

SUPPLEMENT OF A COMMERCIAL MYCORRHIZAL PRODUCT TO IMPROVE THE SURVIVAL AND ECOPHYSIOLOGICAL PERFORMANCE OF OLIVE TREES IN AN ARID REGION

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ABSTRACT

Rainfed olive groves in arid Tunisia face severe water scarcity and a low abundance of native arbuscular mycorrhizal fungi (AMF). We investigated if a supplement of commercial AMF-product at transplantation would improve olive plant survival and ecophysiological performance in an arid region. The commercial AMF product was added to two olive tree cultivars, ‘Meski’ and ‘Zarrazi’. There was an increase in the mycorrhizal intensity in the roots of ‘Meski’. Mycorrhizal symbiosis seems to have improved the survival of ‘Meski’ and the growth rate of ‘Zarrazi’. Plant water status and gas exchanges were enhanced in ‘Meski’. Mycorrhization helped maintain a higher photosynthetic assimilation rate and stomatal conductance in ‘Zarrazi’. AMF-symbiosis exhibited an inter-cultivar difference in the enhancement of the ecophysiological performance of olive trees under aridity. It also improved pre-existent indigenous defense strategies. It reinforced the avoidance strategy of ‘Meski’ but strengthened the tolerance strategy of ‘Zarrazi’.

Key words: drought, gas exchanges, growth, indigenous strategy, inter-cultivar variability

INTRODUCTION

Olive tree cultivation is one of the primary agricultural practices of farmers in Tunisia, and olive oil is the main exported agricultural product, earning the country appreciable revenues [Jackson et al. 2015, Mansour et al. 2018, Arfaoui et al. 2022]. However, during the last few years, the sector has become less reliable due to the extreme fluctuation of national production. This instability is principally caused by a significant decrease in rainfall and an intensification of drought and aridity [Knaepen 2021, Sghaier et al. 2022]. The situation is dire, especially in the south, where arid and Saharan bioclimates dominate, and the olive groves are mainly rainfed [Dhaou et al. 2009, Belgacem et al. 2011]. In addition, in these zones, it is difficult to irrigate the trees because it is costly to extract the deep

groundwater and because it is relatively saline, making it unsuitable for olive tree irrigation. Furthermore, the groundwater resources in southern Tunisia are mostly unrenovable [Ben Alaya et al. 2013].

The scarcity of water for agricultural use and its low quality, the increasing cost of chemical fertilizers and phyto-sanitizers, and the need to increase yield forced the farmers to devise new innovative cultural practices. Adopting a low-cost integrative approach based on biotechnology that respects the environment may be beneficial. Mycorrhizal symbiosis might be a suitable solution for modern agriculture, offering high productivity, respect for the environment, and helping with water shortage. Arbuscular mycorrhizal fungi (AMF) act as bio-fertilizers, bio-protectors, and bio-regula-

tors [Boutaj et al. 2020, Ouledali et al. 2019]. It was previously confirmed that AMF alleviates water stress, improves mineral nutrition, and enhances productivity [Estaún et al. 2003, Ouledali et al. 2018]. In an olive tree, AMF symbiosis influences water relations, stomata functioning control by abscisic acid, aquaporin gene expression, and root hydraulic conductivity [Calvo-Polanco et al. 2016, Ouledali et al. 2019].

In the field, diverse native AMF populations exist naturally in the rhizosphere of olive trees. However, previous studies found a low density of AMF in the rhizosphere of olive trees growing in several orchards in arid southern Tunisia [Chliyeh et al. 2015, Ouledali et al. 2022]. In addition, the association of mycorrhiza with olive tree roots is slow and may take more than six months [Ouledali et al. 2018]. Furthermore, the last few years have been arid, leading to the intensification of aridity. Under these circumstances, adding commercial mycorrhizal products into the planting hole can make olive plants more hardy. We hypothesize that this biotechnological practice will: 1) improve the mycorrhization intensity of the roots, 2) enhance the growth and survival rate of transplanted olive plants, 3) reinforce the resistance of the trees of both drought-sensitive and drought-tolerant olive cultivars to severe aridity. A commercial mycorrhizal product (*Symbivit*, INOCULUMplus, Dijon, France) was added to plants of two local olive tree cultivars during their transplantation in the field under arid climate conditions. The cultivars were ‘Meski’, a drought-sensitive one, and ‘Zarrazi’, a drought-resistant one. The availability of AMF inoculum close to olive plant roots may be more advantageous for the plant and the fungus to proliferate together than in natural conditions where roots need time to extend and reach AMF spore or hypha sparsely present in arid soils. Root mycorrhizal intensity (M%), plant growth and survival, water relations, and gas exchanges were measured on AMF-inoculated (Myc+) or non-inoculated (Myc-) plants subjected to water stress during the dry season (June–July 2017).

