

Effect of mycorrhization on growth and physiology performance of *Quercus* species

Awatef SLAMA^{1*}, Sondes FKIRI¹, Faten MEZNI¹, Boutheina STITI¹,
Julio SALCEDO-CASTRO², Issam TOUHAMI¹,
Marwa KHAMMASSI¹, Abdelhamid KHALDI¹, Zouheir NASR¹

¹University of Carthage, National Research Institute of Rural engineering, Water and Forests INRGREF, Laboratory of Management and Valorization of Forest Resources, BP 10 Ariana 2080, Tunisia; slamaawatef@yahoo.fr (*corresponding author); sondesfkiri@gmail.com; faten-mez@hotmail.com; stiti_b@yahoo.fr; issam_touhami@yahoo.fr; khammassi_marwa@yahoo.fr; khalditn@yahoo.fr; zouheirnasr84@gmail.com

²Institute for Marine and Antarctic Studies, University of Tasmania (UTAS), Australia; julio.salcedocastro@qui.edu.au

Abstract

The development of mycorrhiza could contribute to strengthening the resilience of forest ecosystems to climate change. Several mycorrhizal fungi are known for their valuable effect in increasing plant performances and adaptation to stressful environmental conditions. Thereby, this research aims to investigate how *Terfezia boudieri* (Chatin) mycorrhizal fungi affects the growth (primary root length, above-ground plant weight) and the physiological behaviour (net photosynthesis, responses to intercellular [CO₂] and the intensity of photosynthetically active radiation) of *Quercus* subsp. *coccifera* and *Q. suber* L. Inoculated and non-inoculated seedlings of the two *Quercus* species were grown in one-liter pots in the greenhouse, with a temperature that ranged from 25 to 30 °C, natural lighting and an irrigation applied twice a week with top water. Results revealed that primary root length and the above-ground biomass increased with mycorrhization. In addition, mycorrhization promoted net photosynthesis (at 400 ppm and at saturation point), the apparent quantum yield, the water use efficiency, and the photosynthetic pigments contents. However, inoculation decreased the light compensation point for both species. Effectiveness of *T. boudieri* inoculation on *Quercus* sp. performance, highlights the potential of the mycorrhization process to improve forest management and resilience to climate change.

Keywords: biomass; carbon assimilation; climate change; inoculation; Mediterranean region; *Quercus* sp.; *Terfezia boudieri*

Introduction

Under a changing climate, forest management plays an important role in promoting ecosystem resilience including forestry practices that enhance mycorrhizal networks with several fungal taxa to increase soil aggregation and connectivity, or species (Orlović *et al.*, 2014). Furthermore, the important mycorrhizal

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networks facilitate the survival and the development of trees, helping them withstand and cope with the environmental stress. Access to mycorrhizal networks also improves the physiological response of plants, including carbon assimilation, nutrients, and water supply (Selosse *et al.*, 2006; Simard and Austin, 2010). Hence, mycorrhizal fungal inoculation has the potential to promote forest management (Katanić *et al.*, 2019).

During recent decades, forests have undergone severe degradation, in particular the decline and death of individuals of *Quercus* species around the Mediterranean region and particularly in Tunisia (El Abidine, 2003; Andersson, 2005; Bouachir *et al.*, 2017; Touhami *et al.*, 2020). These forests are threatened mainly by drought and insect attacks (Dupuy *et al.*, 2015; Touhami *et al.* 2023). In view of this decline, the good management of these forests could contribute to their conservation and reduce the intensity of degradation.

Quercus species are mainly distributed in the Mediterranean region for their high socioeconomic value (Sanders *et al.*, 2014). The ecological value of *Quercus* species appears in the soil conservation, fighting in decreasing desertification menace and in their input in water reserves recharge and runoff control (Farguell *et al.*, 2022). Besides to forest decline, *Quercus* species are susceptible to many other challenges such as their low percentage of natural regeneration and their high mortality following field seeding besides the high anthropic effect that threat the natural regeneration essays (Bugalho *et al.*, 2011).

Naturally, Fagaceae species (oak, chestnut, beech) are involved with other plant species to form 80 % of the world's flora that establish mycorrhizal associations (Smith and Read, 2008). Production of mycorrhizal seedlings, in the Mediterranean area constitutes a new approach to increase plant survival, growth, photosynthetic capacity, water efficiency and nutrients supply (Southworth *et al.*, 2009).

