

Diversity of arbuscular mycorrhizal fungi in *Tetraclinis articulata* (Vahl) Masters woodlands in Morocco

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Abstract – A survey of arbuscular mycorrhizal (AM) fungi was conducted in seven *Tetraclinis* woodlands. Microscopic analysis of the mycorrhizal status of *T. articulata* (Vahl) Masters roots revealed that all samples formed only AM, and no ectomycorrhizal fungi were detected. The mycorrhizal colonisation level was generally high (more than 80%), thus reflecting the mycotrophic nature of *T. articulata*. A “Paris-type” mycorrhizal structure was noted in all studied samples. The number of AM fungal spores detected in field-collected soils was relatively high. All recovered spores belonged to the Glomineae order, represented by Glomaceae and Acaulosporaceae families. Two groups were dominant: the first one included small (90 µm), hyaline, white to dark-yellow spores, and the second involved large (295 µm), light orange to dark orange-brown spores. The morphological characters indicated that the spore populations consisted of 3–6 morphotypes. The *Glomus* genus was represented by five species, i.e. *Glomus aggregatum*, *Glomus constrictum*, *Glomus* sp. 1, *Glomus* sp. 2, and *Glomus* sp. 3, while the *Acaulospora* genus was represented by only one unidentified species.

diversity / tetraclinis woodlands / “Paris-type” arbuscular mycorrhizae / *Glomus* / *Acaulospora*

Résumé – Diversité des champignons mycorrhiziens arbusculaires dans les forêts de *Tetraclinis articulata* (Vahl) Masters au Maroc. La présence des champignons mycorrhiziens arbusculaires (CMA) a été étudiée dans sept tetraclinaies marocaines. Les examens microscopiques des racines de *T. articulata* (Vahl) Masters ont révélé la présence, dans tous les échantillons, des endomycorhizes arbusculaires ; aucune ectomycorhize n’a été détectée. Le taux d’infection par les endomycorhizes à arbuscules a été très élevé (plus de 80 %), indiquant le caractère mycotrophique de l’espèce. La structure mycorrhizienne observée dans tous les échantillons analysés est de type « Paris ». Le nombre de spores de CMA isolées à partir des différents sols est relativement élevé. Toutes les spores appartiennent à l’ordre des Glomineae, représenté par deux familles : Glomaceae et Acaulosporaceae. Deux groupes sont dominants : le premier groupe renferme des spores hyalines, blanches à jaunes foncées et de petites tailles (90 µm en moyenne) et le second correspond à des spores orange-claires à orange foncées et de grandes tailles (295 µm en moyenne). Les caractères morphologiques indiquent que les populations de spore comportent 3 à 6 morphotypes selon le site. Le genre *Glomus*, le plus dominant, est représenté par cinq espèces – *Glomus aggregatum*, *Glomus constrictum*, *Glomus* sp. 1, *Glomus* sp. 2, et *Glomus* sp. 3 – alors que le genre *Acaulospora* est représenté par une seule espèce non identifiée.

diversité / tetraclinaies / mycorhizes à arbuscules type « Paris » / *Glomus* / *Acaulospora*

1. INTRODUCTION

Tetraclinis articulata (Vahl) Masters, a member of the Cupressaceae family, is an endemic North African tree species that is widely distributed in Morocco, where it ranges from the eastern part of the country to the western high-Atlas region. The surface area of *Tetraclinis* woodlands is estimated at 565 798 ha, which represents approximately 10% of the total forest cover in Morocco [5, 29].

Tetraclinis articulata is a rustic thermophilous species that thrives in harsh environmental conditions, within the 250–900 mm/year rainfall range [4]. It grows in a wide range of rock

substrates, including limestone, dolomite, granite or schist, but not in habitats with shifting sands.

