 abrupt decrease in the nutrient uptake and growth rate occurs (Meddad-Hamza et al. 2010).
302   Many studies have reported that mycorrhizal colonization can enhance P absorption by plants
303 (Abdel-Fattah et al. 2014; Black et al. 2000; Bücking & Shachar-Hill 2005), including olive plants
304 (Briccoli Bati et al. 2015; Dag et al. 2009; Estaún et al. 2003). Similarly, the results obtained from
305 this study showed that the leaf P content of AMF inoculated plants was higher than that of
306 noninoculated plants (Fig. 3). In addition to dissolving insoluble phosphates into inorganic
307 orthophosphate (Pi) and absorbing Pi from the rhizosphere beyond root depletion zones by AMF
308 mycelium and transport to host plants (Harrison & van Buuren 1995), AMF can also induce the
309 expression of some particular phosphate transporter genes in their host plants under Pi-deficient
310 conditions (Rausch et al. 2001). For example, several AM symbiosis-induced *Pht* genes in plant
311 roots have been identified, such as *LePT3* and *LePT4* in tomato (*Lycopersicon esculentum*) (Xu et
312 al. 2007b); *StPT1*, *StPT3* and *StPT4* in potato (*Solanum tuberosum*) (Nagy et al. 2005); *MtPT1*,
313 *MtPT4* and *PHT2*;1 in alfalfa (*Medicago truncatula*) (Harrison et al. 2002; Versaw & Harrison
314 2002); *OsPT11* and *OsPT13* in rice (*Oryza sativa*) (Paszkowski et al. 2002; Yang et al. 2012b);

315 *ZmPT6* in corn (*Zea mays*) (Wright et al. 2005); *PtPT8*; and *PtPT10* in black cottonwood (*Populus*
316 *trichocarpa*) (Loth-Pereda et al. 2011). The transcripts of one tomato *Pht* gene (*LePT4*) exhibited
317 significantly increased expression levels in low-P treatment and colonized by the *Glomus*
318 *intraradices* (Xu et al. 2007b). Potato's *Pht* genes (*StPT4* and *StPT5*) also exhibited mycorrhiza
319 upregulation when inoculated with *Gigaspora margarita*, and both were highly functionally
320 redundant (Nagy et al. 2005). In our study, the four olive *Pht* genes were differentially regulated,
321 with *OePht1;11* exhibiting mycorrhiza-specific regulation, similar to *LePT4* and *StPT4*; *OePht1;4*
322 only mycorrhiza-upregulated in AMF1; *OePht1;2* were not sensitive to AMF and fertilizer;
323 *OePho3* seemed more effective to fertilizer than AMF (Fig.5). Although *OePht1;11* and *OePht1;2*
324 were closer in the phylogenetic tree (Fig. 4), it remains to be clarified whether they function
325 differently.
326
327
328

329 ## Conclusions

330 Although mycorrhizal networks ubiquitously exist in the soil, in intensively managed fields,
331 mycorrhizal networks are usually absent or low in abundance because of regular soil disturbance
332 destroying the mycelia or the absence of permanent vegetation cover that is needed to maintain
333 mycorrhizal networks. During relatively long periods of development in the nursery, olive plantlets
334 do not have AMF since they are usually grown in inert or fumigated potting media. AMF
335 inoculation at the nursery stages is therefore critical and necessary and can also help plants cope
336 with various stress conditions in the field. In this study, we report the effect of AMF inoculation
337 on growth responses and the expression levels of four phosphate transporter genes in olive
338 plantlets. Research indicates that there is great potential in using native AMF consortia as
339 inoculants for the production of high-quality and robust olive planting stocks, which will help
340 ensure to obtain healthier plantlets being of potentially higher survival rate after transplanting from
341 nursery to the field. Moreover, indigenous AMF are better adapted to local environmental
342 conditions and result in a substantial promotion of plant fitness. The present work concentrated on
343 the response of olive plantlets to AMF inoculation during the nursery process, and further research
344 is required to evaluate the long-term performance of AMF-inoculated plants in the field. In
345 addition, this study also highlights the necessity of further exploring and exploiting the natural
346 diversity of AMF in more olive growing sites and screening for AMF species with a high capacity
347 to alleviate abiotic and biotic stress of olive plants at different growing sites.
348
349

350 ## References

351 Abdel-Fattah GM, Asrar AA, Al-Amri SM, and Abdel-Salam EM. 2014. Influence of arbuscular mycorrhiza
352   and phosphorus fertilization on the gas exchange, growth and phosphatase activity of soybean (Glycine
353   max L.) plants. *Photosynthetica* 52:581-588 DOI 10.1007/s11099-014-0067-0.