MATERIALS AND METHODS

Plant material and treatments. Plants of two indigenous olive cultivars, ‘Meski’ and ‘Zarrazi’, were used in the present study. The choice of these cultivars is based on their contrasted ability to resist

drought. The cultivar ‘Zarrazi’ is drought-resistant, mainly cultivated in southern Tunisia under semi-arid and arid bioclimates [Ouledali et al. 2018]. ‘Meski’ is a drought-sensitive cultivar mainly cultivated in the north under a humid bioclimate [Ennajeh et al. 2008]. Twenty-one-year-old self-rooted cuttings of each cultivar were obtained from the nursery of the National Office of Oil (Bejaoua, Mannouba, Tunisia) to be used in the trial.

An experimental orchard was created in an arid site on the Gabes-Matmata road (33°46’N, 9°58’E) in southern Tunisia (Fig. 1). The plants of each cultivar were subdivided into two lots of ten. The plants of the first lot (Myc+) were inoculated with AMF by adding 120 g/plant of commercial mycorrhizal product *Symbivit* (INOCULUMplus, Dijon, France). ‘*Symbivit*’ contains propagules of six different AMFs: *Glomus etunicatum*, *G. microaggregatum*, *G. intraradices*, *G. claroideum*, *G. mosseae*, and *G. geosporum*. For each plant, half the dose of the AMF commercial product was dispensed as a fine layer in the planting hole beneath the roots, while the second half was added after half the hole was filled with soil. The plants of the second lot (Myc-) were planted without adding the mycorrhizal product. The lot of plants that received the Myc+ treatment was located 24 m away from the lot of plants that received the Myc- treatment to avoid any overlap (Fig. 2). Within each of the two plots, two lines spaced 12 m apart were created; each line contained plants of both cultivars regularly alternated and spaced by 6 m. First, a drip system irrigated all olive plants regularly for two years. In mid-2017, irrigation was withheld for 45 days during the peak of the dry season (June–July) to subject the trees to water stress. Plant water status and gas exchanges were measured every 15 days throughout the water stress period. Plant height and mycorrhizal intensity were determined at the end of the treatment period.

Mycorrhizal colonization. The symbiotic association was assessed on fresh roots from each cultivar’s five *Symbivit*-inoculated (Myc+) and five not inoculated (Myc-) plants. Thirty one-cm-long root fragments were collected from each plant [Trouvelot et al. 1986]. They were stained with trypan blue (0.05%) [Phillips and Hayman 1970]. The microphotographs of AMF colonized roots were taken with a digital camera (Cmex 5, Euromex, Holland) coupled with



Fig. 1. The experimental olive tree orchard (represented by the star) was located on Gabes-Matmata way (33°46' N, 9°58' E) in southern Tunisia

a photonic microscope (OX Range, Euromex, Holland) interfaced to a computer using image manager Zeiss software. The mycorrhizal intensity (M), defined as the proportion of the root invaded by endomycorrhizae, was determined with the MycoCalc program (www.dijon.inra.fr/mychintec/).

$$M = \frac{(95n_5 + 70n_4 + 30n_3 + 5n_2 + n_1)}{\text{total number of observed fragments}} \times 100$$

where: n_5 , n_4 , n_3 , n_2 , and n_1 represent the number of fragments labeled 5, 4, 3, 2, and 1.

Plant growth and survival. The impact of AMF addition on the growth of olive plants was quantified by measuring their height at the end of the experimental period. In addition, the plant mortality rate was recorded at the end of the experimental period by counting the dead plants for each cultivar and treatment.

Plant water status. The plant water status was characterized by taking measurements of predawn leaf water potential (Ψ_{pd}). Ψ_{pd} was measured in the

early mornings before sunrise on small terminal bristles using a Scholander pressure chamber (PMS Instrument Company, Albany, Oregon, USA) [Scholander et al. 1965].

