Arbuscular mycorrhizal fungi enhanced the growth, phosphorus uptake and *Pht* expression of olive (*Olea europaea* L.) plantlets (#71026)

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

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Olive (*Olea europaea* L.) is a highly mycotrophic species that has been introduced and cultivated in China for half a century. However, it is still unclear how native AMF impact growth and mineral nutrients, especially phosphorus absorption. 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 boron; 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. The study results are expected to provide a theoretical basis for analyzing the phosphorus uptake pathway promoted by AMF in olive.

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Keywords

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

Introduction

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

Arbuscular mycorrhizal fungi (AMF) can form mutualistic symbioses with approximately 80% of land plant species. They can acquire nutrients from soil volumes that are inaccessible to roots, and provide the host plant with mineral nutrients and water, in exchange for photosynthetic products (Berruti et al. 2016). Olive is a typical mycotrophic species (Calvente et al. 2004; Hayman et al. 1976; Roldán-Fajardo & Barea 1986), and many studies have reported that the early presence of AMF increases the growth of olive rooted cuttings and micropropagated plants (Estaún et al. 2003; Martín et al. 2006) and enhances olive plant tolerance to stress caused by transplanting (Bompadre et al. 2014; Dag et al. 2009), drought (Ouledali et al. 2018), salinity (Ben Hassena et al. 2021) and disease (Boutaj et al. 2021). Notably, AMF are known to improve P nutrition in plants by making them accessible to unavailable soil P sources (Allen 1990). Thus, AMF show superior prospects as biofertilizers, especially in tropical soils (Igiehon & Babalola 2017), which are usually dominated by iron and aluminum oxides and maintain a lower available P than temperate soils (Tiessen 2005).

In this study, we hypothesized that, after years of planting in China, (1) the olive trees harbored some indigenous AMF; (2) these AMF can produce promising effects on the growth and phosphorus (P) uptake of olive plants, and the expression of genes related to P uptake can also be promoted with the root colonization of AMF. In this context, we collected rhizosphere soil of olive trees from five growing sites in Yunnan as the source of indigenous AMF consortia and compared them with commercial AMF inoculum and fertilizer to study their effects on olive plantlets. Furthermore, the gene expression of P absorption via the formation of AM was also characterized.

Materials & Methods

Plant materials and cultivation conditions

Koroneiki was selected since it is one of the most common olive cultivars in Yunnan province with promising performance. The plantlets used in this study were obtained by mist propagation. The semi-woody cuttings were approximately 10 cm long and had two pairs of leaves at the top

end. The bottom basal end of each cutting was immersed in an ethanol solution containing 5 g of indol-3-butyric acid/L to promote root development. Propagation was performed in an arch tunnel with a controlled glass greenhouse under an air temperature of 20 ± 2 ℃. After three months, the rooting cuttings were transferred to 7.5 cm × 11.5 cm plastic pots filled with growing medium.

The growing medium was a mixture of 50% peat moss and 50% vermiculite (*v*/*v*) with a pH of 6.85, organic matter of 251.83 g/kg, hydrolyzable nitrogen of 406.76 mg/kg, available phosphorus of 43.12 mg/kg, available potassium of 469.38 mg/kg, total nitrogen, phosphorus and potassium of 5.08 g/kg, 0.62 g/kg and 16.57 g/kg, respectively. The growing medium was autoclaved twice for a period of 2 h at 121°C, with a 24 h interval between the two sterilizations.

AMF and fertilization treatments

The native soil inocula were collected from five orchards located in the representative olive introduction and cultivation areas in Yunnan Province. The five growing sites sampled were coded AMF1~AMF5. AMF1 soil was collected from Deqin County, AMF2 and AMF3 soil samples were collected from Yongren County, and AMF4 and AMF5 soil samples were collected from Lijiang County. The spore numbers of AMF1 to AMF5 were 350, 121, 82, 198 and 116 in 20 grams of soil. The dominant AMF species of AMF1, AMF2, AMF3 and AMF5 were *Funneliformis geosporum*, that of AMF4 was *Septoglomus constrictum*. A commercial AMF inoculant (coded AMF6) was assayed as a reference alongside the native soil inocula. AMF6 was produced in the form of granules, spores and roots, *viz*. *Rhizophagus intraradices*, *F. mosseae*, *R. aggregatus* and *Claroideoglomus etunicatum* (without other additives). Three fertilizer treatments were used here to compare their effects with AMF inocula, namely, Fert1 (3 gram/pot of compound (15:15:15) fertilizer), Fert2 (3 gram/pot of earthworm manure, whose main components include 56.2% organic matter, 1.78% total nitrogen, 1.67% total phosphorus, 1.02% total potassium, pH 7.66) and Fert3 (Fert1 + Fert2). Plantlets with neither inoculation nor fertilizer application were used as the control. Six repetitions were performed for each treatment. Inoculation was carried out at the time of transferring the rooted cuttings into the containers. Three grams of inoculum was deposited directly below the roots of the rooted cuttings.

