

Spatiotemporal variation of endomycorrhizae among two populations of *Pistacia terebinthus* L. (Anacardiaceae) in North Africa

Original Article

Abstract:

Arbuscular mycorrhizal fungi (AMF) have a vital role in natural ecosystems, intensifying nutrient cycling, plant competition, and species interactions. These microbes establish symbiotic relationships with a multitude of plant species, promoting optimal nutrient absorption and plant development. This study allowed the characterization of the mycorrhizal status of *Pistacia terebinthus* in Algeria, where we chose two forests located in Djelfa to carry out the sampling, Senelba and Guetia. Four parameters were estimated: infection frequency (F%), colonization intensity (M%), arbuscular content (A%), and vesicular content (V%) to calculate the colonization rate of mycorrhizal fungi in the fine roots of this species. In addition, the physical and chemical soil parameters were analyzed: pH, electrical conductivity, granulometric, calcium carbonate, and organic matter percentage. Results showed that the roots of the *P. terebinthus* trees are colonized by arbuscular mycorrhizal fungi (AMF). Mycorrhization parameters revealed a frequency of colonization (F%) of up to 92%, mycorrhizal intensity (M%) reaching 19.7%, abundance of arbuscules (A%) and vesicles (V%) in the mycorrhizal parts of root fragments (respectively: 15.5% and 11%). Physical and chemical analysis of its ground notes that these soils have an alkaline pH, unsalted, rich in organic matter, and low in total calcium carbonate. A total of 7 species belonging to five genera, three families, and two orders of Glomeromycota were detected in the soil samples. These results indicate that there is a significant relation between this species and mycorrhizal fungi. For future research, isolating and using high-performing strains of arbuscular mycorrhizal fungi (AMF) identified in the semi-arid forests of Algeria, it would be possible to develop effective mycorrhizal inoculants. Such an approach would not only enhance the native *P. terebinthus*-AMF associations but would also contribute to the sustainable cultivation of pistachios in arid and semi-arid regions.

Key words:Arbuscular mycorrhizal fungi, North Africa, *Pistacia terebinthus*, seasons, soil**Apstrakt:****Prostorno-vremenska varijacija endomikorize između dve populacije *Pistacia terebinthus* L. (Anacardiaceae) u Severnoj Africi**

Arbuskularne mikorizne gljive (AMF) imaju ključnu ulogu u prirodnim ekosistemima, jer intenziviraju kruženje hranljivih materija, konkurenciju među biljkama i međusobne interakcije vrsta. Ovi mikroorganizmi uspostavljaju simbiotske odnose sa brojnim biljnim vrstama, čime podstiču optimalnu apsorpciju hranljivih materija i rast biljaka. Ovim istraživanjem izvršena je karakterizacija mikoriznog statusa vrste *Pistacia terebinthus* u Alžiru, pri čemu su za uzorkovanje odabrane dve šume u oblasti Dželfa – Senelba i Guetia. Procijenjena su četiri parametra: učestalost infekcije (F%), intenzitet kolonizacije (M%), sadržaj arbuskula (A%) i sadržaj vezikula (V%), kako bi se izračunala stopa kolonizacije mikoriznih gljiva u finim korenovima ove vrste. Pored toga, analizirani su fizičko-hemijski parametri zemljišta: pH, električna provodljivost, granulometrijski sastav, sadržaj kalcijum-karbonata i procenat organske materije. Rezultati su pokazali da su korenovi stabla *P. terebinthus* kolonizovani arbuskularnim mikoriznim gljivama (AMF). Parametri mikorizacije ukazuju na učestalost kolonizacije (F%) do 92%, intenzitet mikorize (M%) od 19,7%, te prisustvo arbuskula (A%) i vezikula (V%) u mikoriznim delovima korenovih fragmenata (odnosno 15,5% i 11%). Fizičko-hemijska analiza zemljišta pokazala je da ova tla imaju alkalni pH, nisu zasoljena, bogata su organskom materijom i sadrže mali procenat ukupnog kalcijum-karbonata. U uzorcima zemljišta identifikovano je ukupno sedam vrsta koje pripadaju pet rodova, tri porodice i dva reda iz koljena Glomeromycota. Ovi rezultati ukazuju na značajnu povezanost između ove biljne vrste i mikoriznih gljiva. U budućim istraživanjima, izolovanjem i korišćenjem visokopromansnih sojeva arbuskularnih mikoriznih gljiva (AMF) identifikovanih u polusušnim šumama Alžira, moguće je razviti efikasne mikorizne inokulante. Takav pristup ne bi samo unapredio prirodne asocijacije *P. terebinthus*-AMF, već bi doprineo i održivoj kultivaciji pistacija u sušnim i polusušnim regionima.