Desert truffles are edible mushrooms found in the Mediterranean and desert regions forming mycorrhizae in the roots of many plant species. Among these fungi, *Terfezia boudieri* Chatin establish mycorrhizal associations with Cistaceae species in arid and desert areas, but Chatin (1892) specified its connection with oaks, pines, and cedars in the mountains of Algeria. To our knowledge, this association between *Quercus* species and *Terfezia* sp. fungus has not until now been studied or defined neither in the field nor in experimental essays. The difference in geographical distribution based on bioclimatic requirements could explain the lack of approval of the mycorrhizal relationship in the field between these two partners (*Quercus* sp. and *Terfezia* sp.). The confirmation of such symbiotic relationship under controlled conditions could indicate that the association is mediated by external factors and not by internal ones. Otherwise, in which there is no mycorrhization, biological or physiological barriers might be responsible of limiting the establishment of mycorrhization between the two species.

Many truffle species have been widely used in forestry inoculation programs around the world. Currently the mycorrhization method of plants with *Tuber melanosporum* developed firstly by Chevalier and Grente (1979) is exploited industrially (INRA/ANVAR process). However, cultivation of desert truffle in semi-controlled conditions was only studied by Morte *et al.* (2009) and Slama *et al.* (2010) with Cistaceae plant species.

Despite the importance of mycorrhization process with desert truffles on forest species, no studies have yet reported the effects of their association on physiological responses and on the growth of *Quercus* species.

Thus, in the current study, we examined for the first time the hypothesis that *T. boudieri* may be beneficial for the growth and the physiological efficiency of *Q. suber* and *Q. coccifera* plant species by investigating their biomass and gas exchange in response to intercellular CO₂ and light intensity.

Materials and Methods

Materials

Quercus suber and *Q. coccifera* acorns were collected from Ain Drahem and Beja provinces, respectively (Northwestern Tunisia; Table 1). *Terfezia boudieri* fruiting bodies were collected from Ben Guardan region in the Southeast of Tunisia (Table 1).

Table 1. Bioclimatic characteristics of the natural sites of sampled plant and truffle species

Sampled species	Province	Geographic location	Bioclimate	Annual precipitation (mm/year)	Mean annual temperature (°C)	Maximum temperature (°C)	Minimum temperature (°C)
<i>Quercus suber</i>	Ain Drahem	36°46'06.6"N 8°47'43.8"E	Superior humid	1534	15.6	31 - July	4.4 - January
<i>Q. coccifera</i>	Beja	37°04'12.9"N 8°57'43.8"E	Lower humid	685	15.2	37.2 - July	5.4 - February
<i>Terfezia boudieri</i>	Ben Guardan	33°15'39.3"N 10°50'12.0"E	Arid	196.4	22.3	36.2 - August	10.3 - January

Experimental essay

T. boudieri ascocarps were dried and then stored at 20 °C until the experiment. *Quercus coccifera* and *Q. suber* acorns were soaked for 48 hours in the dark. Three acorns for each plant species were then sown in a single pot. For each plant species, experiment was conducted using ten plastic pots (volume of 1 L, 7.5 cm base and 12 cm top diameters) corresponding to 30 seedlings in each treatment per plant species in total. Used pots were previously sterilized with a diluted bleach solution (12%), washed then filled with a sterile equal mixture of peat and sand. Pots were perforated at the bottom to ensure water drain.

Terfezia boudieri inocula were prepared according to Chevalier and Grente (1979). Inoculated plants were prepared by adding inoculums in the soil mixture. While, the none inoculated or control seedlings were prepared similarly but without any fungal inocula addition. Mycorrhizal (M) and controls (NM) plants were grown in a greenhouse (with a temperature ranged from 25 to 30 °C; 40-60% of humidity and natural lighting) for 6 months. Irrigation was applied twice a week with tap water. No nutrient solution or minerals addition were carried out throughout this experiment.

Determination of mycorrhization percentage

For each species, ten inoculated seedlings were harvested randomly at the end of the experiment (after 6 months of sowing date). Sampled roots were washed free of soil and each plant root system was treated and coloured with 0.5 % acidic Fuchsin according to the method of Phillips and Hayman (1970). Stained roots were observed using Leica DMLS microscope (Leica Microsystems, Bannockburn, IL) and the frequency of mycorrhizal colonization in the root system was determined by the equation of Trouvelot *et al.* (1986):

$$F(\%) = \left(\frac{n}{N}\right) * 100$$
 ; Where N is the total number of observed root fragments and n is the number of mycorrhized root fragments.

Physiological survey

Gas exchange measurements

Gas exchanges were measured for M and NM seedlings, with a Li-Cor Li-6400XT portable photosynthesis system (Li-Cor, Lincoln, NE, USA) based on the Infra-Red Gas Analysis (IRGA principle). Three plants of *Quercus suber* and *Q. coccifera* were chosen randomly for each treatment. The experiments were carried at 1500 $\mu\text{mol. m}^{-2} \cdot \text{s}^{-1}$ of photosynthetic active radiation (PAR), 25°C of leaf temperature and 60 % of humidity.