To expose the cuttings in every treatment to an equal nutritional level, for the AMF1 treatment, 3 grams of sterilized rhizosphere soil from AMF2 to AMF5 were added to the pot, and for AMF2, 3 grams of sterilized rhizosphere soil from AMF1, AMF3 to AMF5 were added to the pot, and so on. For the AMF6, Fert1, Fert2, Fert3 and CK treatments, the rhizosphere sterilized soil of AMF1 to AMF5 was added to the pot 3 grams each.

The plantlets were grown in the greenhouse of the Yunnan Academy of Forestry and Grassland and watered manually with tap water depending on the environmental conditions prevailing inside the greenhouse. After six months of growth, the shoots and roots of the olive plantlets were harvested separately.

Detection of AMF colonization

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

Plantlet growth measurements

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

qRT–PCR of phosphate transporter genes

The wild olive assembly and annotated genome (Unver et al. 2017) were used as queries for phosphate transporter protein genes. Total RNA of olive roots was isolated using an RNAprep Pure Plant Kit (Tiangen, China). DNase was used to eliminate the potential trace of genomic DNA in RNA samples. Then, 1.0% agarose gel and a NanoDrop ND-2000 spectrophotometer (Thermo Fisher, USA) were used to evaluate and quantify RNA, respectively. RNA samples were reverse-transcribed into cDNA with the FastKing RT Kit (Tiangen, China), and synthesized cDNAs were used as templates for qRT–PCR with the SuperReal PreMix Plus Kit (Tiangen, China). *O. europaea* translation elongation factor-1 alpha (*OeEF1α*) served as the internal control for the qRT–PCR (Ray & Johnson 2014). All the primer sequences are listed in Table 1.

A 20 μL reaction solution containing 1 μL cDNA (20 ng), 1 μL of each primer (10 μM), 10 μL SYBR Green I Master mix reagent (Tiangen) and 7 μL ddH_2O was amplified with a LightCycler96 (Roche, Switzerland). The qRT–PCR program was as follows: 95°C for 15 min, followed by 45 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 biological replicates, and the data are shown as the mean ± SD. The relative transcript level was calculated using the $2^{-\Delta\Delta Ct}$ method.

Statistical analysi

Values of mycorrhizal parameters were summarized for each native and commercial AMF using some descriptive statistics (mean ± standard error (SE). Data related to olive plantlet growth parameters per treatment were visualized using boxplots. One-way analysis of variance (ANOVA) was applied to test the variation in seedling traits. Each ANOVA was followed by Tukey's honest significance test (HSD) to distinguish homogeneous groups among AMF inoculation and fertilizer application.

Results

Root colonization by AMF

No mycorrhizal colonization was detected, as expected, in the roots of plantlets under fertilizer application (treatment Fert1, Fert2 and Fert3) and noninoculated ones (treatment CK). Except for AMF4, all other treatments showed similar or higher root AMF colonization percentages compared with the commercial AMF inoculum (AMF6). AMF hyphae colonization in roots of olive plantlets ranged from 40.20% in AMF4 to 93.78% in AMF3. The lowest mean arbuscule colonization was noted in AMF4 (3.32%), whereas the highest average was recorded in AMF5 (42.46%). For the abundance of vesicles, the mean varied between 2.07% in AMF4 and 49.56% in AMF5 (Table 2). Some typical structures of AMF colonizing the roots of olive plantlets are presented in Figure 1.

Effect of AMF inoculation on the growth of olive plantlets

Six months after inoculation, all AMF inoculation treatments had a positive influence on plant growth in terms of height, diameter, biomass and leaf area (Fig. 2). Compared to the noninoculated CK, the commercial AMF inoculum increased the shoot and root fresh weights of plantlets by 66.27% and 91.90%, respectively. However, the native AMF inocula achieved higher effects (Fig. 2).

AMF3 showed the highest effect on aboveground growth, and the shoot dry weights of inoculated plantlets were 2.16 times, 1.41 times, 2.02 times, 1.38 times and 1.24 times those of the CK, Fert1, Fert2, Fert3 and AMF6 treatments, respectively. Whereas AMF1 showed the highest effect on enhancing the belowground growth of the plantlets, the plantlets inoculated with AMF1 had root dry weights 2.84 times, 2.17 times, 2.71 times, 2.19 times and 1.35 times those of treatment CK, Fert1, Fert2, Fert3 and AMF6 treatments, respectively. The root to shoot ratios of olive plantlets inoculated with AMF1, AMF2, AMF3, AMF4, AMF5, AMF6, Fert1, Fert2, Fert3 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.

Effect of AMF inoculation on the N, P, K and B content of olive plantlets

The leaf nitrogen (N) contents of all the AMF inoculated plantlets were lower than the CK, except AMF2, which had a similar value to the control. Fertilization significantly increased the foliar N content compared with AMF and CK (Fig. 3a). The foliar P contents of olive plantlets inoculated with AMF1 and AMF2 were significantly higher than those of all other treatments

(Fig. 3b). AMF inoculation marginally enhanced the potassium (K) uptake of the plantlets, and the leaf K contents of plantlets treated with AMF1 to AMF6 were 8.34%, 15.54%, 9.33%, 12.07%, 9.20% and 16.29% higher than those of the control; however, the differences were not significant (Fig. 3c). The leaf boron (B) content changed slightly among the different treatments, and the difference was not significant (Fig. 3d).