Ključne reči:Arbuskularne mikorizne gljive, Severna Afrika, *Pistacia terebinthus*, sezone, zemljište

Zahra Robā Bouabdelli

Centre de Recherche en Agropastoralisme (CRAPAST),
Djelfa, Algeria
bzaharoba@gmail.com (corresponding author)

Abdelghafour Doghbage

Centre de Recherche en Agropastoralisme (CRAPAST),
Djelfa, Algeria

Safia Belhadj

Ziane Achour University, Faculty of Science of Nature
and Life, Djelfa 17007, Algeria

Hafidh Zemour

ISTA, University of Larbi Ben M'hidi, Oum El Bouaghi,
Algeria

Fathi Abdellatif Belhouadjeb

Centre de Recherche en Agropastoralisme (CRAPAST),
Djelfa, Algeria

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Introduction

The cultivation of pistachio (*Pistacia vera* L.) has gained increasing attention due to the fruit’s proven antioxidant and anti-inflammatory properties, making it valuable both nutritionally and economically (Ağlar et al., 2020). Pistachio fruits are rich in fibers, minerals, and unsaturated fats. However, its annual yield often fluctuates, influenced by the selection of appropriate rootstocks and varying weather conditions (Aznarte-Mellado et al., 2014). Rootstocks play a critical role in the growth, development, and quality of pistachio trees, affecting yield, precocity, and nutrient uptake (Ozturk & Ozturk, 2014; Yıldız et al., 2018). Pistachios are economically propagated by grafting onto *Pistacia terebinthus* L. trees, whose adaptability to diverse ecological conditions enhances species resilience (Moriani et al., 2018).

A crucial factor in pistachio cultivation is the symbiotic relationship between plants and arbuscular mycorrhizal fungi (AMF). Mycorrhizae improve nutrient uptake, particularly nitrogen and phosphorus, which are otherwise inaccessible to plants (Genre et al., 2023; Ioanna & Stavropoulos, 2023). AMF also play a protective role by reducing disease incidence in host plants through mechanisms such as competition for carbon sources, pathogen inhibition, and the activation of plant biochemical and molecular defenses (Tian et al., 2021; Balestrini et al., 2015). This partnership not only enhances nutrient acquisition but also increases the pistachio tree’s resilience in various climates and soils, ultimately contributing to sustainable agriculture practices. In exchange, it receives the plant’s reduced carbon (Luginbuehl, 2017; Oldroyd & Downie, 2017). Both partners benefit from this mutual exchange, which helps to boost plant productivity. These responses enhance plant performance, photosynthetic efficiency, and biomass production under abiotic stress. AMF helps maintain resilient ecosystems by improving soil structure, nutrient cycling, and carbon sequestration (Menge, 2023).

Biotic and abiotic constraints in semi-arid Mediterranean ecosystems have significant impacts on crop yields and agricultural sustainability. Understanding interactions between AMF and plant is crucial for effective conservation strategies aimed at preserving biodiversity and ecosystem services provided by natural habitats. The field of arbuscular mycorrhizal fungi (AMF) and their ecological role in agricultural ecosystems has experienced

notable advancements in recent years. Nevertheless, despite these advancements, numerous unanswered questions persist, compelling the need for additional research to delve further into the intricacies of AMF-mediated interactions. It is widely recognized that AMF can have an impact on plant competition and promote the coexistence of various species (Wang et al., 2022).

The study reported here aimed to conduct a detailed examination of the seasonal variation of AMF root colonization of *Pistacia terebinthus*, the relationship between edaphic factors, and AMF spore morphological identification. Two natural forest study sites in Djelfa, Algeria, were selected for this study. This is the first comprehensive study of the mycorrhization of *P. terebinthus* in these regions. The results of this study could give us an idea of the behavior of *P. vera* rootstock for the purpose of growing them in semi-arid regions.

Materials and Methods

Study area

The present study was conducted in the province of Djelfa, located in north-central Algeria. Within this region, two forest sites were selected: Guetia (34°33’N, 2°48’E) and Senelba (34°53’N, 3°03’E). Root samples were of *Pistacia terebinthus* were collected in 2014. Both sites represent typical forest ecosystems of the semi-arid zone. Meteorological data for the studied period were obtained from the National Office of Meteorology (ONM, Djelfa). To define the bioclimatic stage of these forests, climatic parameters such as maximum temperature (MT), minimum temperature (mT), and rainfall were analyzed over a 14-year period (1990–2014). The main geographical and ecological characteristics of these forests are summarized in **Tab. 1**.

Roots material,

Collection and preparation of roots

Roots were collected concurrently with soil samples at a depth of 20 cm. For each station, five distinct *Pistacia terebinthus* individuals were randomly selected. Surface litter was removed, and samples were taken by digging in three locations around each tree, arranged in a triangular pattern with angles at least one meter apart to ensure a representative sampling of the root system. The collected samples

Table 1. Determination of the bioclimatic levels of the study sites

	Latitude	Longitude	Altitude (m)	MT (°C)	mT (°C)	P (mm/an)	Q3	Bioclimatic levels
Guetia	34°33’N	2°48’E	1320	33.21	-0.44	336.1	33.05	Semi-arid (cold winter)
Senelba	34°53’N	3°03’E	1363	32.91	-0.63	344.7	35.25	Semi-arid (cold winter)

MT: Maximum Temperature, **mT:** Minimum Temperature, **P:** Pluviometry, **Q:** pluviothermic quotient

were placed in plastic bags and transported to the laboratory. Sampling was conducted during the spring, summer, and autumn seasons.

Endomycorrhizal coloration

The harvested roots are gently washed in a beaker on a shaker to remove adhering soil particles. The modified method of Philips & Hayman (1970) is used to highlight the light microscope mycorrhizal infection. The roots are cut into pieces about 1 cm long, placed in a 10% potassium hydroxide (KOH) solution, and then oven-dried at 90 °C for 1 hour. To remove the rest of the pigments, the roots were transferred to a 10% solution of H₂O₂ at 90 °C for 20 minutes until the roots turned white. The roots are rinsed several times with water, passed through a 10% lactic acid bath, and then transferred to a trypan blue solution, which is placed in the oven at 90 °C for 1 hour. After removing the roots from the dye solution, they were rinsed with tap water and placed in a Petri dish containing glycerol.