Plant gas exchange. Net photosynthetic assimilation rate (P_n , $\mu\text{mol m}^{-2} \text{s}^{-1}$), stomatal conductance (g_s , $\text{mol m}^{-2} \text{s}^{-1}$), and transpiration rate (E , $\text{mmol m}^{-2} \text{s}^{-1}$) were measured on mature leaves using an LCi portable gas exchange system (ADC BioScientific Ltd, Hoddesdon, UK). The measurements were conducted between 9:30 and 10:30 under saturating light conditions at 20–30°C ambient air temperature. The measurements were repeated three times for each of the three leaves per plant. A total of three plants per treatment were used.

Statistics. The data reported in the current study were the averages of at least five replicates. Analysis of variance was performed using the GLM procedure of SAS software [SAS Institute 1999] with a level of significance $P = 0.05$. Means separation was carried out with Duncan's post-hoc test.

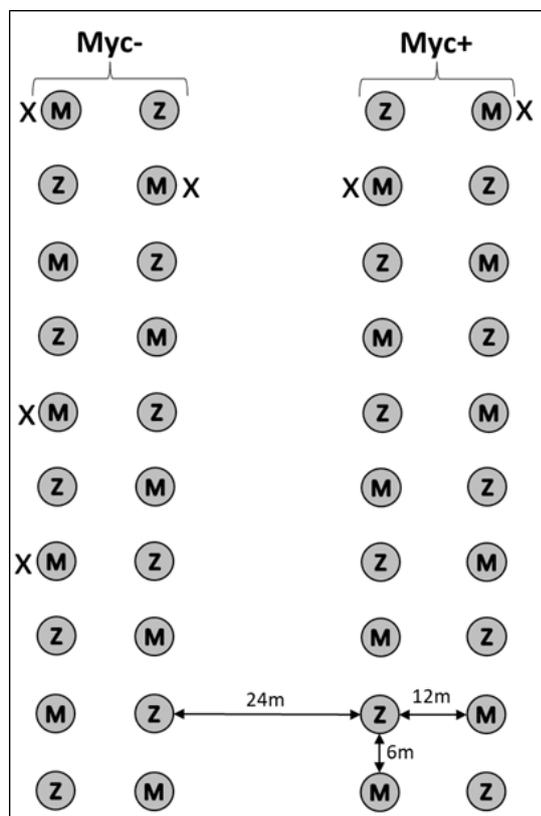


Fig. 2. The layout of the experimental field of two indigenous olive tree cultivars ‘Meski’ (M) and ‘Zarrazi’ (Z) inoculated (Myc+) or not (Myc-) by arbuscular mycorrhizal fungi. The plants marked with an “x” were dead at the end of the experiment

RESULTS

Two years after transplanting, the microscopic observations of fines roots stained with trypan blue proved that roots of all olive plants were colonized by endomycorrhizal fungus regardless of the treatment (Fig. 3). However, the mycorrhizal intensity (M) varied significantly ($P = 0.02$) between cultivars and AMF-treatments (Fig. 4). Indeed, AMF-inoculated plants of ‘Meski’ cultivar (MMyc+) exhibited the highest M value. MMyc+ and MMyc- were significantly different ($P = 0.04$) but not ZMyc+ and ZMyc- ($P = 0.83$).

The effect of AMF-inoculation on the growth of the trees was characterized by measuring plant height (Fig. 5). There were significant inter-cultivar differences in plant height ($P = 0.009$). Inoculated plants

of ‘Zarrazi’ (ZMyc+) were taller than not-inoculated ones (ZMyc-) ($P = 0.02$), while ‘Meski’ plants had approximately the same height ($P = 0.06$). The survival rate of transplanted olive plants under severe arid conditions varied depending on the cultivar and AMF treatment. We remarked that all transplanted plants of the ‘Zarrazi’ cultivar survived regardless of whether they were AMF-inoculated (Fig. 2). Whereas AMF treatment highly influenced the post-transplanting survival rate of ‘Meski’ plants. Indeed, we recorded the death of two plants out of the ten transplanted in the MMyc+ lot. The mortality rate was twice higher (4/10) in the MMyc lot.

The changes in Ψ_{pd} in plants of both olive tree cultivars during the peak of the dry season exhibited appreciable variability between cultivars depending on mycorrhization treatment (Fig. 6). Ψ_{pd} in all olive plants AMF-inoculated or not decreased significantly during the dry season. However, this decrease was more acute in Myc- plants than Myc+ ones, especially when the water stress was still moderate (June 30). The mycorrhization alleviated the impact of water stress on Ψ_{pd} more significantly in the drought-sensitive cultivar ‘Meski’ than in the drought-resistant cultivar ‘Zarrazi’. Indeed, on June 30, the $\Delta\Psi_{pd}$ between Myc+ and Myc- plants was -1.53 MPa and -0.5 MPa in ‘Meski’ and ‘Zarrazi’, respectively.