354   Affokpon A, Coyne DL, Lawouin L, Tossou C, Dossou Agbèdè R, and Coosemans J. 2011. Effectiveness of
355   native West African arbuscular mycorrhizal fungi in protecting vegetable crops against root-knot
356   nematodes. *Biology and Fertility of Soils* 47:207-217 DOI 10.1007/s00374-010-0525-1.
357   Aganchich B, Wahbi S, Yaakoubi A, El-Aououad H, and Bota J. 2022. Effect of arbuscular mycorrhizal fungi
358   inoculation on growth and physiology performance of olive tree under regulated deficit irrigation and partial
359   rootzone drying. *South African Journal of Botany* 148:1-10 DOI 10.1016/j.sajb.2022.03.051.
360   Allen MF. 1996. The ecology of arbuscular mycorrhizas: a look back into the 20th century and a peek into the
361   21st. *Mycological Research* 100:769-782 DOI 10.1016/S0953-7562(96)80021-9.
362   Angelard C, Colard A, Niculita-Hirzel H, Croll D, and Sanders IR. 2010. Segregation in a Mycorrhizal Fungus
363   Alters Rice Growth and Symbiosis-Specific Gene Transcription. *Current Biology* 20:1216-1221 DOI
364   10.1016/j.cub.2010.05.031.
365   Ben Hassena A, Zouari M, Trabelsi L, Decou R, Ben Amar F, Chaari A, Soua N, Labrousse P, Khabou W, and
366   Zouari N. 2021. Potential effects of arbuscular mycorrhizal fungi in mitigating the salinity of treated
367   wastewater in young olive plants (*Olea europaea* L. cv. Chetoui). *Agricultural Water Management*
368   245:106635 DOI 10.1016/j.agwat.2020.106635.
369   Berruti A, Lumini E, Balestrini R, and Bianciotto V. 2016. Arbuscular mycorrhizal fungi as natural biofertilizers:
370   let's benefit from past successes. *Frontiers in Microbiology* 6:1559 DOI 10.3389/fmicb.2015.01559.
371   Black KG, Mitchell DT, and Osborne BA. 2000. Effect of mycorrhizal-enhanced leaf phosphate status on carbon
372   partitioning, translocation and photosynthesis in cucumber. *Plant, Cell & Environment* 23:797-809 DOI
373   10.1046/j.1365-3040.2000.00598.x
374   Bompadre MJ, Pérgola M, Bidondo L, Colombo R, Silvani V, Pardo A, Ocampo J, and Godeas A. 2014.
375   Evaluation of arbuscular mycorrhizal fungi capacity to alleviate abiotic stress of olive (*Olea europaea* L.)
376   plants at different transplant conditions. . *The Scientific World Journal* 30389 DOI 10.1155/2014/378950.
377   Boutaj H, Chakhchar A, Meddich A, Wahbi S, El Alaoui-Talibi Z, Douira A, Filali-Maltouf A, and El Modafar
378   C. 2021. Mycorrhizal autochthonous consortium induced defense-related mechanisms of olive trees against
379   Verticillium dahliae. *Journal of Plant Diseases and Protection* 128:225-237 DOI 10.1007/s41348-020-
380   00365-3.
381   Briccoli Bati C, Santilli E, and Lombardo L. 2015. Effect of arbuscular mycorrhizal fungi on growth and on
382   micronutrient and macronutrient uptake and allocation in olive plantlets growing under high total Mn levels.
383   *Mycorrhiza* 25:97-108 DOI 10.1007/s00572-014-0589-0.
384   Bücking H, and Shachar-Hill Y. 2005. Phosphate uptake, transport and transfer by the arbuscular mycorrhizal
385   fungus *Glomus intraradices* is stimulated by increased carbohydrate availability. *New Phytologist* 165:899-
386   912 DOI 10.1111/j.1469-8137.2004.01274.x.
387   Calvente R, Cano C, Ferrol N, Azcón-Aguilar C, and Barea JM. 2004. Analysing natural diversity of arbuscular
388   mycorrhizal fungi in olive tree (*Olea europaea* L.) plantations and assessment of the effectiveness of native
389   fungal isolates as inoculants for commercial cultivars of olive plantlets. *Applied Soil Ecology* 26:11-19 DOI
390   10.1016/j.apsoil.2003.10.009.
391   Castillo P, Nico AI, Azcón-Aguilar C, Del Río Rincón C, Calvet C, and Jiménez-Díaz RM. 2006. Protection of
392   olive planting stocks against parasitism of root-knot nematodes by arbuscular mycorrhizal fungi. *Plant
393   Pathology* 55:705-713 DOI 10.1111/j.1365-3059.2006.01400.x.
394   Chenchouni H, Mekahlia MN, and Beddiar A. 2020. Effect of inoculation with native and commercial arbuscular
395   mycorrhizal fungi on growth and mycorrhizal colonization of olive (*Olea europaea* L.). *Scientia
396   Horticulturae* 261:108969 DOI 10.1016/j.scienta.2019.108969.
397   Dag A, Yermiyahu U, Ben-Gal A, Zipori I, and Kapulnik Y. 2009. Nursery and post-transplant field response
398   of olive trees to arbuscular mycorrhizal fungi in an arid region. *Crop & pasture science* 60(5):427-433 DOI
399   10.1071/CP08143.
400   Dela Cruz RE, Manalo MQ, Aggangan NS, and Tambalo JD. 1988. Growth of three legume trees inoculated
401   with VA mycorrhizal fungi and Rhizobium. *Plant and Soil* 108:111-115 DOI 10.1007/BF02370105.
402   Estaún V, Camprubí A, Calvet C, and Pinochet J. 2003. Nursery and field response of olive trees inoculated with
403   two arbuscular mycorrhizal fungi, *Glomus intraradices* and *Glomus mosseae*. *Journal of the American
404   Society for Horticultural Science jashs* 128:767-775 DOI 10.21273/JASHS.128.5.0767.