Estimation of Mycorrhization Colonization

Root fragments are carefully crushed and observed by light microscopy at magnifications of 100 and 400. Several scoring systems are used to assess the importance of mycorrhizal infection. The system proposed by Trouvelot et al. (1986) was adopted in this study. Using this method of colonization, five key parameters were calculated as follows:

Frequency of Mycorrhizal Colonization

(F%): This represents the percentage of root fragments colonized by endomycorrhizae, indicating the extent of root system colonization.

Intensity of Mycorrhizal Colonization in the Root System (M%): This measures the degree of colonization within the root system, providing insight into the depth of the symbiotic relationship.

Abundance of Arbuscules in the Root System

(A%): This parameter reflects the proportion of the root system containing arbuscules, which are specialized structures formed by mycorrhizal fungi for nutrient exchange.

Abundance of Vesicles in the Root System

(V%): This measures the presence of vesicles, storage structures formed by the fungi, within the root system.

These parameters help assess the overall extent and functional efficiency of mycorrhizal colonization in the sampled roots. Calculation of these parameters was performed using the software program MYCOCALC available at www.dijon.inra.fr (INVAM).

Physico-Chemical Analyses of Soils

We collected approximately 1 to 2 kg of soil around *P. terebinthus* roots in 3 different places (from a depth of 20 cm). Soils were taken to the laboratory for physico-chemical analysis. We used the methods described by Aubert (1978) in his book “*Method of Soil Analysis*.” We analyzed: pH and electrical conductivity (EC) on a soil-water suspension (1/5), granulometric composition, calcium carbonate content (TCaCO₃), and organic matter percentage.

Statistical Analysis

STATISTICA Version 10 software was used to conduct descriptive statistics. Subsequently, the assessed parameters were analyzed for significant differences using analyses of variance (ANOVAs) based on the specified factors and their interactions. All ANOVAs were conducted with a significance level set at $p=0.05$. This was followed by principal component analysis (PCA).

Results and Discussion

Mycorrhizal fungi

Observation of the roots through the binocular magnifying glass reveals the absence of a fungal mantle. The roots are slightly transformed, slightly swollen at the end, and have absorbent hairs on their surface. Nevertheless, at the Guetia station (spring season), we noticed the presence of some structures origin of the deformation of the rootlets in some trees (3 trees), this can be due to a colonization of other fungi forming ecto-endomycorrhiza (double colonization) or to rhizo-endophytes (mycoendophytes) which can generate ecto-mycorrhizoidal structures (**Fig. 1**). Two forms were recorded during our observations: the simple form characterized by a swelling of the lateral rootlets at the end (**Fig. 1, a and b**) and the coralloid or dichotomous form characterized by the branching of the lateral rootlets (**Fig. 1, c and d**), which are surrounded by an extra-matrix network of mycelial cords (branched aggregates of hyphae) (**Fig. 1, d and e**).

Microscopic observations of root fragments of *P. terebinthus*, collected during the three seasons (summer, autumn, and spring) and crushed and stained with trypan blue, allowed us to highlight the presence of arbuscular mycorrhizae at the studied stations. This type of mycorrhiza is characterized by the presence of three different fungal structures: hyphae, vesicles, and arbuscules (**Fig. 2, a and b**). The different characteristics of the three structures are presented in **Tab. 2**.

Tab. 3 presents the mycorrhization parameters of *Pistacia terebinthus* at two forest stations, Senelba and Guetia, across three sampling seasons. Overall,

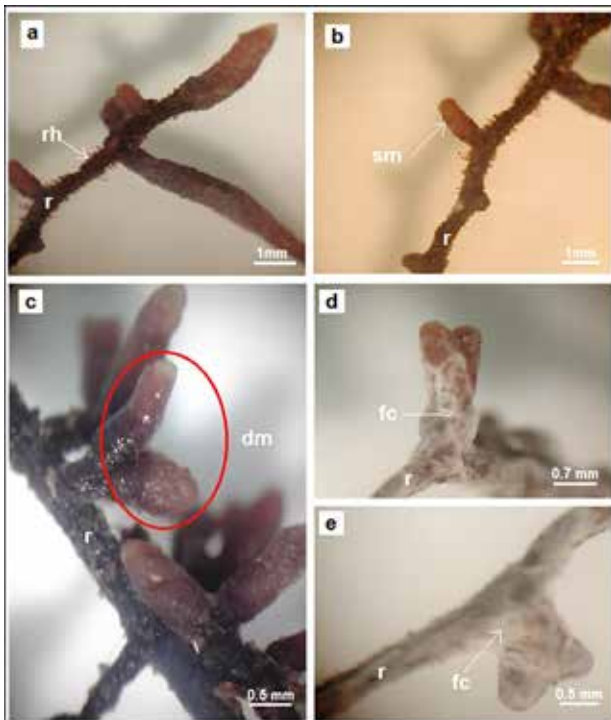


Fig. 1. Stereomicroscope observation of roots (**r**) morphology of *Pistacia terebinthus* (Goutia forest spring): **a-b**: simple mycorrhiza - **sm**, with root hairs - **rh**, **c**: coralloid or dichotomous mycorrhiza - **dm**, **d-e**: fungal coat - **fc**.