Effect of AMF inoculation on expression levels of phosphate transporter genes

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

A phylogenetic tree was constructed using a multiple DNA sequence alignment of four olive *Pht* genes and *Pht* transporters of other plants (Fig. 5). The four olive *Pht* genes were clustered into three main groups, *OePht1;11* and *OePht1;2* were clustered together with *PT4* genes of tomato (*LePT4*), potato (*StPT4*) and alfalfa (*MtPT4*); *OePho3* and *OePht1;4* were individually clustered into a separate group. The two *Pht* genes of monocotyledonous plants, *OsPT13* of rice and *ZmPT6* of maize, were distinct from those of all dicotyledonous plants.

Discussion

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

In this study, we found that the effects of native AMF were generally higher than those of the commercial AMF inoculum (Fig. 2). Many studies have pointed out the higher efficiency of native AMF compared to nonnative, introduced AMF (Affokpon et al. 2011; Briccoli Bati et al.

2015; Estrada et al. 2013; Labidi et al. 2015). Our results are in consistent with those found by Chenchouni et al. (2020), demonstrating that the effects of local AMF strains on increasing different growth parameters of olive plantlets were better than those of commercial AMF species (Chenchouni et al. 2020). Several studies have highlighted that different isolates within the same species, rather than different species, can cause large variations in plant response (Angelard et al. 2010; Gai et al. 2006; Munkvold et al. 2004). A study conducted earlier by Calvente et al. in 2004, showed that the *G. intraradices* strain, isolated from the olive rhizosphere, was more effective than the exotic *G. intraradices* from the culture collection (Calvente et al. 2004). More importantly, exploring and exploiting native AMF can avoid any potential problems related to the application of nonnative AMF inoculum in terms of biodiversity losses and homogenization as a result of anthropogenic translocation of biota between biogeographic regions (Pellegrino et al. 2012; Schwartz et al. 2006).

There is evidence that AMF play a role in the uptake of nitrate and ammonium which are assimilated and transported within the mycelium as arginine (Olsson et al. 2005), but compared with ectomycorrhizas, rates of N uptake by the hyphae of AMF are too small to contribute substantially to the N nutrition of plants (Smith & Read 2008). Accordingly, N uptake was not significantly different in AMF inoculated plants compared to noninoculated plants in this study (Fig. 3). The lower N concentration of inoculated olive plantlets can be explained by a 'dilution' effect commonly observed in plants growing well in infertile conditions (Steenbjerg & Jakobsen 1963), as Dela Cruz et al. (1988) reported previously in *Albizia falcataria* seedlings (Dela Cruz et al. 1988). In the present study, the N concentrations of olive plantlets inoculated with AMF1 to AMF6 were 0.8346%, 0.9927%, 0.7827%, 0.7528%, 0.7730% and 0.6939% respectively, whereas the noninoculated poorly growing olive plantlets (CK) showed the highest N concentration (1.0047%) among all treatments (Fig. 2, 3).

Mycorrhizal symbiosis was found to be important for root system development, which is critical for better mineral nutrition and stress resistance of seedlings. Tobar et al. (1994) conclusively demonstrated that the root-to-shoot ratio reflects the degree of efficiency of AM fungi (Tobar et al. 1994). In the present study, inoculation with AMF3 significantly enhanced the aerial parts of olive plantlets, resulting in a low ratio of roots to shoots, whereas the plantlets treated with the other four inocula achieved root to shoot ratios higher than those of the control. In addition, the average root to shoot ratio of the plantlets treated with fertilizer was the lowest among all the treatments (Fig. 2). Under chemical fertilization, minerals are immediately available for the plant, which reduces the need and triggering for extensive root development, resulting in a low ratio of root to shoot. Later, when the young plants are transplanted in the field without fertilizers, an abrupt decrease in the nutrient uptake and growth rate occurs (Meddad-Hamza et al. 2010).

Many studies have reported that mycorrhizal colonization can enhance P absorption by plants (Abdel-Fattah et al. 2014; Black et al. 2000; Bücking & Shachar-Hill 2005), including olive plants (Briccoli Bati et al. 2015; Dag et al. 2009; Estaún et al. 2003). Similarly, the results obtained from this study showed that the leaf P content of AMF inoculated plants was higher