405   Estrada B, Aroca R, Barea JM, and Ruiz-Lozano JM. 2013. Native arbuscular mycorrhizal fungi isolated from
406   a saline habitat improved maize antioxidant systems and plant tolerance to salinity. *Plant Science* 201-
407   202:42-51.   DOI 10.1016/j.plantsci.2012.11.009.
408   Fabbri A, Lambardi M, and Ozden-Tokatli Y. 2009. Olive Breeding. In: Jain SM, and Priyadarshan PM, eds.
409   *Breeding Plantation Tree Crops: Tropical Species*. New York, NY: Springer New York, 423-465.
410   Gai JP, Feng G, Christie P, and Li XL. 2006. Screening of arbuscular mycorrhizal fungi for symbiotic efficiency
411   with sweet potato. *Journal of Plant Nutrition* 29:1085-1094 DOI 10.1080/01904160600689225.
412   Gutierrez AP, Ponti L, and Cossu Q. 2009. Effects of climate warming on Olive and olive fly (*Bactrocera oleae*
413   (Gmelin)) in California and Italy. *Climatic Change* 95:195-217 DOI 10.1007/s10584-008-9528-4.
414   Harrison MJ, Dewbre GR, and Liu J. 2002. A phosphate transporter from Medicago truncatula involved in the
415   acquisition of phosphate released by arbuscular mycorrhizal fungi. *The Plant Cell* 14:2413-2429 DOI
416   10.1105/tpc.004861.
417   Harrison MJ, and van Buuren ML. 1995. A phosphate transporter from the mycorrhizal fungus Glomus
418   versiforme. *Nature* 378:626-629 DOI 10.1038/378626a0.
419   Hayman DS, Barea J-M, and Azcon R. 1976. Vesicular-arbuscular mycorrhiza in Southern Spain: its distribution
420   in crops growing in soil of different fertility. *Phytopathologia Mediterranea* 15:1-6.
421   Horden P, and Purcell N. 2012. *The corrupting sea: a study of Mediterranean history*. Malden: Blackwell
422   Publishing.
423   Igiehon NO, and Babalola OO. 2017. Biofertilizers and sustainable agriculture: exploring arbuscular mycorrhizal
424   fungi. *Applied Microbiology and Biotechnology* 101:4871-4881 DOI 10.1007/s00253-017-8344-z.
425   Jing Y, Mao J, and Li R. 2022. Arbuscular mycorrhizal fungal diversity associated with *Olea europaea* L.
426   growing in Yunnan province, southwestern China. *Phyton-International Journal of Experimental Botany*
427   DOI 10.32604/phyton.2022.021989.
428   Labidi S, Jeddi FB, Tisserant B, Yousfi M, Sanaa M, Dalpé Y, and Sahraoui AL-H. 2015. Field application of
429   mycorrhizal bio-inoculants affects the mineral uptake of a forage legume (*Hedysarum coronarium* L.) on a
430   highly calcareous soil. *Mycorrhiza* 25:297-309 DOI 10.1007/s00572-014-0609-0.
431   Loth-Pereda V, Orsini E, Courty P-E, Lota F, Kohler A, Diss L, Blaudez D, Chalot M, Nehls U, Bucher M, and
432   Martin F. 2011. Structure and expression profile of the phosphate Pht1 transporter gene family in
433   mycorrhizal Populus trichocarpa. *Plant Physiology* 156:2141-2154 DOI 10.1104/pp.111.180646.
434   M'barki N, Chehab H, Aissaoui F, Dabbaghi O, Attia F, Mahjoub Z, Laamari S, Chihaoui B, del Giudice T,
435   Jemai A, Boujnah D, and Mechri B. 2018. Effects of mycorrhizal fungi inoculation and soil amendment
436   with hydrogel on leaf anatomy, growth and physiology performance of olive plantlets under two contrasting
437   water regimes. *Acta Physiologiae Plantarum* 40:116 DOI 10.1007/s11738-018-2692-x.
438   Martín MLS, Azcón R, Barea JM, Porras Soriano A, Goldaracena IM, and Piedra AP. 2006. Reduction of the
439   juvenile period of new olive plantations through the early application of mycorrhizal fungi. *Soil Science*
440   171 DOI 10.1097/01.ss.0000187348.31987.b6.
441   McGonigle TP, Miller MH, Evans DG, Fairchild GL, and Swan JA. 1990. A new method which gives an
442   objective measure of colonization of roots by vesicular—arbuscular mycorrhizal fungi. *New Phytologist*
443   115:495-501.   DOI 10.1111/j.1469-8137.1990.tb00476.x.
444   Meddad-Hamza A, Beddiar A, Gollotte A, Lemoine M, Kuszala C, and Gianinazzi S. 2010. Arbuscular
445   mycorrhizal fungi improve the growth of olive trees and their resistance to transplantation stress. *African
446   Journal of Biotechnology* 9:1159-1167 DOI 10.5897/AJB09.1282.
447   Mohamed Oussouf F, Essahibi A, Benhiba L, and Qaddoury A. 2014. Effectiveness of arbuscular mycorrhizal
448   fungi in the protection of olive plants against oxidative stress induced by drought. *SPANISH JOURNAL OF
449   AGRICULTURAL RESEARCH* 12:763-771 DOI 10.5424/sjar/2014123-4815.
450   Munkvold L, Kjøller R, Vestberg M, Rosendahl S, and Jakobsen I. 2004. High functional diversity within species
451   of arbuscular mycorrhizal fungi. *New Phytologist* 164:357-364 DOI 10.1111/j.1469-8137.2004.01169.x.
452   Nagy R, Karandashov V, Chague V, Kalinkevich K, Tamasloukht MB, Xu G, Jakobsen I, Levy AA, Amrhein
453   N, and Bucher M. 2005. The characterization of novel mycorrhiza-specific phosphate transporters from
454   *Lycopersicon esculentum* and *Solanum tuberosum* uncovers functional redundancy in symbiotic phosphate
455   transport in solanaceous species. *The Plant Journal* 42:236-250 DOI 10.1111/j.1365-313X.2005.02364.x.
456   Nussaume L, Kanno S, Javot H, Marin E, Nakanishi TM, and Thibaud M-C. 2011. Phosphate Import in Plants:
457   Focus on the PHT1 Transporters. *Frontiers in Plant Science* 23(2):83 DOI 10.3389/fpls.2011.00083