P. terebinthus exhibited a very high infection frequency (F%), averaging 92%, which indicates that arbuscular mycorrhizal fungi (AMF) are well established in its rhizosphere. The frequency reached 100% at Senelba for all seasons, while Guetia showed slightly lower and more variable values (80–92%). This confirms a strong spatial influence, with Senelba providing more favorable edaphic or climatic conditions for AMF colonization.

The intensity of colonization (M%) was moderate overall (mean=19.7%) and significantly higher at Senelba (26%) than at Guetia (13%), suggesting denser intraradical fungal development in Senelba soils. Seasonally,

colonization intensity was greatest in summer (≈30%) and lowest in autumn (≈7-18%), reflecting the positive effect of warmer, drier conditions on fungal activity and plant dependence on the symbiosis.

The arbuscular content (A%), representing functional exchange structures, followed a similar trend—higher at Senelba (up to 25%) than at Guetia (≈15%)—with maximum development during spring and summer, indicating periods of active nutrient transfer between the host and the fungus.

The vesicle content (V%), which reflects storage structures within roots, was also more pronounced at Senelba (up to 18%) than at Guetia (≈10%), particularly in summer and autumn. This suggests enhanced fungal storage activity under seasonal water stress.

Statistical analysis revealed that both station and season factors influenced mycorrhizal parameters, with Senelba consistently showing higher values. These results demonstrate that *P. terebinthus* maintains a strong and functionally diverse AMF association, modulated by environmental conditions, especially temperature and soil moisture.

Identification of AMF of *P. terebinthus*

The morphological identification of the spores allowed us to observe various morphotypes that differ among themselves in size, color, and shape (rounded or oblong) (Fig. 3). A total of 7 species belonging to five genera, three families, and two orders of Glomeromycota were detected in the soil samples. Two species for each of the genera *Glomus* and *Gigaspora*, one species for each of the genera *Septoglomus*, *Funneliformis*, and *Acaulospora*. Two major families have been listed, *Glomeraceae* (Glomerales) and *Gigasporaceae* (Diversisporales) (Tab. 4). Shannon’s diversity index varies between 1.04 and 1.61 and follows the Guetia > Senelba gradient. Despite the climatic homogeneity of the two stations (cool semi-arid). The Guetia forest is richer in AMF species.

Table 2. Different AMF structures observed in *P. terebinthus* in the two stations

Vesicules	Arbuscules	Hyphae
<p>Two shapes Oval (+++) Round (++) Irregular</p>	<p>Arum type «Senelba and Guetia» (+++)</p> <p>Paris type «Guetia» (+/-)</p>	<p>Thick siphoned intercellular hyphae forming a skeleton and much branched (+++)</p>
<p>Two locations Intracellular (++) Extracellular</p>		<p>Coiled thick intracellular hyphae (Pelotons) (+)</p>
<p>Varied size Large (+) Small</p>		<p>Hyphae with arbuscules or vesicles at their ends (+)</p>