The external CO₂ partial pressure was changed in 12 steps. Photosynthesis was initially induced at an ambient CO₂ concentration of 400 μmol mol⁻¹ until the net photosynthesis (A) stabilization (i.e., A varied by less than ± 2%) to ensure the steady-state activation of Rubisco (Long and Bernacchi, 2003). Before data logging, leaves were subjected to equilibrate during at least 5 min in every step. Net photosynthesis (A), degree of stomatal pore opening, or stomatal conductance (g_s) and transpiration (E) were measured. Water-use efficiency (WUE) was estimated as the ratio of the net photosynthesis to the transpiration rate ($\frac{A}{E}$).

Net photosynthesis response to intercellular [CO₂] (A/Ci)

The A/Ci curves (where A is the net photosynthesis response and Ci intercellular [CO₂]) was carried out on a well-developed and healthy leaf which has reached a state of equilibrium at 400 μmol mol⁻¹ CO₂ concentration. The first point of the A/Ci curve refers to the plant acclimatization to temperature, PAR, flux, ventilation rate and CO₂ concentration that lasts 30 minutes. In this state, the enrichment concentration of CO₂ has been decreased to reach 40 μmol. mol⁻¹ by divert air through a soda-lime column. Beyond, each new concentration registered A value. The applied CO₂ external concentrations were 300, 200, 100 and 50 μmol CO₂. mol⁻¹ consecutively. By the end, external CO₂ concentration was then increased stepwise to 600, 700, 1000, 1200, 1500, 2000 and 2200 ppm.

The PAR and the temperature values were fixed at 1500 μmol.m⁻². s⁻¹ and 25 °C respectively during the experiment and the relative humidity was maintained around an average value of 60%. To estimate the CO₂ saturated rate of photosynthesis (Asat), CO₂ response curves were fitted using the three components exponential function (Choi *et al.*, 2017) according to the following equation:

$$Asat = a(1 - e - bx) + c$$

Where A is the leaf net photosynthetic rate and x is the intercellular CO₂ concentration or Ci. Using this equation, Asat was calculated as: a+c.

Measurements were accomplished for three leaves from three different plants for each species and treatment. The A-Ci curve was obtained using regression. Carboxylation efficiency (CE) was calculated as the initial slope (dA / dCi) of the A-Ci curve when Ci ranged from 0 to 200 μmol CO₂. mol⁻¹. Stomatal limitation of photosynthesis (Ls) was calculated as $100 * \left(1 - \frac{A}{A'}\right)$; where A' is the rate when Ci = [CO₂]_e and A is the rate under conditions of normal CO₂ concentration (Choi *et al.*, 2017).

Light curves (A/PPFD)

For each studied species and applied treatment (M or NM), the variation of photosynthesis to the intensity of photosynthetically active radiation (A/PPFD) was recorded. This experiment does not require an acclimatization period of more than 10 minutes, in contrast to the A / Ci curves. The temperature was set at 25 °C and a CO₂ capsule was used as a reference with an average concentration of 400 μmol CO₂. mol⁻¹. For each leaf, the PAR was varied starting with a value of 1000 μmol. m⁻². s⁻¹ and increasing to 2200 μmol CO₂. m⁻². s⁻¹, and then value was decreased to 50 μmol. m⁻². s⁻¹. Photosynthetic capacity was estimated as eqn.1 (Meng *et al.*, 2014).

The apparent quantum yield based on incident light (Φ) was calculated as the initial slope at the 3 lowest PPFD values (between 0 and 100 μmol. m⁻². s⁻¹). Light compensation point (LCP) was expected when A = 0 using the line representing the initial slope.

Photosynthetic pigments

Leaves used for the measurement of gas exchange were sampled for the assessment of chlorophylls and carotenoids contents. Each leaf was incubated in 80% acetone until visibly extraction of all pigments. Spectrophotometer readings of the extracts were conducted at 750, 663, 645 and 453 nm to determine chlorophyll a (Chla), b (Chlb), total chlorophylls and carotenoids contents using the equations of Porra *et al.* (1989).

*Growth survey*Primary root length measurements

Four mycorrhizal (M) and non-mycorrhizal (NM) *Q. suber* and *Q. coccifera* seedlings were sampled randomly after 6 months from the sowing date. All root parts were washed, and the length of the primary root was measured with a graduated ruler. Values were presented as the mean of 4 repetitions.