Figure 7 depicts the changes in P_n as a function of Ψ_{pd} . For the cultivar ‘Meski’, P_n of Myc+ plants was higher than that of Myc- plants under relatively high Ψ_{pd} ($\Psi_{pd} > -1.33$ MPa). Nevertheless, P_n decreased significantly and similarly in both lots of ‘Meski’ cultivar when water stress became severe. For ‘Zarrazi’, Myc+ and Myc- plants exhibited similarly high P_n values under favorable water availability. However, water stress caused a significant reduction in P_n in both plant lots, but more so in the Myc- lot than Myc+ lot. Indeed, for the same leaf tissue hydration ($\Psi_{pd} = -3.8$ MPa), Myc+ plants exhibited higher P_n than Myc- plants.

During the dry season, the stomatal function behaved similarly to the photosynthetic activity in both cultivars, AMF-inoculated or not (Fig. 8). In ‘Meski’, stomatal conductance was higher in Myc+ plants than Myc- ones under moderate water stress. However, it was similarly low in both lots under acute water stress. In ‘Zarrazi’, g_s was high and comparable for the two treatments with no water stress. When the water

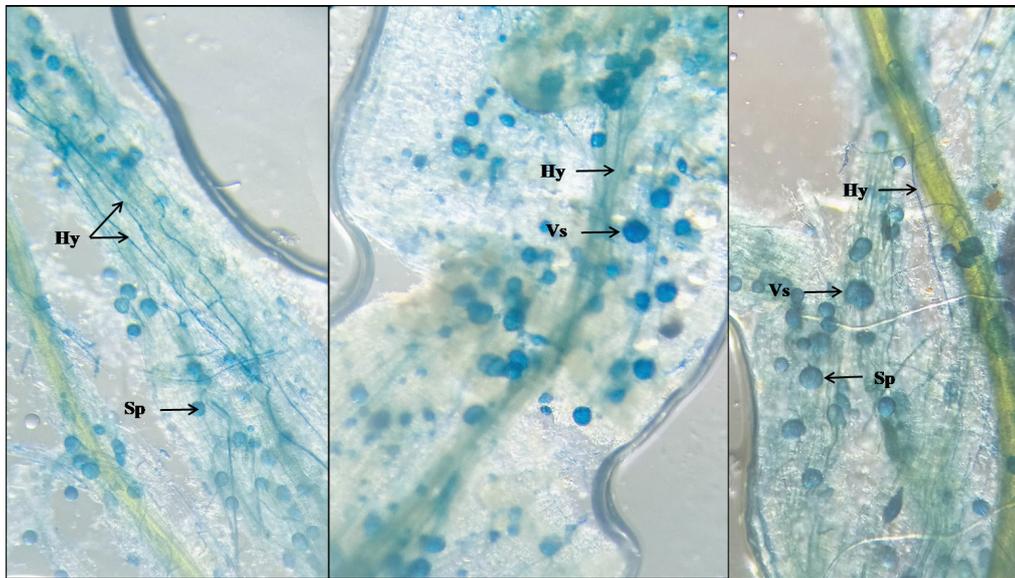


Fig. 3. Arbuscular mycorrhizal colonization of the roots of ‘Meski’ and ‘Zarrazi’ trees: spore (Sp) appears attached to intercellular hyphae (Hy); vesicles (Vs) have formed between cells in the root cortex of olive plants (G:400×)