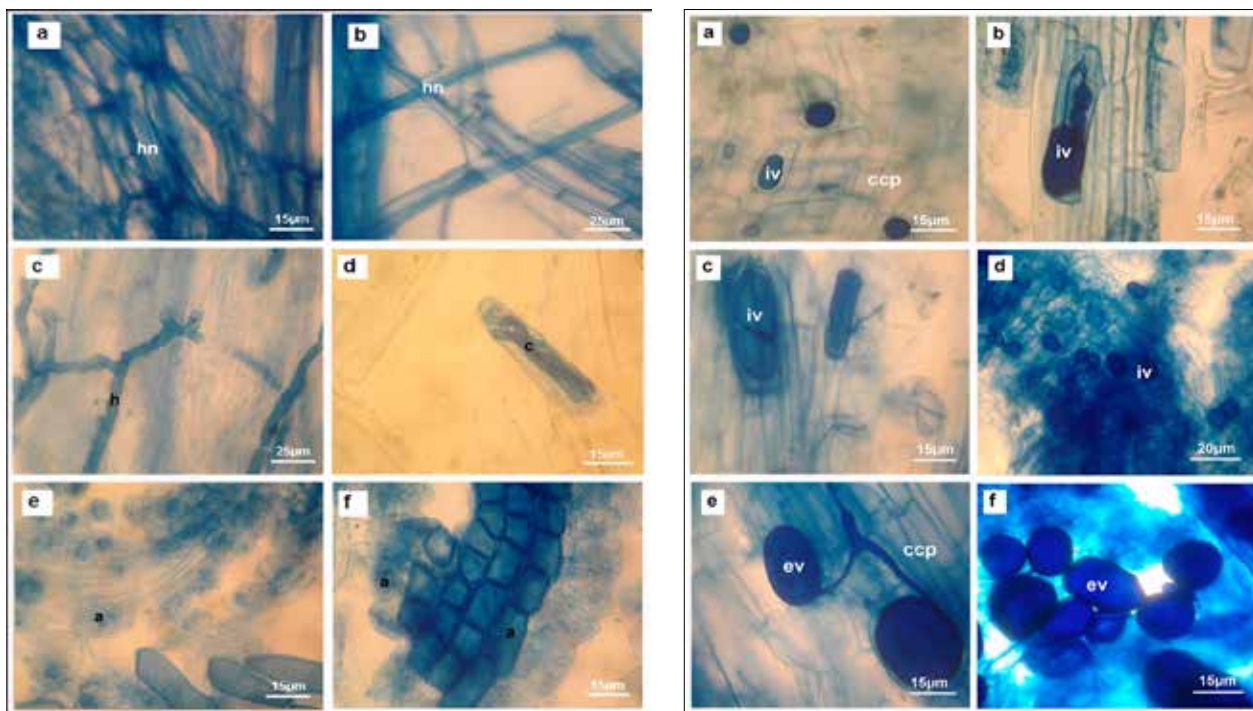


Fig. 2a. Microscopic observations of stained root of *Pistacia terebinthus* showing different mycorrhizal structures. **a–b:** The hartig network - **hn** formed between cortical cells' parenchyma illustrates the intercellular colonization typical of ectoendomycorrhizal associations (Guetia forest, spring), **c:** Fungal hyphae - **h**, extending through the root tissues (Guetia forest, spring). **d:** Coiled structure - **c** observed within cortical cells parenchyma, indicating an intracellular fungal interaction (Senelba forest, autumn). **e–f:** developed arbuscules - **a** inside the cortical cells of the parenchyma, characteristic of arbuscular mycorrhizal symbiosis. Magnification (x 40).

Fig. 2b. Microscopic observations of the stained root of *Pistacia terebinthus* showing vesicular structures formed during arbuscular mycorrhizal colonization: **a–d:** Intracellular vesicles - **iv**, localized within the cortical cell parenchyma - **ccp**, indicating lipid or nutrient storage structures characteristic of mature arbuscular mycorrhizal symbioses. **e–f:** Extracellular vesicles - **ev**, located between cortical cells. **a and b:** Guetia forest, summer, **c and e:** Senelba forest, summer, **d:** Senelba forest, autumn, **f:** Senelba forest, spring. Magnification (x 40).

Table 3. Mycorrhization parameters of *P. terebinthus* (Senelba and Guetia)

Parameters	Season	Frequency of infection F (%)	Intensity of colonization M (%)	Arbuscular content of infection A (%)	Vesicle content of infection V (%)
Senelba (S)	Spring	100±0	28.8±20.5	10.0±4.2	6.1±2.9
	Summer	100±0	30.9±12.1	25.5±12.9	18.7±15.5
	Autumn	100±0	18.7±10.4	13.5±9	10.3±7.6
	Mea. S±SD	100 ^{NS} ±0	26.1 ^{NS} ±14.9	16.3 ^{NS} ±11.1	11.2 ^{NS} ±10.2
Guetia (T)	Spring	80±44.7	16±9.6	30.4±36.7	22.8±33.4
	Summer	92.0±17.9	16.4±14.5	11.6±14	7.9±10.3
	Autumn	80±44.7	7.4±4.8	2±1.5	1.4±0.9
	Mea. S±SD	84 ^{NS} ±35.6	13.3*±10.6	14.6 ^{NS} ±24.3	10.7 ^{NS} ±20.9
Season factor	Mea. E±SD	92 ^{NS} ±26.1	19.7 ^{NS} ±14.3	15.5 ^{NS} ±18.6	11 ^{NS} ±16
Station factor	Mea. E±SD	92 ^{NS} ±26.1	19.7*±14.3	15.5 ^{NS} ±18.6	11 ^{NS} ±16
Season-Station Interaction	Mea. E±SD	92 ^{NS} ±26.1	19.7 ^{NS} ±14.3	15.5 ^{NS} ±18.6	11 ^{NS} ±16

Mea. S±SD: Mean Station, **Mea. E±SD:** Mean species, **Mea. G±SD:** Mean Genus, **SD:** standard deviation, Statistical significance level at $p=0.05$, * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **NS:** No Significant

Table 4. AMF species isolated from the rhizosphere of *P. terebinthus*

Class	Order	Family	Genus	Identified species	Unidentified species	Stations	
						Senelba (S)	Guetia (T)
Glomeromycètes	Glomerales	Glomeraceae	Glomus		Glomus sp.1		*
Glomeromycètes	Glomerales	Glomeraceae	Glomus		Glomus sp.2	*	*
Glomeromycètes	Glomerales	Glomeraceae	Septoglomus	<i>S. constrictum</i>		*	*
Glomeromycètes	Glomerales	Glomeraceae	Funneliformis	<i>F. mosseae</i>			*
Glomeromycètes	Diversisporales	Gigasporaceae	Gigaspora		Gigaspora sp.1		*
Glomeromycètes	Diversisporales	Gigasporaceae	Gigaspora		Gigaspora sp.4	*	*
Glomeromycètes	Diversisporales	Acaulosporaceae	Acaulospora		Acaulospora sp.1	*	*
Total	2	3	5	2	5	4	5