458 Olsson PA, Burleigh SH, and Van Aarle IM. 2005. The influence of external nitrogen on carbon allocation to
459   *Glomus intraradices* in monoxenic arbuscular mycorrhiza. *New Phytologist* 168:677-686 DOI
460   10.1111/j.1469-8137.2005.01532.x.
461 Ouledali S, Ennajeh M, Zrig A, Gianinazzi S, and Khemira H. 2018. Estimating the contribution of arbuscular
462   mycorrhizal fungi to drought tolerance of potted olive trees (*Olea europaea*). *Acta Physiologiae Plantarum*
463   40:81 DOI 10.1007/s11738-018-2656-1.
464 Paszkowski U, Kroken S, Roux C, and Briggs SP. 2002. Rice phosphate transporters include an evolutionarily
465   divergent gene specifically activated in arbuscular mycorrhizal symbiosis. *Proceedings of the National*
466   *Academy of Sciences* 99:13324 DOI 10.1073/pnas.202474599.
467 Pellegrino E, Turrini A, Gamper HA, Cafà G, Bonari E, Young JPW, and Giovannetti M. 2012. Establishment,
468   persistence and effectiveness of arbuscular mycorrhizal fungal inoculants in the field revealed using
469   molecular genetic tracing and measurement of yield components. *New Phytologist* 194:810-822 DOI
470   10.1111/j.1469-8137.2012.04090.x.
471 Porras-Soriano A, Soriano-Martín ML, Porras-Piedra A, and Azcón R. 2009. Arbuscular mycorrhizal fungi
472   increased growth, nutrient uptake and tolerance to salinity in olive trees under nursery conditions. *Journal*
473   *of Plant Physiology* 166:1350-1359 DOI 10.1016/j.jplph.2009.02.010.
474 R Core Team, 2019. R: A Language and Environment for Statistical Computing. Available at: R Foundation for
475   Statistical Computing, Vienna, Austria. http://www.r-project. org.
476 Raghothama KG, and Karthikeyan AS. 2005. Phosphate acquisition. *Plant and Soil* 274:37. 10.1007/s11104-
477   004-2005-6
478 Rausch C, Daram P, Brunner S, Jansa J, Laloi M, Leggewie G, Amrhein N, and Bucher M. 2001. A phosphate
479   transporter expressed in arbuscule-containing cells in potato. *Nature* 414:462-465 DOI 10.1038/35106601.
480 Ray DL, and Johnson JC. 2014. Validation of reference genes for gene expression analysis in olive (*Olea*
481   *europaea*) mesocarp tissue by quantitative real-time RT-PCR. *BMC Res Notes* 7:304 DOI 10.1186/1756-
482   0500-7-304.
483 Rodrigues MÂ, Piroli LB, Forcelini D, Raimundo S, da Silva Domingues L, Cassol LC, Correia CM, and
484   Arrobas M. 2021. Use of commercial mycorrhizal fungi in stress-free growing conditions of potted olive
485   cuttings. *Scientia Horticulturae* 275:109712 DOI 10.1016/j.scienta.2020.109712
486 Roldán-Fajardo B, and Barea J. 1986. Mycorrhizal dependency in the olive tree (*Olea europaea* L.). In:
487   Gianninazzi-Pearson V, and Gianninazzi S, eds. *Les mycorhizes: physiologie et gènètique*. Paris: INRA,
488   323-326.
489 Schwartz MW, Hoeksema JD, Gehring CA, Johnson NC, Klironomos JN, Abbott LK, and Pringle A. 2006. The
490   promise and the potential consequences of the global transport of mycorrhizal fungal inoculum. *Ecology*
491   *Letters* 9:501-515 DOI 10.1111/j.1461-0248.2006.00910.x.
492 Smith SE, and Read D. 2008. *Mycorrhizal Symbiosis (Third Edition)*. New York, London, Burlington, San
493   Diego: Academic Press.
494 Steenbjerg F, and Jakobsen ST. 1963. Plant Nutrition and Yield Curves. *Soil Science* 95:69-88 DOI
495   10.1097/00010694-196301000-00012.
496 Tiessen H. 2005. Phosphorus Dynamics in Tropical Soils. In: Sims T, and Sharpley AN, eds. *Agronomy*
497   *Monographs*: American Society of Agronomy, 253-262.
498 Tobar RM, Azcón R, and Barea JM. 1994. The improvement of plant N acquisition from an ammonium-treated,
499   drought-stressed soil by the fungal symbiont in arbuscular mycorrhizae. *Mycorrhiza* 4:105-108 DOI
500   10.1007/BF00203769.
501 Unver T, Wu Z, Sterck L, Turktas M, Lohaus R, Li Z, Yang M, He L, Deng T, Escalante FJ, Llorens C, Roig
502   FJ, Parmaksiz I, Dundar E, Xie F, Zhang B, Ipek A, Uranbey S, Erayman M, Ilhan E, Badad O, Ghazal H,
503   Lightfoot DA, Kasarla P, Colantonio V, Tombuloglu H, Hernandez P, Mete N, Cetin O, Van Montagu M,
504   Yang H, Gao Q, Dorado G, and Van de Peer Y. 2017. Genome of wild olive and the evolution of oil
505   biosynthesis. *Proceedings of the National Academy of Sciences* 114:E9413 DOI 10.1073/pnas.1708621114.
506 Versaw WK, and Harrison MJ. 2002. A chloroplast phosphate transporter, PHT2;1, influences allocation of
507   phosphate within the plant and phosphate-starvation responses. *The Plant Cell* 14:1751-1766 DOI
508   10.1105/tpc.002220.