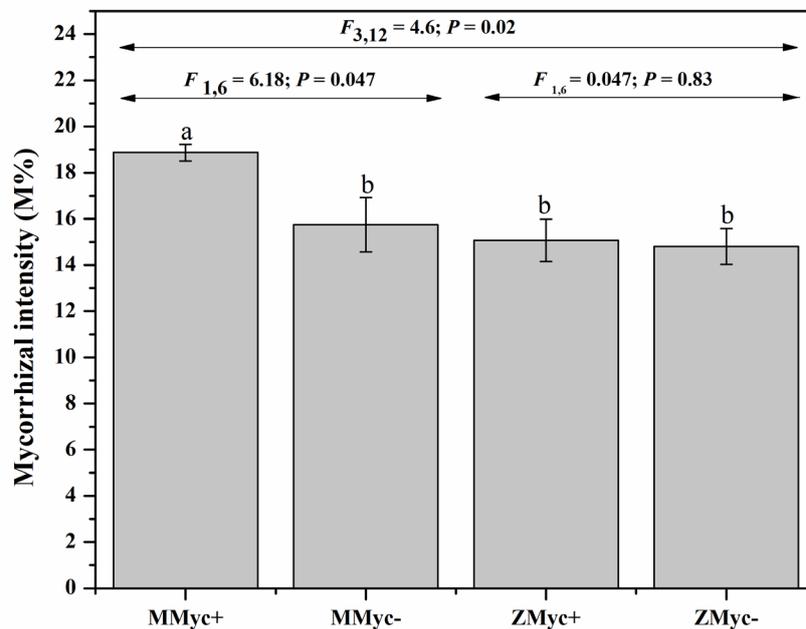


Fig. 4. Mycorrhizal intensity (%) in roots of the tree of two olive cultivars ‘Meski’ and ‘Zarrazi’ inoculated (MMyc+, ZMyc+) or not (MMyc-, ZMyc-) with arbuscular mycorrhizal fungi. Bars represent mean \pm SE ($n = 5$ per cultivar per treatment). Different letters indicate significant differences between the cultivars and the treatment levels ($P \leq 0.05$)

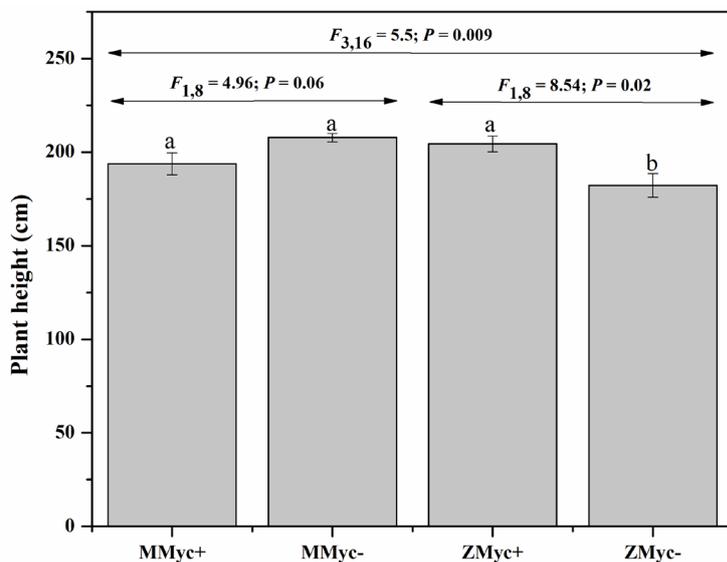


Fig. 5. Plant height of two olive tree cultivars ‘Meski’ and ‘Zarrazi’ inoculated (Myc+) or not (Myc-) with arbuscular mycorrhizal fungi measured after two years of field planting. Bars represent mean \pm SE ($n = 5$ per cultivar per treatment). Different letters indicate significant differences between the cultivars and treatment levels ($P \leq 0.05$)