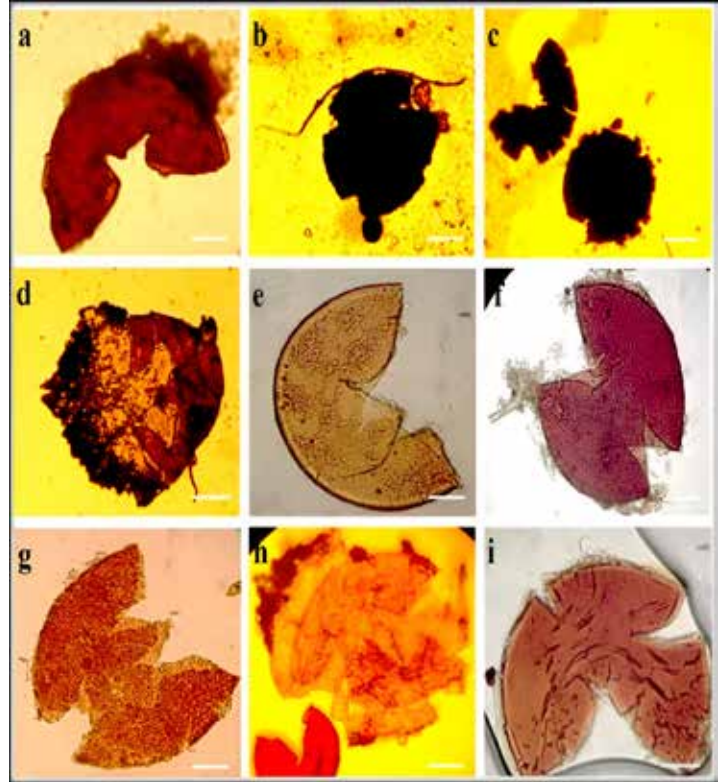


Fig. 3. Spores of different AMF species isolated from the rhizosphere under *P. terebinthus*: a-d: spores isolated from Guetia forest. e-i: spores isolated from the Senelba forest. Magnification (x 40). Bar - 30 µm.

Physico-chemical analysis of the soils

The average pH of the two stations is 8.2 (alkaline soil). A higher value (8.4) is recorded at Senelba (with 8.0 for Guetia). The three seasons, spring, autumn, and summer, have pH-water values between 8.4, 8.2, and 8.1, respectively. For electrical conductivity, the average rate is 0.2 mS/cm. The soils are non-saline, with electrical conductivity values of 0.23 mS/cm in Senelba and 0.15 mS/cm in Guetia. ECs vary little between seasons (0.22, 0.21, and 0.15 mS/cm for spring, summer, and autumn, respectively). For organic matter, the two stations are rich (7.9% for Guetia and 4.8% for Senelba, with an average of 6.3%). Spring and summer have high rates of 8.4% and 7.8% respectively, while autumn is the season when this rate is low (2.8%). The average total limestone value is 10.6% for both stations, representing a low amount of total CaCO₃ (12% and 9.2% for Guetia and Senelba, respectively). It is noted that the highest values are marked in spring (12.5%), and decrease in summer (12.0%), then in autumn (7.2%) (Tab. 5).

The soil at Guetia is characterized by a loam texture, with a relatively balanced composition of sand (44.2%), silt (42.8%), and clay (13%). In contrast, the Senelba site exhibits a sandy-loam texture, dominated by a high proportion of silt (81.33%) and a lower content of sand (13.77%) and clay (4.9%) (Tab. 6).

Table 5. Physico-chemical analysis of soils (Senelba and Guetia)

Parameters	Season	pH-water	Electrical Conductivity (mS/cm)	Organic matter (%)	Total CaCO3 (%)
Senelba (S)	Spring			2.4±1.3	5.6±1.4
	Summer	8.7±0.1	0.3±0.1	7.9±2.2	11.2±6.7
	Autumn	8.2±0.1	0.3±0.1	4.2±1	10.8±6.3
	Mea. S±SD	8.4***±0.3	0.2*±0.1	4.8***±2.8	9.2 ^{NS} ±5.6
Guetia (T)	Spring	8.1±0.0	0.1±0.01	3.2±0.7	8.8±6.5
	Summer	8.1±0.1	0.2±0.02	9±1.2	13.8±6.6
	Autumn	7.9±0.2	0.2±0.02	11.4±2.2	13.3±6.6
	Mea. S±SD	8.0 ^{NS} ±0.1	0.2*±0.02	7.9***±3.8	12 ^{NS} ±6.5
Season factor	Mea. E±SD	8.2*±0.3	0.2*±0.1	6.3***±3.6	10.6 ^{NS} ±6.1
Station factor	Mea. E±SD	8.2***±0.3	0.2***±0.1	6.3*±3.6	10.6 ^{NS} ±6.1
Season-Station Interaction	Mea. E±SD	8.2***±0.3	0.2***±0.1	6.3***±3.6	10.6 ^{NS} ±6.1

Mea. S±SD: Mean Station, **Mea. E±SD:** Mean species, **Mea. G±SD:** Mean Genus, **SD:** standard deviation, Statistical significance level at $p=0.05$, * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **NS:** No Significant

Data analysis

The correlation matrix between the mycorrhization soil and climatic parameters shows several significant correlations (**Tab. 7**): between arbuscular (A) and vesicular (V) content ($r=0.98$); between conductivity (EC) and pH ($r=0.57$), and between colonization intensity (M) and electrical conductivity (EC) ($r=0.63$).

The correlation circle shows that the majority of the variables (except F, OM, and TL) are correlated with the two axes, explaining 69.53% of the variance. The two soil variables (pH and EC) and all the climatic variables are correlated with axis 1; the two mycorrhization variables (A and V) are correlated with axis 2, while the M variable is correlated with two axes (**Fig. 4A**). Consequently, this interaction made it possible to group all the trees of the two populations into two distinct groups (**Fig. 4B**). Axis 1 seems to reflect an increasing aridity index (Q3), where the different stations are discriminated according to their bioclimates into two distinct groups.