509   Wang J, Zhang D, Farooqi TJA, Ma L, Deng Y, and Jia Z. 2019. The olive (*Olea europaea* L.) industry in China:
510   its status, opportunities and challenges. *Agroforestry Systems* 93:395-417 DOI 10.1007/s10457-017-0129-
511   y.
512   Wright DP, Scholes JD, Read DJ, and Rolfe SA. 2005. European and African maize cultivars differ in their
513   physiological and molecular responses to mycorrhizal infection. *New Phytologist* 167:881-896 DOI
514   10.1111/j.1469-8137.2005.01472.x.
515   Xu GH, Chague V, Melamed-Bessudo C, Kapulnik Y, Jain A, Raghothama KG, Levy AA, and Silber A. 2007.
516   Functional characterization of LePT4: a phosphate transporter in tomato with mycorrhiza-enhanced
517   expression. *Journal of Experimental Botany* 58:2491-2501 DOI 10.1093/jxb/erm096.
518   Yang SY, Grønlund M, Jakobsen I, Grotemeyer MS, Rentsch D, Miyao A, Hirochika H, Kumar CS,
519   Sundaresan V, Salamin N, Catausan S, Mattes N, Heuer S, and Paszkowski U. 2012. Nonredundant
520   regulation of rice arbuscular mycorrhizal symbiosis by two members of the phosphate transporter1 gene
521   family. *The Plant Cell* 24:4236-4251 DOI 10.1105/tpc.112.104901.

Primer sequences of four *Pht* genes and the internal control gene *OeEF1α* in the root of *Olea europaea*

Gene name	Accession number	Sequences (5'-3')	Produce size / bp
Pht1;11	NC_036237	F–ATCCACTTGCCACTCACTGA	201
		R–ATATCTCCTCCAGCGACAGC	
Pht1;4	XM_023017225	F–GACTGCGATCTACATGCCATG	164
		R–GCCTAACACGATGAGCGAATT	
Pht1;2	XM_023031284	F–GCTCAAGAATCAACGAGGTCA	159
		R–CGAGTTGGCTGAGACGCATTA	
Pho	XM_023005125	F–AGCACATATTGGGACATTGTA	143
		R–CAGGCTAACCTTAACAAGACA	
OeEF1α	XM_002527974	F–GAATGGTGATGCTGGTTTCG	191
		R–CCCTTCTTGGCAGCAGACTTG	

Root colonization parameters of olive plantlets inoculated with six AMF inocula

Values are means ± SE ($n = 4$), columns marked with different letters differed significantly ($p < 0.05$).

Treatment	AMF hypha colonization (H%)	AMF arbuscule colonization (A%)	AMF vesicle colonization (V%)
AMF1	73.78±2.35b	17.07±4.40c	12.11±3.14b
AMF2	78.30±9.76ab	20.80±2.84c	10.91±1.88b
AMF3	93.78±2.13a	37.69±5.23ab	47.35±8.17a
AMF4	40.20±6.51c	3.32±0.53d	2.07±1.05c
AMF5	93.38±4.19a	42.46±7.62a	49.56±11.95a
AMF6	67.17±5.23b	28.79±4.18bc	11.53±2.92b

Figure 1

Typical structures of AMF colonizing the roots of olive plantlets.