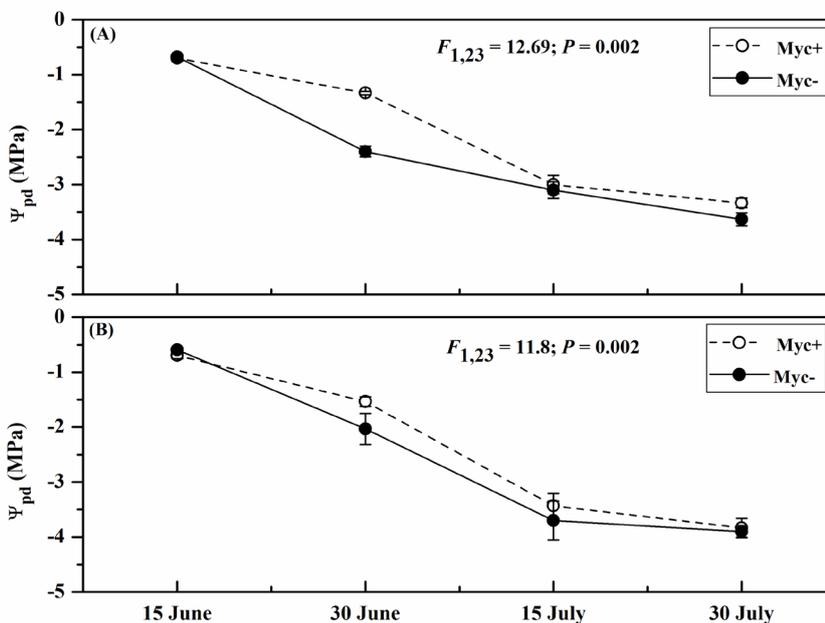


Fig. 6. Changes of predawn leaf water potential (Ψ_{pd} , MPa) during the peak of the dry season (June–July) measured at two-week intervals in the trees of two olive cultivars (A) ‘Meski’ and (B) ‘Zarrazi’ inoculated (Myc+) or not (Myc-) with arbuscular mycorrhizal fungi and subjected to water stress by withholding irrigation. Each symbol represents the mean \pm SE ($n = 5$ per cultivar per treatment)

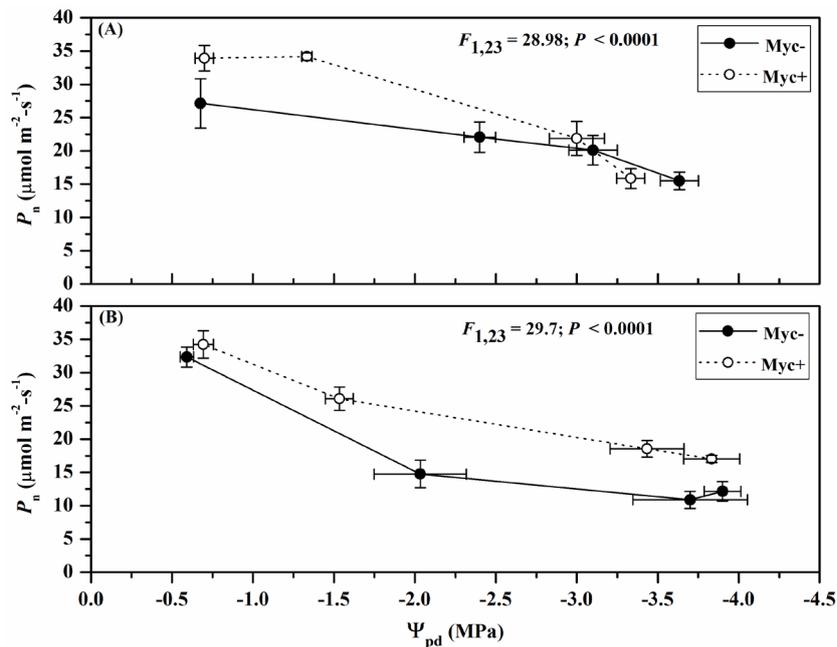


Fig. 7. Variation of net photosynthetic assimilation rate (P_n , $\mu\text{mol m}^{-2} \text{s}^{-1}$) as a function of predawn leaf water potential (Ψ_{pd} , MPa) in the trees of two olive cultivars (A) 'Meski' and (B) 'Zarrazi' inoculated (Myc+) or not (Myc-) with arbuscular mycorrhizal fungi and subjected to water stress by withholding irrigation. Each symbol represents the mean \pm SE ($n = 5$ per cultivar per treatment)