Plant above-ground biomass

All the external parts (stems and leaves) of the last sampled plants used for roots measurement were dried (65 °C / 48h). The external parts (for M and NM seedlings of both *Quercus* species) were weighted to evaluate the plant above ground biomass. Data are the mean of four repetitions.

Statistical analysis

The variance of multiple parameters (net photosynthesis An, WUE, Tr, Asat, Amax, CE, Rd, Φ, LCP, Ls, chlorophyll a, chlorophyll b, total chlorophyll, carotenoids, primary root length and plant above-ground biomass) was analyzed with the generalized linear model (GLM) using the SAS statistical software (version 9.0). Multiple comparisons of means were performed using the SNK test with a threshold p value of 0.05.

Results*Mycorrhization rate*

Mycorrhization rate between *T. boudieri* fungus and *Q. suber*, and *Q. coccifera* seedlings were respectively $64.7 \pm 1.3\%$ and $37.73 \pm 1.084\%$ (Table 2). Highly significant differences for the mycorrhization rate between the two *Quercus* species was registered at ($p < 0.001$). After 6 months of growth, *Q. suber* seedlings were more mycorrhized than *Q. coccifera* ones. Results showed higher mycorrhization efficiency of *T. boudieri* in *Q. suber* than *Q. coccifera* roots.

Table 2. Roots mycorrhization rate (%), maximum net photosynthesis (Amax), net photosynthesis (A) in 400 ppm [CO₂], Carboxylation efficiency (CE) and stomatal limitation value (Ls) in leaves of *Q. suber* and *Q. coccifera* mycorrhizal (M) and control (NM) seedlings

Species /Treatment	Mycorrhization rate (%)	Amax ($\mu\text{mol.m}^{-2}.\text{s}^{-1}$)	A (400 ppm)	CE	Ls (%)
<i>Q. suber</i> M	64.7 ± 1.3 a	13.2 ± 0.067 a	6 ± 0.11 a	0.6 ± 0.067 a	54.54 ± 0 c
<i>Q. suber</i> NM	0 c	3.5 ± 0.011 d	1.2 ± 0.5 b	0.02 ± 0 c	66.67 ± 0.007 b
<i>Q. coccifera</i> M	37.73 ± 1.8 b	12.6 ± 0.067 b	5 ± 0.61 a	$0.2^b \pm 0$ b	60.32 ± 0.008 a
<i>Q. coccifera</i> NM	0 c	6.87 ± 0.007 c	1.5 ± 0.58 b	0.005 ± 0.001 c	78.17 ± 0.009 b

The different letters denote significant differences by the student-Newman-Keuls test ($\alpha < 0.05$); \pm : SD.

Gas exchange at 400 ppm

The gas-exchange parameters of M and NM seedlings leaves were compared at an ambient CO₂ concentration of 400 ppm (Figure 1A). Results confirmed that the net photosynthesis (A) was higher in M seedlings than NM ones for both *Quercus* species. An extremely low p value ($p < 0.0001$) indicates high significant difference between net photosynthesis variation according to mycorrhization treatment and *Quercus* species was recorded using statistical analysis.

WUE_i and gs trends (Figures 1B and D) followed the same trend of net photosynthesis ones. Mycorrhizal association promoted gas exchange (gs) for both *Quercus* species (Figure 1C). All parameters were highest in *Q. suber* than *Q. coccifera* seedlings species except WUE_i which was relatively similar in mycorrhizal

plants of both species. Statistical analysis confirmed the highly significant difference ($p < 0.01$) of WUEi, E, and gs according to mycorrhization. It is important to mention that the WUEi for NM seedlings was higher in *Q. coccifera* than *Q. suber* species which highlights the mycorrhization effect passed with a major consequence on *Q. suber* compared to *Q. coccifera* one.