Plantlets inoculated with AMF1 (a), AMF2 (b and c), AMF3 (d), AMF4 (e), AMF5 (f), and AMF6 (g). Plantlets with compound fertilize application, the treatment Fert1 (h). Uninoculated plantlets, the treatment Control (i). H: hypha, HC: hyphal coil, A: arbuscule, V: vesicle.
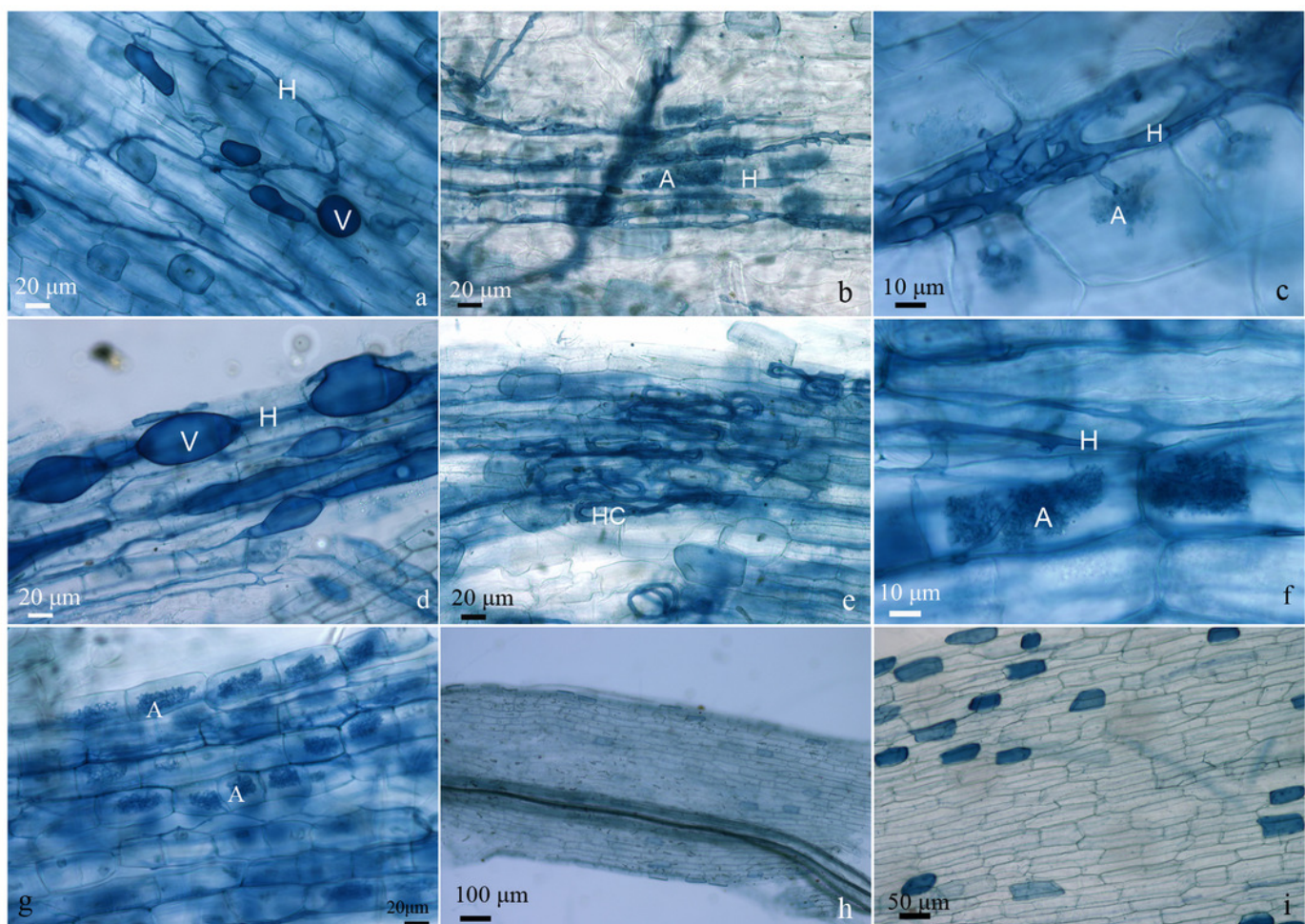
Figure 2

Box plots representing growth parameters of six-month-inoculated plantlets by native and commercial AMF, fertilizers, control (uninoculated).

(a) Plant height; (b) Root collar diameter; (c) Shoot fresh weight; (d) Shoot dryweight; (e) Root fresh weight; (f) Root dry weight; (g) Leaf area; (h) Root:shootratio. Solid white circles indicate the means, bold black line indicates the median, whereas black dots are outliers. Letters on white circles are the results of Tukey's HSD tests, where different letters indicate significant differences at $P \leq 0.05$.

(a) Shoot height (cm)

(b) Root collar diameter (mm)

(c) Shoot fresh weight (g)

(d) Shoot dry weight (g)

(e) Root fresh weight (g)

(f) Root dry weight (g)

(g) Leaf area (cm²)