Table 6. Particle size analysis of soils in the studied populations of *P. terebinthus*

Element	Guetia (T)	Senelba(S)
Sand (%)	44.2	13.77
Silt (%)	42.8	81.33
Clay (%)	13.0	4.90
Texture	Loam	Sandy-loam

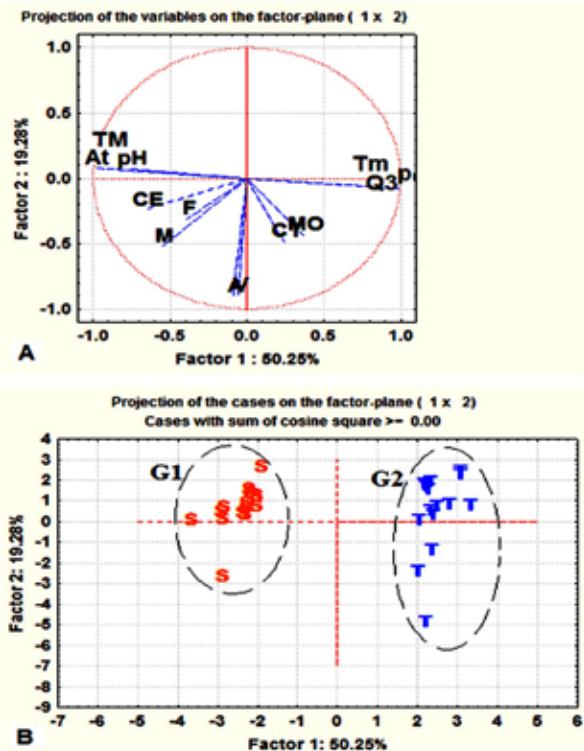


Fig. 4. Principal component analysis, **A:** Circle of correlation of variables for *P. terebinthus*; **F** - frequency of mycorrhization. **M** - intensity of colonization of the root cortex. **A** - arbuscular content. **V** - vesicular content. **pH** - pH-water. **EC** - electrical conductivity. **MO** - organic matter. **CT** - total limestone. **Alt** - altitude. **Q3** - rainfall quotient. **TM** - mean maximum temperature of the hottest month in degrees Celsius. **Tm** - mean minimum temperature of the coldest month in degrees Celsius. **B:** Scatterplot of samples, **S** - Senelba, **T** - Guetia, **G1** - group 1, **G2** - group 2.

Table 7. Pearson correlation matrix ($p < 0.05$) for mycorrhization, soil and climatic variables studied of *P. terebinthus*

	F%	M%	A%	V%	pH	EC (mS/cm)	OM%	TL%	P (mm/y)	MT (°C)	mT (°C)	At (m)	Q3
F%	1	0.42	0.26	0.21	0.2	0.22	-0.05	-0.16	-0.31	0.31	-0.31	0.31	-0.31
M%		1	0.36	0.33	0.34	0.63	0.12	0.07	-0.46	0.46	-0.46	0.46	-0.46
A%			1	0.98	-0.05	0.1	0.16	0.29	-0.05	0.05	-0.05	0.05	-0.05
V%				1	-0.07	0.07	0.13	0.3	-0.02	0.02	-0.02	0.02	-0.02
pH					1	0.57	-0.08	-0.14	-0.71	0.71	-0.71	0.71	-0.71
EC (mS/cm)						1	0.18	-0.02	-0.57	0.57	-0.57	0.57	-0.57
OM%							1	0.28	0.43	-0.43	0.43	-0.43	0.43
TL%								1	0.23	-0.23	0.23	-0.23	0.23
P (mm/y)									1	-1	1	-1	1
MT (°C)										1	-1	1	-1
mT (°C)											1	-1	1
At (m)												1	-1
Q3													1

F%: Frequency of Mycorrhizal Colonization, **M%:** Intensity of Mycorrhizal Colonization in the Root System, **A%:** Abundance of Arbuscules in the Root System, **V%:** Abundance of Vesicles in the Root System, **pH:** pH water, **EC:** Electrical Conductivity (millisiemens per centimeter), **OM:** Organic Matter, **TL:** Total Limestone, **MT:** Maximum temperature, **mT:** Minimum temperature, **P:** Pluviometry (mm per year), **Q3:** Pluviometric quotient, **At:** Altitude (meter)

Group 1 is made up of the station of Senelba (S), characterized by high values of pH, EC, TM, and At. Group 2 includes the station of Guetia (T), which has high values of P, mT, and Q3. For the *P. terebinthus* species, the matrix of correlations between the parameters of mycorrhization and those of the soils shows significant correlations between all the parameters of mycorrhization: between the pH and the total limestone ($r=0.73$), and between the pH and colonization intensity ($r=-0.57$) (**Tab. 7**).

Discussion

There has been no research on mycorrhizae assessment in *Pistacia terebinthus* rhizospheres' soil; however, many other studies on other species, such as *Pistacia atlantica*, have been conducted (Hamada et al., 2013; Alguacil et al., 2016). In our study, rhizospheric roots of *P. terebinthus* were colonized by both arbuscular mycorrhizal fungi (AMF) and ectoendomycorrhizae (ECM) types. According to Brundrett (2004), the same types are found in the *Quercus suber* species sampled in Mediterranean forests.