than that of noninoculated plants (Fig. 3). In addition to dissolving insoluble phosphates into inorganic orthophosphate (Pi) and absorbing Pi from the rhizosphere beyond root depletion zones by AMF mycelium and transport to host plants (Harrison & van Buuren 1995), AMF can also induce the expression of some particular phosphate transporter genes in their host plants under Pi-deficient conditions (Rausch et al. 2001). For example, several AM symbiosis-induced *Pht* genes in plant roots have been identified, such as *LePT3* and *LePT4* in tomato (*Lycopersicon esculentum*) (Xu et al. 2007); *StPT1*, *StPT3* and *StPT4* in potato (*Solanum tuberosum*) (Nagy et al. 2005); *MtPT1*, *MtPT4* and *PHT2*;1 in alfalfa (*Medicago truncatula*) (Harrison et al. 2002; Versaw & Harrison 2002); *OsPT11* and *OsPT13* in rice (*Oryza sativa*) (Paszkowski et al. 2002; Yang et al. 2012); *ZmPT6* in corn (*Zea mays*) (Wright et al. 2005); *PtPT8*; and *PtPT10* in black cottonwood (*Populus trichocarpa*) (Loth-Pereda et al. 2011). The transcripts of one tomato *Pht* gene (*LePT4*) exhibited significantly increased expression levels in low-P treatment and colonized by the *Glomus intraradices* (Xu et al. 2007). Potato's *Pht* genes (*StPT4* and *StPT5*) also exhibited mycorrhiza upregulation when inoculated with *Gigaspora margarita*, and both were highly functionally redundant (Nagy et al. 2005). In our study, the four olive *Pht* genes were differentially regulated, with *OePht1;11* exhibiting mycorrhiza-specific regulation, similar to *LePT4* and *StPT4*; *OePht1;4* only mycorrhiza-upregulated in AMF1; *OePht1;2* were not sensitive to AMF and fertilizer; *OePho3* seemed more effective to fertilizer than AMF (Fig. 4). Although *OePht1;11* and *OePht1;2* were closer in the phylogenetic tree (Fig. 5), it remains to be clarified whether they function differently.

Conclusion

Although mycorrhizal networks ubiquitously exist in the soil, in intensively managed fields, mycorrhizal networks are usually absent or low in abundance because of regular soil disturbance destroying the mycelia or the absence of permanent vegetation cover that is needed to maintain mycorrhizal networks. During relatively long periods of development in the nursery, olive plantlets do not have AMF since they are usually grown in inert or fumigated potting media. AMF inoculation at the nursery stages is therefore critical and necessary and can also help plants cope with various stress conditions in the field. In this study, we report the effect of AMF inoculation on growth responses and the expression levels of four phosphate transporter genes in olive plantlets. Research indicates that there is great potential in using native AMF consortia as inoculants for the production of high-quality and robust olive planting stocks. The present work concentrated on the response of olive plantlets to AMF inoculation during the nursery process, further research is required to evaluate the long-term performance of AMF-inoculated plants in the field. In addition, this study also highlights the necessity of further exploring and exploiting the natural diversity of AMF in more olive growing sites.

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Table 1(on next page)

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	

1

Table 2(on next page)

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

1

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.