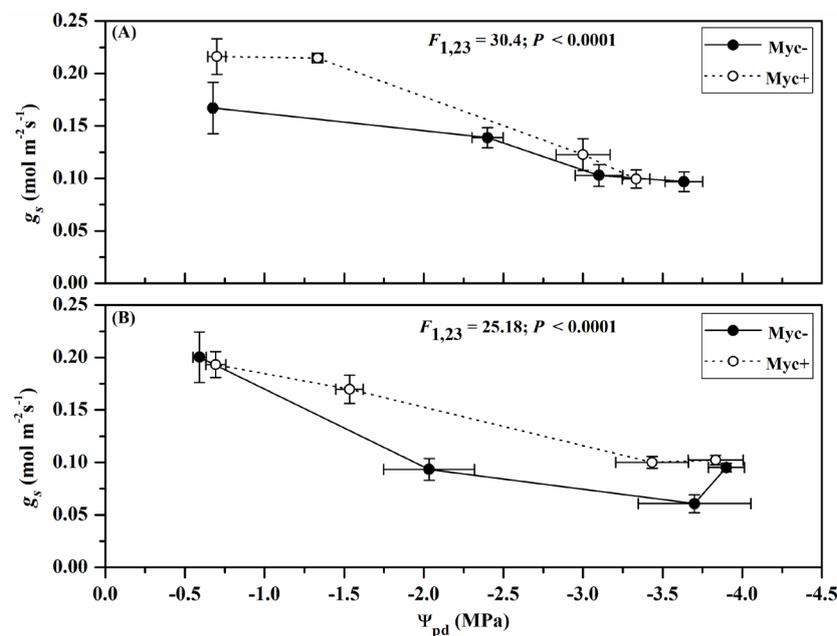


Fig. 8. Variation of stomatal conductance (g_s , $\text{mol m}^{-2} \text{s}^{-1}$) as a function of predawn leaf water potential (Ψ_{pd} , MPa) in the trees of two olive cultivars (A) 'Meski' and (B) 'Zarrazi' inoculated (Myc+) or not (Myc-) with arbuscular mycorrhizal fungi and subjected to water stress by withholding irrigation. Each symbol represents the mean \pm SE ($n = 5$ per cultivar per treatment)

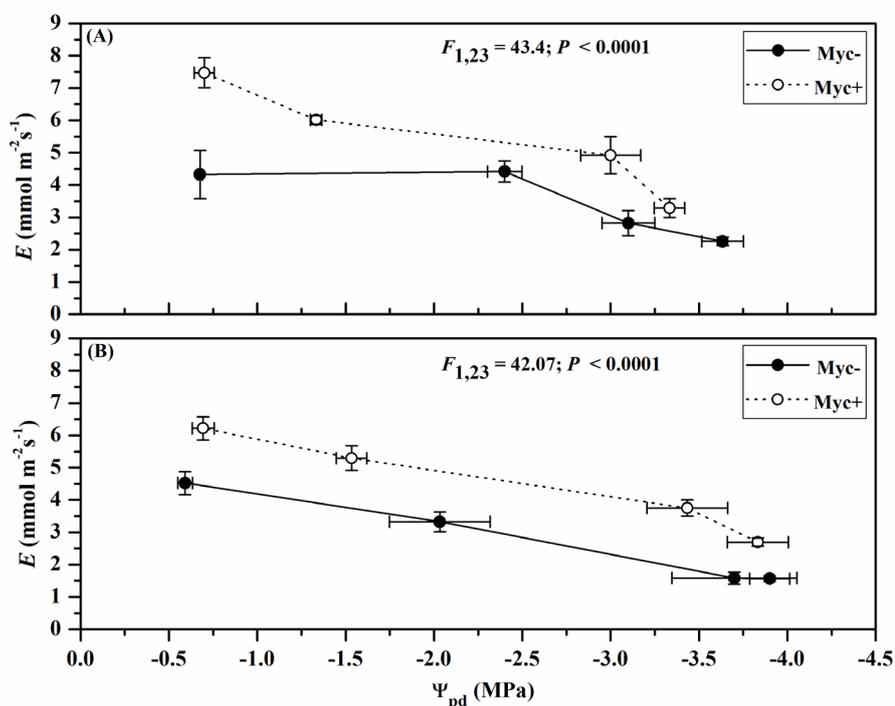


Fig. 9. Variation of transpiration rate (E , $\text{mmol m}^{-2} \text{s}^{-1}$) as a function of predawn leaf water potential (Ψ_{pd} , MPa) in the trees of two olive cultivars (A) ‘Meski’ and (B) ‘Zarrazi’ inoculated (Myc+) or not (Myc-) with arbuscular mycorrhizal fungi and subjected to water stress by withholding irrigation. Each symbol represents the mean \pm SE ($n = 5$ per cultivar per treatment)

became severe, g_s decreased significantly, especially in Myc- plants.

During the dry season, leaf water loss by transpiration varied between the two cultivars and was influenced by mycorrhization (Fig. 9). In ‘Meski’, E of Myc+ plants was greater than that of Myc- plants in the absence of water stress, but it decreased progressively as Ψ_{pd} declined. In Myc- ‘Meski’ plants, E persisted relatively high and unchanged in the absence and under moderate water stress, then it fell remarkably under severe water stress. In the ‘Zarrazi’ cultivar, E was higher in Myc+ plants than Myc- plants for the whole Ψ_{pd} interval studied. The water deficit caused a progressive decrease of E in plants of both lots.