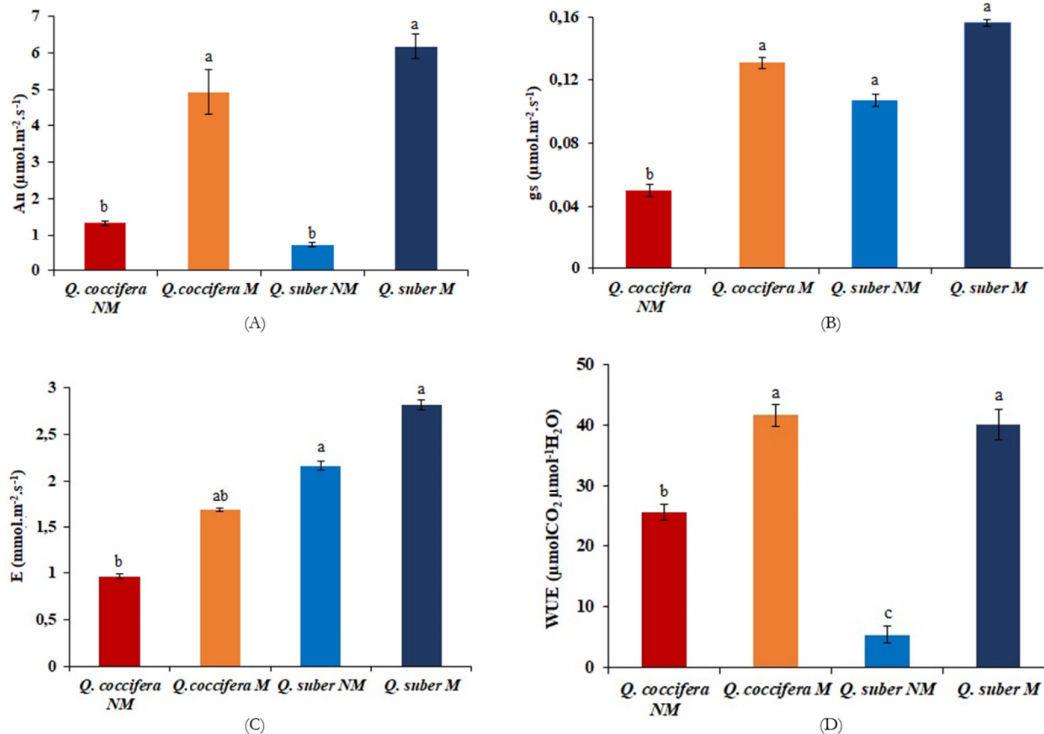


Figure 1. Gas exchanges measures of mycorrhizal (M) and control (NM) seedlings of *Q. coccifera* and *Q. suber*. A: Net photosynthesis (An) measurements; B: Stomatal conductance (gs) measurements; C: Transpiration measurements (E); D: Water use efficiency (WUE) measurements
Errors bars represented the SD of triplicate leaves (A: net photosynthesis; gs: stomatal conductance; E: transpiration; WUE: water use efficiency)

Stomatal limitation (L_s) was higher for M seedlings and varies significantly according to *Quercus* species ($p < 0.0001$). Non inoculated seedlings differed with their L_s (54.54 % and 60.32 % respectively for *Q. suber* and *Q. coccifera*).

At 400 ppm, (A) the difference between M and NM seedlings of *Q. suber* and *Q. coccifera* (Figure 1A) showed the positive effect of mycorrhizal association with *T. boudieri*. Net photosynthesis values were more significant in M than NM seedlings. The *Q. suber* presented the highest ones of up to $13.2 \pm 0.067 \mu\text{mol. m}^{-2}.\text{s}^{-1}$.

Gas exchange to CO_2 intercellular concentration

Variability of net photosynthesis (A) according to CO_2 intercellular concentrations (C_i) of M and NM seedlings of both *Quercus* species showed two phases. In the first part, the net photosynthesis increased with C_i then continues unchanged (Figure 2). M seedlings showed advanced response to the increase in C_i than NM. A_{max} for M plants was higher than the NM ones (Table 2). A_{max} was about 3.7 times higher in mycorrhized *Q. suber* seedlings than NM and 1.83 times superior in M *Q. coccifera* than NM. Variation of A_{max} data

according to mycorrhization and oak species factors was highly significant ($p < 0.0001$). Mycorrhization enhanced the photosynthetic performance of seedling plants of the two *Quercus* species in response to elevated internal CO₂ concentrations.

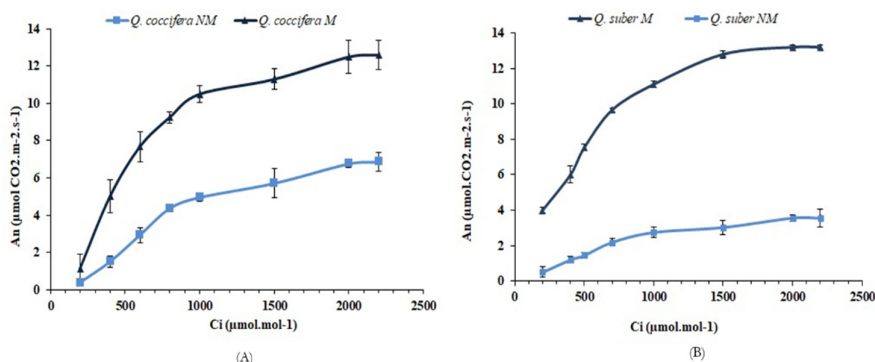


Figure 2. Net photosynthesis response (A) to intercellular CO₂ intensities (Ci) of mycorrhized (M) and control (NM) seedlings of *Q. coccifera* (A) and *Q. suber* (B). Errors bars represented the SD of triplicate leaves)