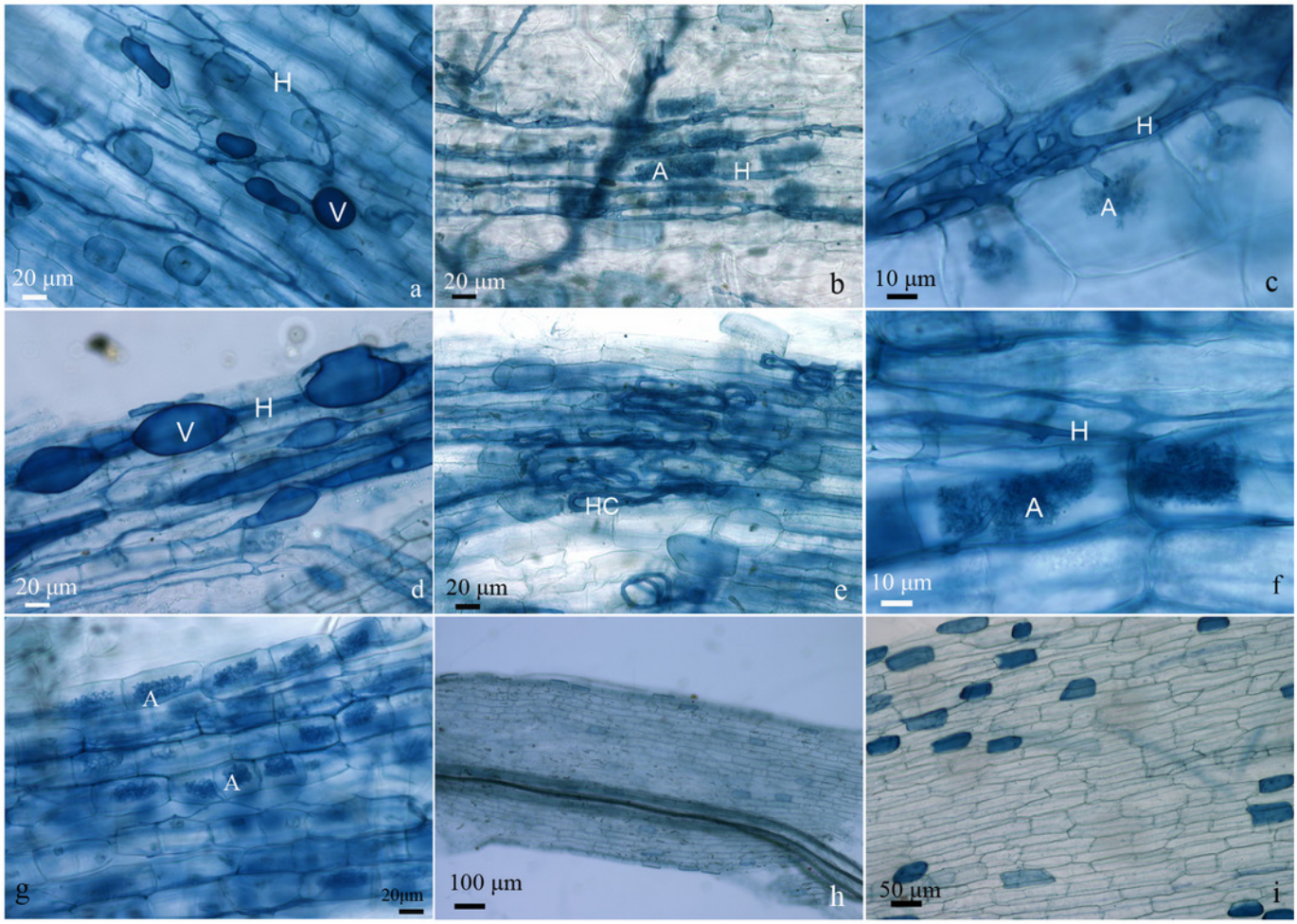


Figure 2

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 dry weight; (e) Root fresh weight; (f) Root dry weight; (g) Leaf area; (h) Root:shoot ratio. 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 the same letters are not significantly different at $p>0.05$.

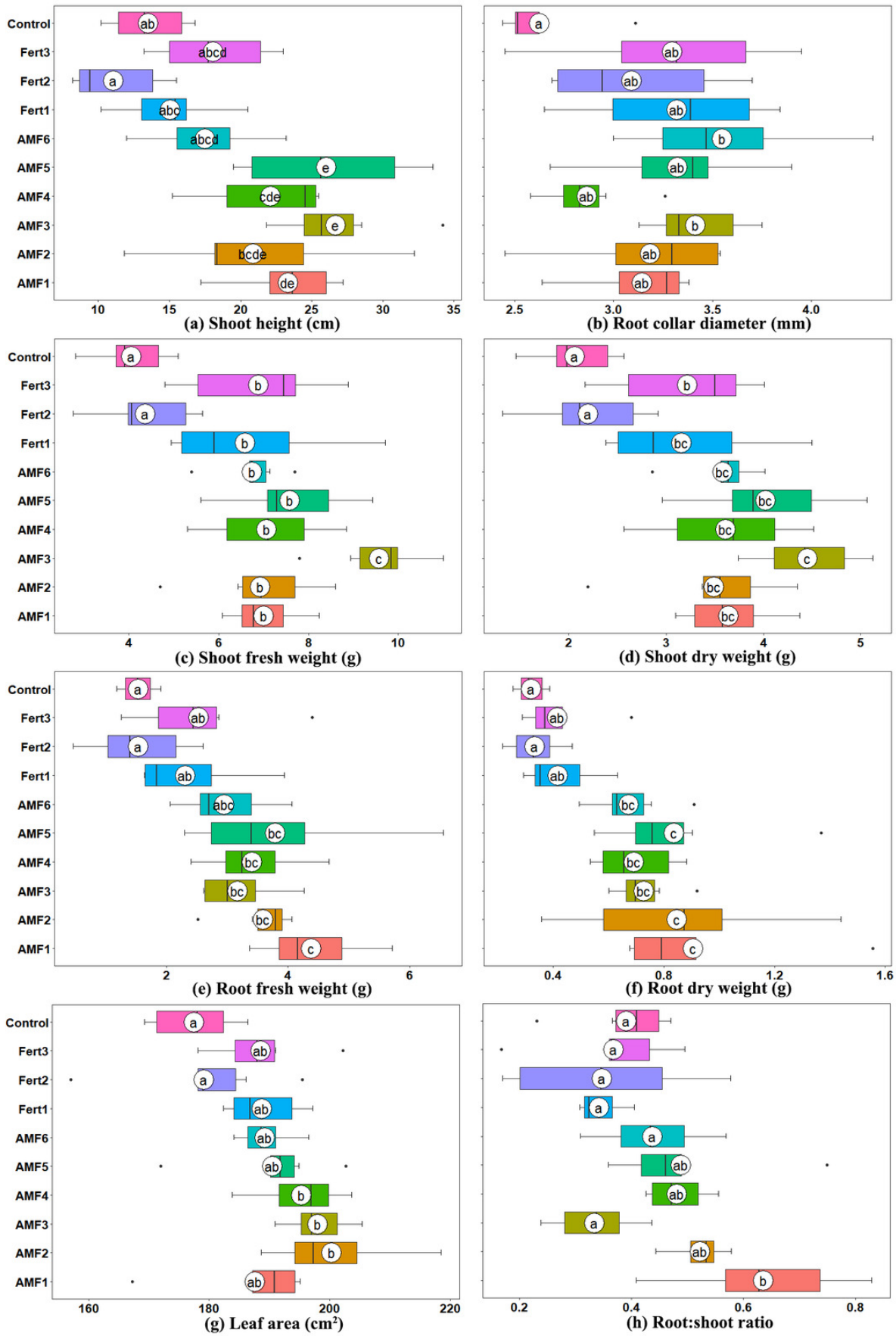

Control
Fert3
Fert2
Fert1
AMF6
AMF5
AMF4
AMF3
AMF2
AMF1
(a) Shoot height (cm)
(b) Root collar diameter (mm)
(c) Shoot fresh weight (g)
(d) Shoot dry weight (g)
(e) Root fresh weight (g)
(f) Root dry weight (g)
(g) Leaf area (cm²)
(h) Root:shoot ratio

Figure 3

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

(a) Nitrogen content (Ncon); (b) Phosphorus content (Pcon); (c) Potassium content (Kcon); (d) Boron content (Bcon). Labeling is the same as in Figure 2.