DISCUSSION

AMF inoculation biotechnology has been widely adopted at the nursery stage and when planting in the

field to improve plant growth and survival [Estaún et al. 2003, Chenchouni et al. 2020, Wu et al. 2022]. However, less is known about the effectiveness of this technology under arid climates, such as in the olive groves of southern Tunisia, where an arid climate is coupled with low soil fertility. In Southern Tunisia, like in most arid regions south of the Mediterranean Sea, the olive tree rhizosphere is characterized by a low abundance of native AMF [Chliyeh et al. 2015, Ouledali et al. 2022]. Supplements of commercial AMF products at transplantation may improve olive tree survival and ecophysiological performance under arid climates. Our results showed vesicular-arbuscular mycorrhizae formation in roots specific to AMF-inoculated olive plants [Barbaro and Basso 2022]. There was also an increase in AMF-colonization intensity (M%) in the roots following the addition of the commercial AMF-product (*Symbivit*) in the drought-sensitive cultivar, ‘Meski’, mainly cultivated under the

humid bioclimate of northern Tunisia. However, M was similar in the *Symbivit*-inoculated and not-inoculated trees of the drought-resistant cultivar, ‘Zarrazi’. The weak intra-cultivar difference (Myc-/Myc+) in M during the study (after two years of field transplantation) may be due to the reduction in colonization of the roots by indigenous AMF after adding commercial AMF. Similar results were reported by Estaún et al. [2003]. The inter-cultivar difference in M may be related to the higher compatibility of the *Symbivit*-AMF strains, originating from a humid region (Dijon, France), with the root of the cultivar ‘Meski’ compared to those of ‘Zarrazi’. Indeed, AMF infection has a high host specificity in olive trees, and edaphic and environmental conditions significantly influence it [Montes-Borrego et al. 2014].

AMF-inoculation during field transplantation significantly improved survival in ‘Meski’, but it enhanced shoot growth in ‘Zarrazi’. Similarly, previous studies have shown that inoculation of olive plants with native AMF in the nursery or at the time of transplanting in the field increases early growth and yield in various olive tree cultivars [Estaún et al. 2003, Dag et al. 2009, Chenchouni et al. 2020]. In addition, using native AMF consortia at the nursery stage may confer a high survival rate after transplanting of olive plantlets [Wu et al. 2022].

Furthermore, our results suggest that AMF inoculation mitigated the impact of water stress on the ecophysiology of the two cultivars characterized by contrasted drought stress responses in different ways. Indeed, in the drought-sensitive cultivar, ‘Meski’, the mycorrhizal symbiosis maintained higher plant water status in the absence and when water stress was moderate. AMF inoculation reinforced the avoidance strategy in response to water stress for this drought-sensitive cultivar. This finding may also be confirmed by the apparent involvement of AMF in the improvement of gas exchange parameters (P_n , g_s , E) when the water stress was moderate. However, in the case of the drought-tolerant cultivar, ‘Zarrazi’, the mycorrhizal symbiosis played a role mainly when water stress became severe. Similar results were observed with potted plants of ‘Zarrazi’ inoculated with AMF and subjected to dehydration [Ouledali et al. 2018]. In contrast to ‘Meski’, plant water status and gas exchange parameters of ZMyc+ were not improved in the absence

or under moderate water stress, but they were significantly ameliorated under severe water deficit. This ecophysiological behavior of ‘Zarrazi’ may suggest the implication of mycorrhizal symbiosis in reinforcing its tolerance strategy in response to drought stress.

CONCLUSIONS

The post-transplanting survival and ecophysiological performance of olive plants inoculated with a commercial AMF product exhibited inter-cultivar variability. Indeed, mycorrhization improved the survival rate for the drought-sensitive cultivar ‘Meski’ and reinforced the avoidance strategy adopted by the cultivar in response to drought. However, for the drought-tolerant cultivar ‘Zarrazi’, the AMF-inoculation strengthened its tolerance-strategy. Thus, the AMF-inoculation may be used to ameliorate the ecophysiological performance of olive trees under arid conditions depending on the pre-existing indigenous response of the cultivar. Inoculation with AMF appears to be a promising biotechnology that fits well with a sustainable agroecosystem vision to increase the odds of success of olive grove establishment on marginal soils in arid regions. Furthermore, our study demonstrated the effectiveness of AMF-supplement to olive plants, a relatively new promising practice that does not require specific technical skills or equipment and can be done by the growers themselves.

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