Gas exchange to light intensity

Photosynthesis variation in inoculated and control seedlings of the two *Quercus* species according to active radiation (PAR) revealed a significant effect for mycorrhization in plants behavior (Figure 3). The photosynthetic assimilation increased with incident light intensities for all tested seedlings. Amax was constantly higher in M than NM seedlings up to the stationary level discernible by a constant value of photosynthesis. The Asat varied significantly ($p < 0.0001$) between species and treatment (Table 2). The Amax level was distinguishable with mycorrhization for *Q. suber* species. For *Q. coccifera* seedlings, the Asat value was $4.7 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ and $4.24 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ in M and NM ones respectively. For *Q. suber* plants, Amax was $6.13 \pm 0.16 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ and $1.333 \pm 0.22 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ for M and NM seedlings respectively. These results revealed that mycorrhization promoted photosynthesis efficacy of *Quercus* species. Likewise, dark respiration values (Rd) increased in seedlings with fungal association (Table 3). Dark respiration data showed a high significant difference due to mycorrhization effect ($p < 0.0001$). Apparent quantum yield (Φ) was also higher in M plants for the two studied species (Table 3) without spotting a significant difference.

LCP values showed decreases from $150 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ in *Q. suber* species control seedlings to $50 \mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ in mycorrhizal ones. The LCP of mycorrhizal *Q. coccifera* seedling was also $50 \mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ as for mycorrhizal *Q. suber* ones but LCP of *Q. coccifera* NM was $75 \mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ (Table 3). Results showed a decrease of LCP in relation to inoculation. However, both *Quercus* species reached the same LCP. Variation on LCP was highly significant between treatments and species ($p < 0.0001$).

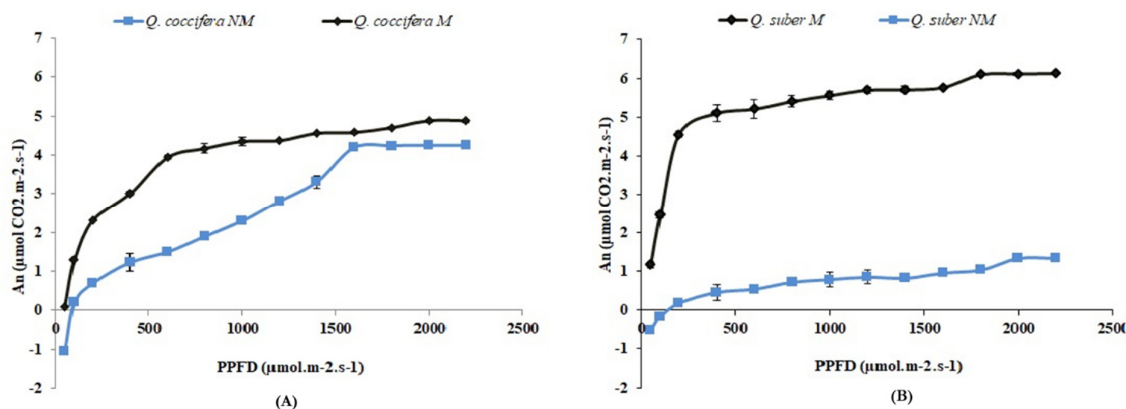


Figure 3. Photosynthesis variation with photosynthetically active radiations intensity (A/PPFD) for mycorrhizal (M) and control (NM) seedlings of *Q. coccifera* (A) and *Q. suber* (B).

Errors bars represented the SD of triplicate leaves)

Table 3. Light-saturated photosynthesis (Asat), apparent quantum yield (Φ), dark respiration (Rd) and light compensation point (LCP) in leaves of *Q. suber* and *Q. coccifera* mycorrhizal and control seedlings

Species /Treatment	Asat ($\mu\text{mol CO}_2 \cdot \text{m}^{-2} \cdot \text{s}^{-1}$)	Φ ($\text{mol CO}_2 \cdot \text{mol}^{-1}$ photon)	Rd ($\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$)	LCP ($\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$)
<i>Q. suber</i> M	6.13 \pm 0.007 a	0.0025 \pm 0.01 a	-0.2 \pm 0.005 a	50 \pm 0 c
<i>Q. suber</i> NM	1.333 \pm 0.006 d	0.000183 \pm 0 c	-0.527 \pm 0.001 b	150 \pm 0 a
<i>Q. coccifera</i> M	4.7 \pm 0.067 b	0.0013 \pm 0 b	-0.3 \pm 0.013 a	50 \pm 0 c
<i>Q. coccifera</i> NM	4.24 \pm 0.007 c	0,0007 \pm 0 c	-1.05 \pm 0.033 c	75 \pm 0 b

The different letters denote significant differences by the student-Newman-Keuls test ($\alpha < 0.05$); \pm : SD.