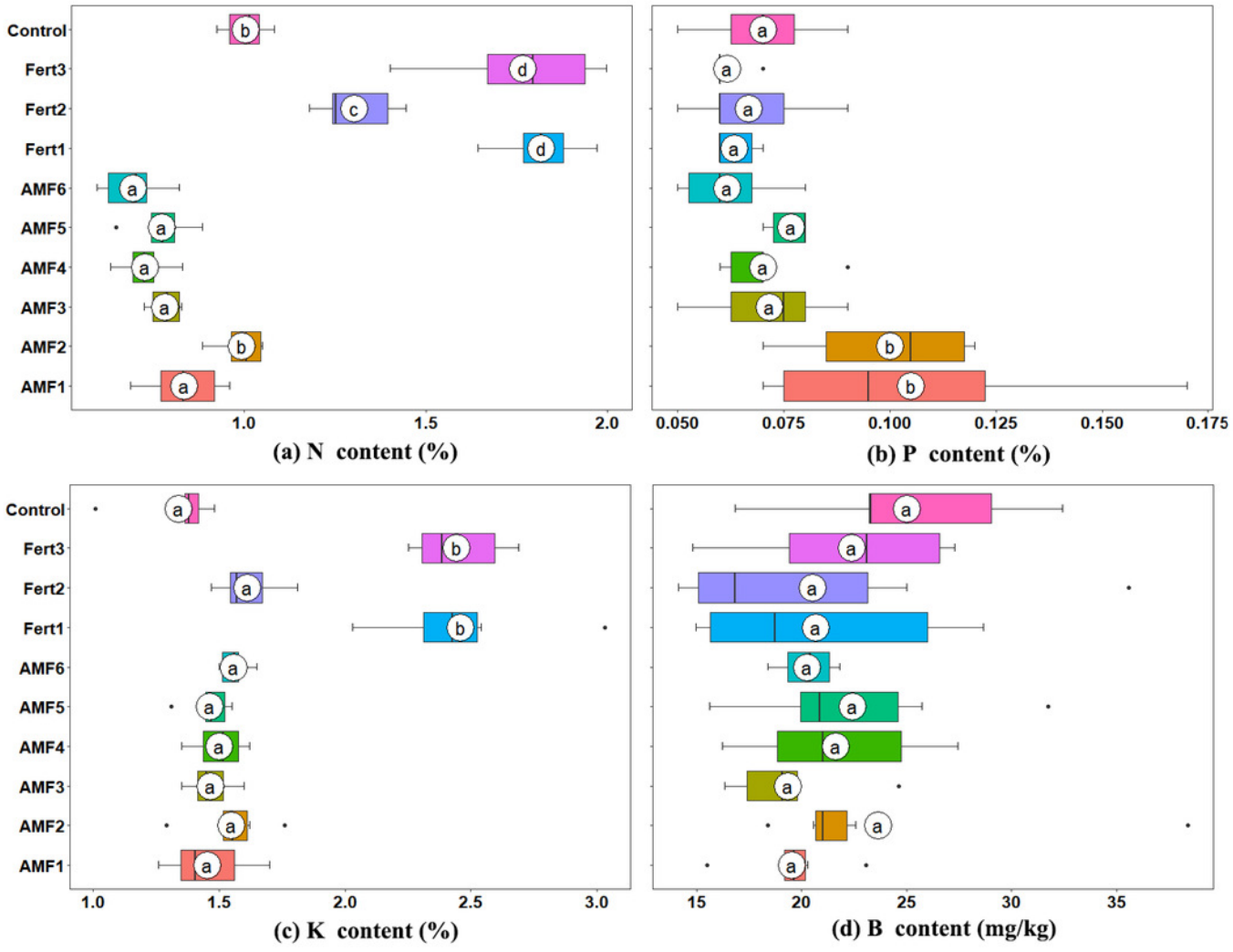
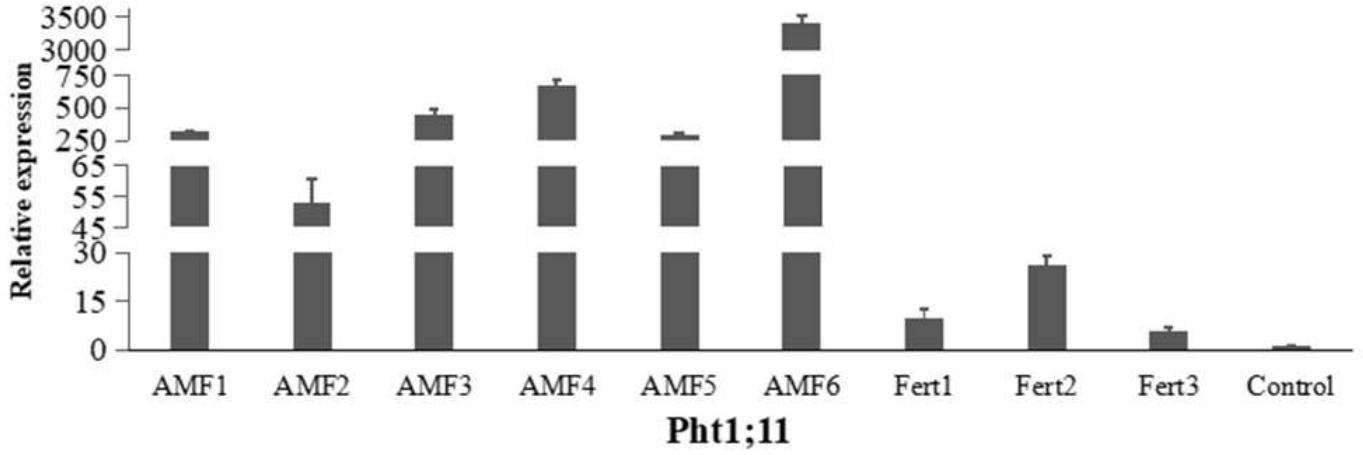
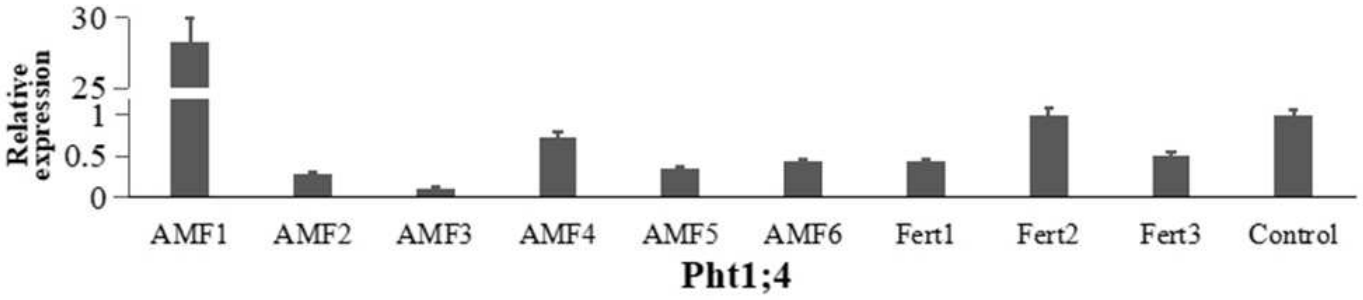