The root deformation observed in some trees of *P. terebinthus* is of particular ecological significance, as it indicates active interactions between roots and diverse fungal partners. Such morphological modifications reflect the establishment of complex symbiotic structures that can enhance nutrient exchange, improve root surface area, and contribute to the functional diversity and adaptability of the mycorrhizal association. Previous studies have demonstrated that single colonization by either endophyte fungi (DSE) or ecto-endomycorrhizal fungi (ECM) can negatively affect plant growth, whereas dual colonization tends to mitigate or neutralize this inhibitory effect (Jumpponen & Trappe, 1998; Püschel et al., 2013). This synergistic effect is attributed to the complementary ecological functions of both fungal groups: DSE contribute

to the mineralization of organic matter and nutrient mobilization, while ECM fungi enhance nutrient uptake efficiency and regulate endophytic colonization (Mandyam & Jumpponen, 2005; Smith & Read, 2008; Kariman et al., 2018).

We found high total AMF colonization rates in the roots. This rate reached up to 100%. Our results are similar to those of Ortas et al. (2013) on four forest trees, where the colonization rate ranged from 62.07% to 100%. Our results suggest that *P. terebinthus* is well adapted to these two forest conditions and promotes arbuscular mycorrhizal symbiosis.

Data for the winter season were not collected due to limited accessibility to the study area during this period. Harsh climatic conditions and restricted access to the forest made sampling difficult, preventing the collection of root and soil samples. Consequently, only data from spring, summer, and autumn were included in this study.

Arbuscular mycorrhizal (AM) fungi play crucial functional roles in ecosystems, including facilitating nutrient uptake, modifying the physical properties of soil, and altering plant interactions with other biota (Smith & Read, 2008). However, the composition, specificity, and abundance of these fungal communities in semiarid conditions, particularly in tropical regions, are poorly understood (Gai et al., 2006).

The soils in this study area are alkaline and calcareous, which, according to Ortas et al. (2013), leads to phosphorus precipitation, making it less available to plants. By interacting with the abiotic environment, mycelium can directly impact ecosystem functioning (Leifheit et al., 2014). Furthermore, Hoeksema et al. (2010) discovered that the presence of AM fungi reduced variation in community productivity across strong gradients of phosphorus and nitrogen limitations while also increasing plant community-level stoichiometric homeostasis. The particle size analysis revealed clear differences in soil texture between the two studied sites. A loam texture characterizes the soil at Guetia, and this composition suggests a medium-textured soil that favors good aeration and moderate water retention, suitable for root development. In contrast, the Senelba site exhibits a sandy-loam texture, dominated by a high proportion of silt (81.33%) and a lower content of sand (13.77%). This texture indicates lighter soil with better drainage but lower nutrient-holding capacity. Such variation in soil texture between sites may influence water availability, nutrient dynamics, and consequently, the mycorrhizal colonization of *Pistacia terebinthus* roots.

Notably, AMF colonization intensity was significantly and negatively correlated with electrical conductivity. Previous studies have also demonstrated this correlation (Koide & Kabir, 2000; Lekberg & Koide, 2005; Munkvold et al., 2004). However, no significant correlation was observed between AMF colonization rate and other soil parameters in our study.

Our results differ from those of Ortas et al. (2013), who found that AMF colonization intensity was significantly and negatively correlated with soil total carbon and organic matter content.

Selecting the rootstock in pistachio orchards is one of the most critical decisions, mainly in conditions of water scarcity. However, there are only a few studies that report the response of pistachio to water stress according to rootstock. According to Trotta et al. (1996), mycorrhiza-treated plants achieved approximately 80% positive grafts using *P. terebinthus* as a rootstock for *P. vera* buds.

Our results suggest a great affinity between these fungi and *P. terebinthus*, making it a suitable rootstock for *P. vera* buds in semi-arid forests for two reasons:

1. The rehabilitation and restoration of degraded forests in these regions.
2. Increasing almond production in our country.

Symbiotic arbuscular mycorrhizal fungi (AMF) represent a cost-effective and non-destructive strategy for enhancing crop yield and promoting a sustainable agricultural system with low inputs. These fungi efficiently absorb low-level soil nutrients, significantly impacting ecosystem functioning and stability (Giovannetti & Avio, 2002). As vital components of soil microbiology, AMFs can be harnessed to improve crop development, potentially reducing or eliminating the need for chemical fertilizers and pesticides in organic agriculture (Gianinazzi et al., 2010; Veresoglou et al., 2012).

Previous research by Hartnett & Wilson (1999) indicated that the application of benomyl in dry grassland plots suppressed AM fungi, leading to reduced temporal stability in community productivity. AMF-colonized plants exhibit superior soil utilization, enhanced nutrient absorption, and greater resilience to drought, salinity, and heavy metals. Additionally, they offer benefits as plant growth promoters and biological control agents against phytopathogens (Pozo & Azcón-Aguilar, 2007).

Conclusion

Given the key role of mycorrhizal fungi in the poor soils of fragile ecosystems in arid and semi-arid areas, identifying some of these fungi has enabled us to understand their ecology across different soil types. The use of molecular techniques for the identification of AMF species as a complement to the morphological tool is desired in order to proceed with the isolation of the most efficient strains which plays a key role in the ecosystem productivity and stability with the aim of their use on a large scale for mycorrhization of young pistachio trees to be planted in favorable areas for the reforestation of the green dam.

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