Photosynthetic pigments

Variation of pigment content was revealed between *Quercus* species. Chla increased with mycorrhization in *Q. coccifera* leaves but decreases in mycorrhizal *Q. suber* ones. With mycorrhization, Chlb varied in reverse for the two *Quercus* species. This difference in Chla and b generated variance on total Chl amounts (Table 4). Carotenoid levels were promoted in *Q. suber* species by mycorrhization but not in *Q. coccifera* species. This dissimilarity on pigment content and arrangement between M and NM *Quercus* species made the absence of statistical differences ($p > 0.1$) between pigments amounts variation with treatment and species which was revealed by statistical analysis.

Table 4. Chlorophylls a, b, total and carotenoids contents (mg/mg MS) in leaves; above-ground biomass (g) and primary root length (mm) of *Q. suber* and *Q. coccifera* mycorrhizal and control seedlings

Analysed parameter	<i>Q. suber</i> M	<i>Q. suber</i> NM	<i>Q. coccifera</i> M	<i>Q. coccifera</i> NM
Chla	0.055 ^b \pm 0.008 b	0.0891 \pm 0.012 a	0,0316 \pm 0,004 c	0,0243 \pm 0,001 c
Chlb	0.0637 \pm 0.011 a	0.0530 \pm 0.008 a	0,0193 \pm 0,000 b	0,0440 \pm 0,006 a
Total Chlorophyll (a+b)	0.1308 \pm 0.037 a	0.1421 \pm 0.111 a	0,051 \pm 0,022 b	0,0547 \pm 0,026 b
Carotenoids	0.0552 \pm 0.007 a	0.0266 \pm 0.002 b	0,0098 \pm 0,009 c	0,0312 \pm 0,008 b
Above-ground biomass	1.3 \pm 0.02 a	0.59 \pm 0.02 c	0.736 \pm 0.03 b	0.39 \pm 0.02 c
Primary root length	110 \pm 3.5 b	91 \pm 2 c	162 \pm 3 b	87 \pm 3.5 c

The different letters denote significant differences by the student-Newman-Keuls test ($\alpha < 0.05$). \pm : SD.

Seedling growth

M seedlings showed longer primary root for both studied species compared to NM (Table 4). Mycorrhizal *Quercus coccifera* seedlings have more extended primary roots than *Q. suber* ones conversely to NM plants where roots were longer in *Q. suber* than *Q. coccifera*. A root rolling up was recorded in both species. This winding was more accentuated in *Q. coccifera* species, and it increase with inoculation treatment. Statistical analysis proved a high significant difference ($p < 0.0001$) with mycorrhization and species factors.

Above-ground biomass plants increased with mycorrhization. This difference was highly significant ($p < 0.001$) according to treatment and species. M seedlings of *Q. suber* showed heavier weight than *Q. coccifera* ones (Table 4).

Discussion

Several studies proved that mycorrhizal symbioses promoted the performance of *Quercus* seedlings (Savoie and Largeteau, 2011; Dib and Fortas, 2019; Khrizi *et al.*, 2022; Sisti *et al.*, 2022). Thus, mycorrhization is an important tool in developing the management of forest trees. According to our results, *Quercus* sp. seedlings were larger and grow better with mycorrhization. In fact, mycorrhization improved net photosynthesis and consequently seedlings growth of both *Quercus* species (*Q. suber* and *Q. coccifera*).

This study verified that mycorrhization increased *Quercus* seedlings transpiration. Indeed, transpiration levels were known to reduce in stressed conditions (Vignes, 1988). Ksontini *et al.* (1998) have explained that the distribution openings of the stomata are less uniform in *Q. coccifera* than *Q. suber* and correlated this proof to the ecological adaptation of *Q. coccifera* to arid lands. Thus, mycorrhization may promote resistance of plants to drought (Jia-Dong *et al.*, 2019, Wang *et al.*, 2023). Furthermore, stomatal limitation (L_s) was promoted by the association of the fungus in *Q. suber* seedlings. As known, stomata play a critical role in the regulation of gas exchange which confirms our hypothesis on the role of mycorrhiza on plant adaptation under harsh conditions (Bueno *et al.*, 2022; Madouh and Quoreshi, 2023).