Figure 4

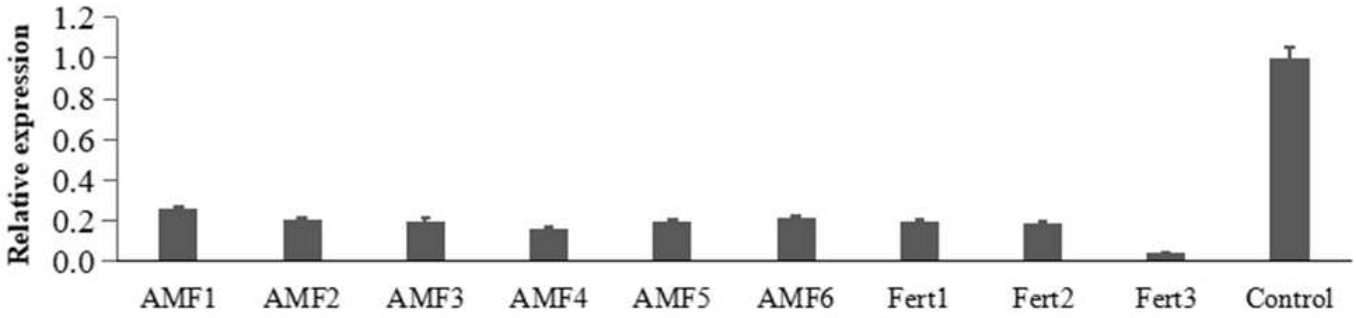
Expression levels of *Pht1;11*, *Pht1;4*, *Pht1;2* and *Pho3* in olive mycorrhizal roots by qRT-PCR analysis.