(h) Root:shoot ratio
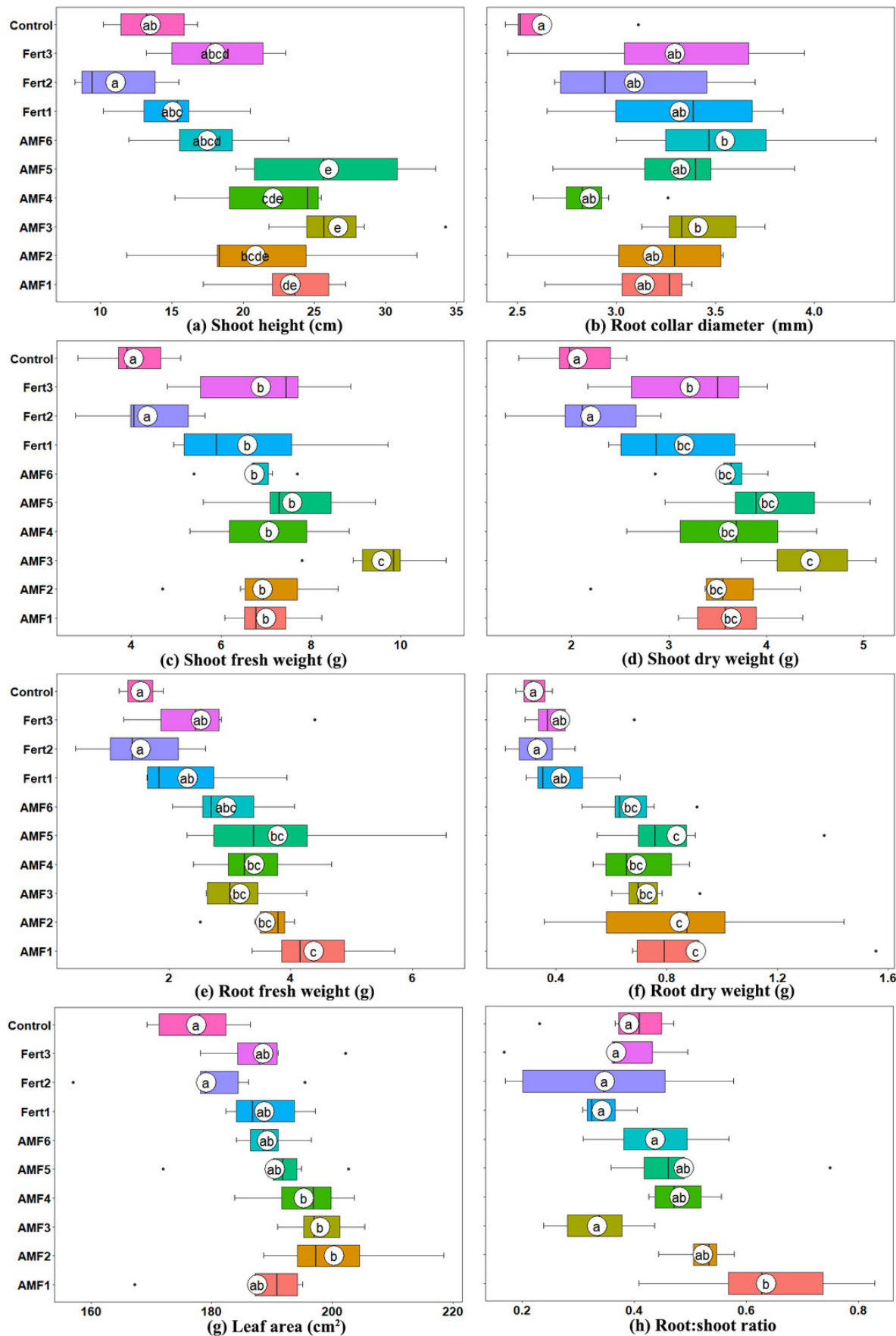
Figure 3

Box plots representing the foliar nutrient content of olive plantlets treated with AMF, fertilizers and the control.

(a) Nitrogen content (N content); (b) Phosphorus content (P content); (c) Potassium content (K content). Labeling is same as in Figure 2.
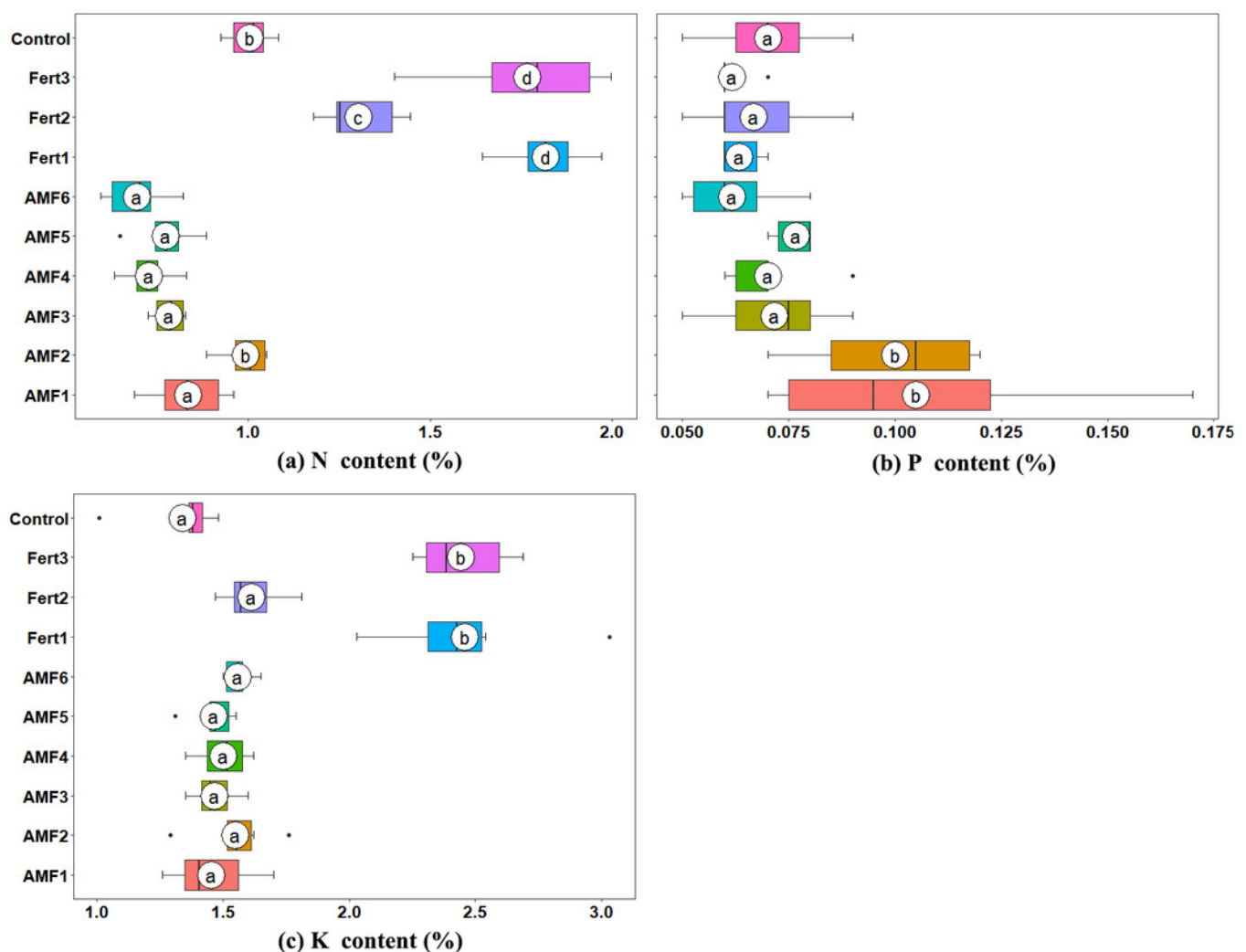
Figure 4