Typically, associations between the mycorrhizal fungi and the roots of Angiosperm plants influence the water status of the host plant (Morte *et al.*, 2000). This study induces that with mycorrhization, *Quercus* seedlings develop morpho-physiological adjustments of the roots and leaves to those strongly tapered and developed (Ksontini 1998; Slama *et al.*, 2021) to allow for better water and growth efficiency. In fact, WUEi measurements were higher in mycorrhized than non-mycorrhized *Quercus* seedlings. Thus, Augé and Stodola (1990) confirmed that mycorrhized plants require more water than non-mycorrhized ones since high growth is related to higher water requirement. Vignes (1988) also demonstrated differences in the water consumption of leaf potentials of these two oak species and concluded that the greater water uptake capacity could be associated with faster and more efficient osmotic adjustment. This uptake capacity, leaf photosynthetic performance, and carbon/water balance varied by treatment and/or species (Medrano *et al.*, 2015; Mikiciuk *et al.*, 2019).

Mycorrhization contributes to improve *Quercus* photosynthesis balance sheet by the equilibration of transpiration on CO_2 intercellular increase. Indeed, carboxylation efficiency and Apparent Quantum Yield for both *Quercus* species increased with inoculation that may explain the increasing in net photosynthesis (Singsaas *et al.*, 2001; Shi *et al.*, 2021). Mycorrhized seedlings showed a higher response to increased intercellular CO_2 intensities than control ones for both *Quercus* species. As well, chlorophyll pigments might corroborate this useful effect of mycorrhization on the photosynthetic performance of *Quercus* species (Fester *et al.*, 2002; Shi *et al.*, 2017; Li *et al.*, 2018; Chandrasekaran *et al.*, 2019). Physiological response generally is related to morphological characteristics. In fact, root length is considered as an important parameter to evaluate root functions and plant vigor (Gaiser *et al.*, 2013). Primary root length was more important in M plants of *Q. suber*

and *Q. Coccifera* species compared to NM ones. Beltrano *et al.* (2013) stated that mycorrhizal plant of pepper (*Capsicum annuum* L.) maintained greater root system and biomass compared to NM plants. Comas *et al.* (2002) reported that plants transfer carbon to fine roots to amplify the absorbing area by improving specific fine root length then increasing plant biomass by the assimilation of sufficient elements by roots added to a higher photosynthesis rate (Taylor *et al.*, 2014). These confirmations corroborate our results about *T. boudieri* part in root formation and support therefore water and nutrient uptake principle in mycorrhizal seedlings (Chen *et al.*, 2017; Morte *et al.*, 2000). Hence, photosynthesis performance has been correlated to plant biomass and total root length in response to intercellular CO₂ concentrations (Ellsworth *et al.*, 2012). Accurately, the positive effect of mycorrhizal fungi on roots growth, on nutrient availability and thus plant biomass was demonstrated (Lü and Wu, 2017).

Quercus sp. / *Terfezia boudieri* association defined for the first time after the study of Malençon (1952), attests to the ability of the desert fungus to establish a mycorrhizal relationship with a broad spectrum of vegetation outside of the climatic requirements of both partners. However, with the same fungus species, the colonization rate reaches 89% in the plant species *H. sessiliflorum* (Slama *et al.*, 2012). The difference seems to depend on the nature of the species, biology, or expression of the “myc” factor (Kagan-Zur *et al.*, 2013). Dissimilarity on root structure of plant species can also generate diversity on mycorrhization response expressed by special colonization rate and fungal forms (Qin and Feng, 2022). Thus, *T. boudieri* hyphae invade plant roots differently. It appears that the thickness of the roots along with the tannin content generates a difference in colonization rates between *Quercus* (tree) and *Helianthemum* (shrub) species when inoculated with the same fungal species.

Conclusions

The net mycorrhization impact in promoting plant water, nutrient supply, growth, and physiological performances was established. This study confirms, for the first time in Tunisia, the ability of plants and fungi to establish a mycorrhizal association despite that they belong to two different natural conditions. The benefits of the mycorrhization with *T. boudieri* can be attributed to various specific physiological (gas exchange, pigment contents...) and morphological (roots and plant biomass) adjustments in associated plants. Mycorrhization of *Quercus* sp. with edible desert truffles could be a useful tool to improve forest productivity and preserve plant species against limiting factors, especially drought. Our results could be promising for forest management in selecting specimen for reforestation. However, it would be important to determine the cycling and carbon content of mycorrhized plants involving water and nutrient supplies from various growing substrates, as well as water efficiency under stressful conditions to clarify the role of mycorrhiza facing climate change especially drought in a warming future.

Authors' Contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by AS and SF. The manuscript was written by AS, and SF. IT, FM, BS, MK, JSC, AK, and ZN revised the manuscript. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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