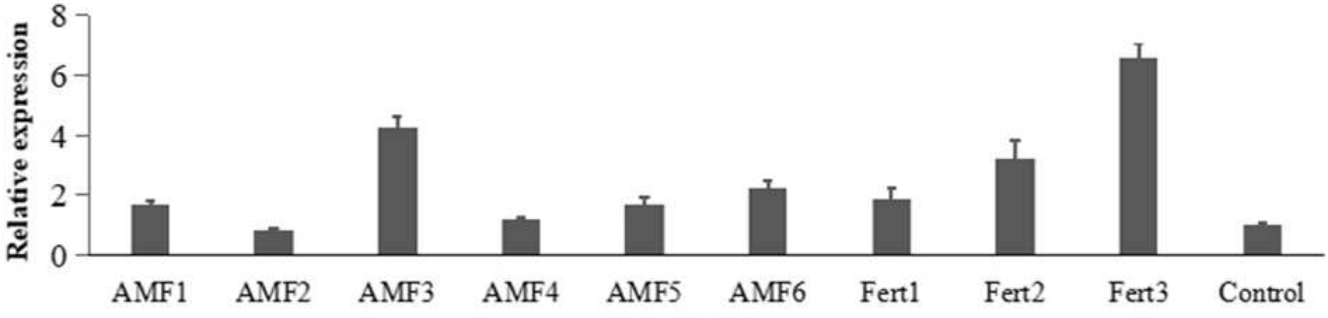
Pht1;11



Pht1;4



Pht1;2

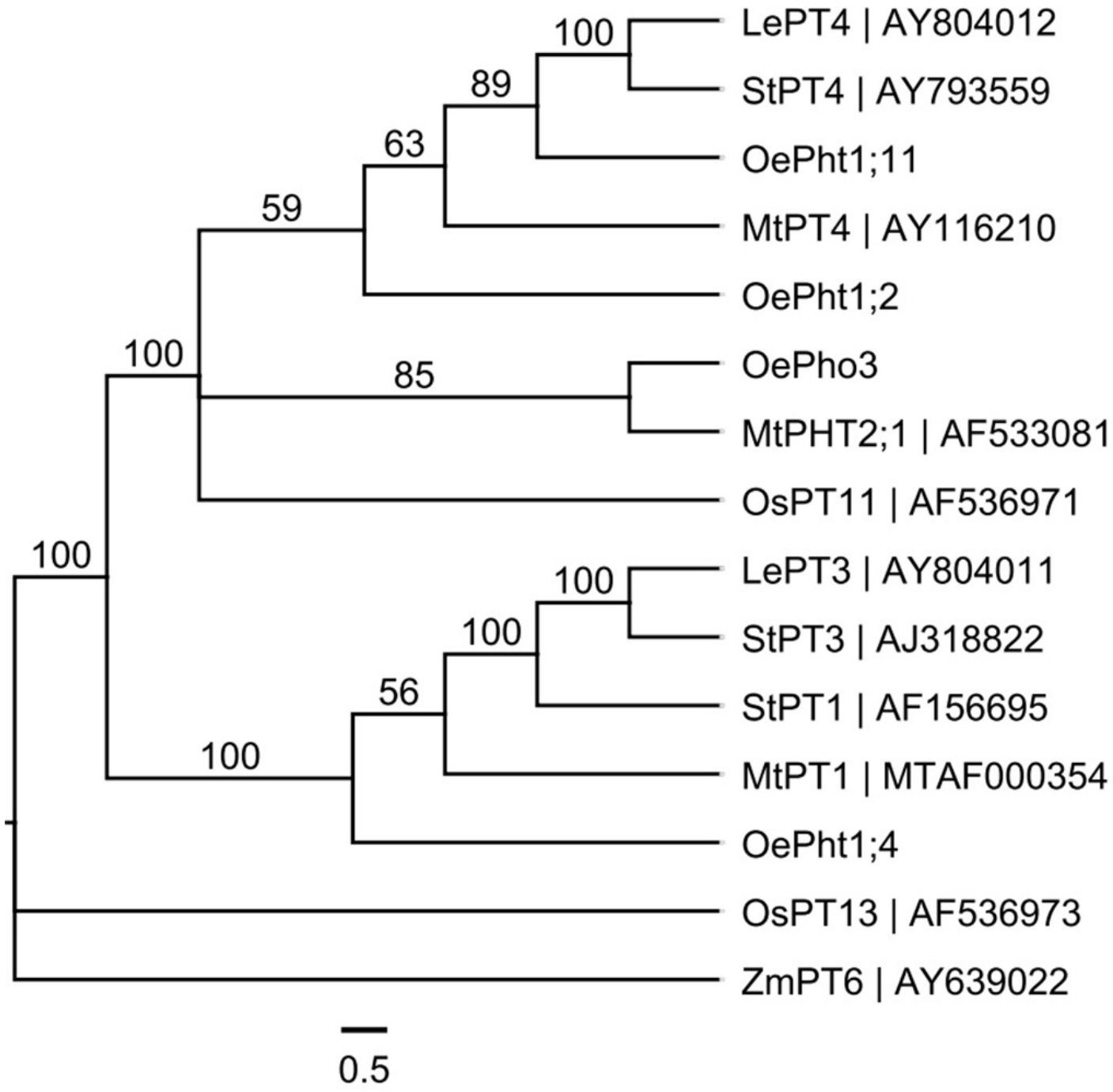


Pho3

Figure 5

Phylogenetic relationships of the *Pht* DNA sequences in different plants built with the GTR substitution model.

Le: *Lycopersicon esculentum*; Mt: *Medicago truncatula*; Oe: *Olea europaea*; Os: *Oryza sativa*; St: *Solanum tuberosum*; Zm: *Zea mays*.


LePT4 | AY804012
StPT4 | AY793559
OePht1;11
MtPT4 | AY116210
OePht1;2
OePho3
MtPHT2;1 | AF533081
OsPT11 | AF536971
LePT3 | AY804011
StPT3 | AJ318822
StPT1 | AF156695
MtPT1 | MTAF000354
OePht1;4
OsPT13 | AF536973
ZmPT6 | AY639022
100
89
63
59
100
85
100
100
100
56
100
0.5