Phylogenetic relationships of the *Pht* DNA sequences in different plants built using MrBayes method based on the GTR substitution model, supported by bootstrap resampling with 1,000 replications.

Le: *Lycopersicon esculentum*; Mt: *Medicago truncatula*; Oe: *Olea europaea*; Os: *Oryza sativa*;

St: *Solanum tuberosum*; Zm: *Zea mays*.
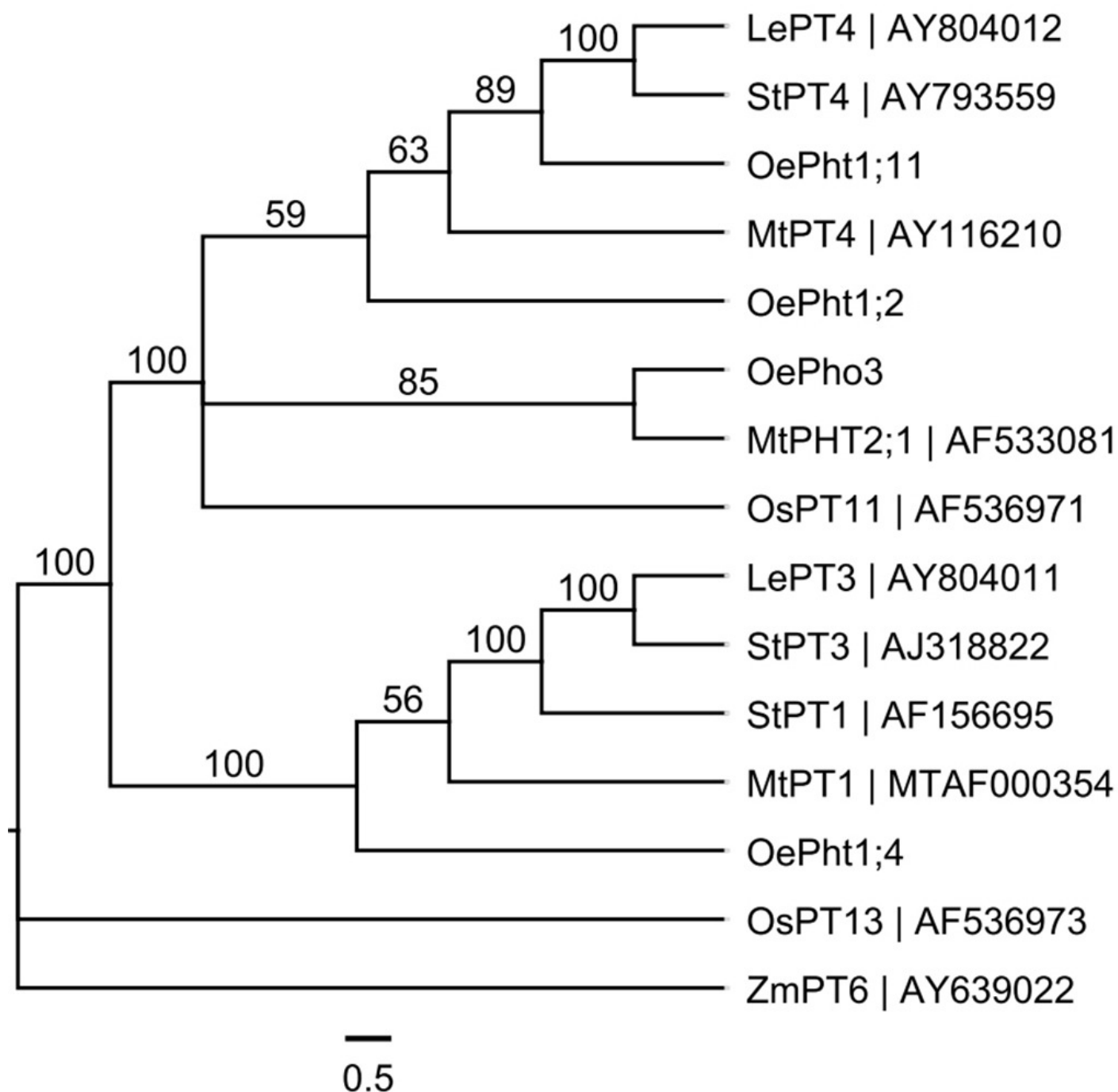
Figure 5

Expression levels of *Pht1;11*, *Pht1;4*, *Pht1;2* and *Pho3* in olive mycorrhizal roots by qRT-PCR analysis.

Pht1;11

Pht1;4

Pht1;2

Pho3