Tetraclinis articulata is of high interest, for both the value and diversity of its products (timber, wood tar, firewood, charcoal, sandarac gum, etc.). It is considered as a precious species because its wood is highly appreciated in inlaid work and for making decorative items. Art crafts activities developed around this species generates considerable income for local populations. *Tetraclinis* woodlands also produce fodder (6.2% of total fodder input). Unfortunately, drought and high grazing pressure limit the natural regeneration of the species and restrict its distribution range.

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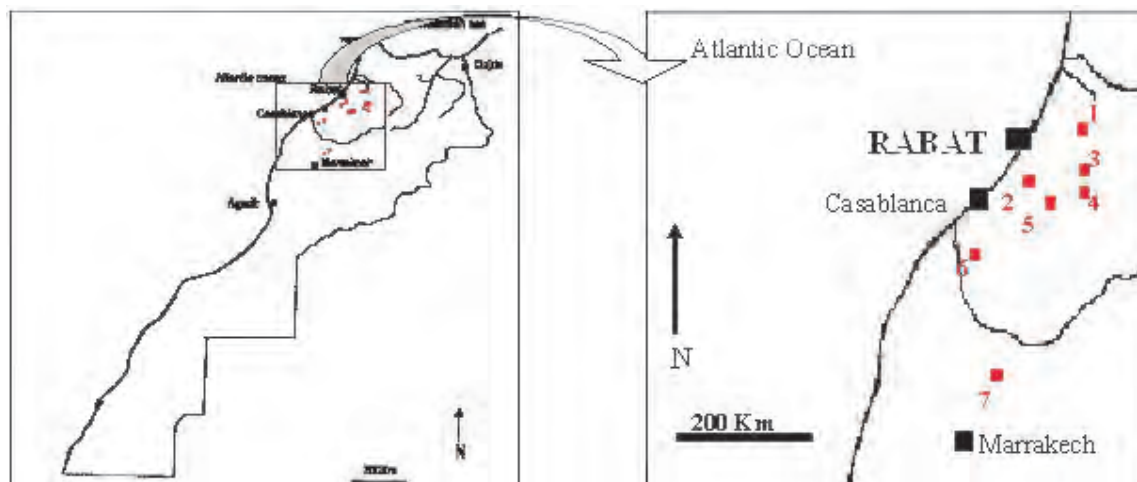


Figure 1. Location of the sample sites. 1: Oued Beht, 2: Oued Cherrat, 3: Korifla, 4: Maghchouch, 5: BenSlimane, 6: El Kantour, 7: Sidi Jaber.

Tableau I. Pedological soil characters of 7 Moroccan *Tetraclinis* woodlands.

Site number	pH	Clay (%)	Silt (%)	Sand (%)	Organic matter (%)	N total (‰)	Assimilable P (mg/100)
1	7.55	27.73	40.73	31.4	3.43	0.80	2.10
2	6.8	29.00	26	45	2.29	1.02	1.85
3	6.9	33.13	40.96	25.8	5.14	0.99	1.49
4	7.6	17.4	22.1	48.1	0.80	0.02	1.91
5	6.7	12.3	27.70	54.1	4.81	0.25	1.41
6	7.8	0	20.5	79.8	1.20	0.015	1.45
7	7.7	8.6	46.4	45	1.20	0.025	1.01

1: Oued Beht; 2: Oued Cherrat; 3: Korifla; 4: Maghchouch; 5: Ben Slimane; 6: El Kantour and 7: Sidi Jaber.

Artificial regeneration of *T. articulata*, principally in the eastern region, started in the 1960s. This species ranks first under the current federal reforestation programme [2], with 14.3% of the overall 5 Mha to be reforested in Morocco. Unfortunately, this reforestation initiative is hampered by severe problems of seedling survival, poor growth and even total planting failures. The low quality of seedlings produced in forest nurseries is among the factors responsible for the failures observed in the field.

In some cases, mycorrhizae improve plant growth, mineral nutrient status and resistance to transplanting stress [19]. Arbuscular mycorrhizae are the most widespread plant symbiosis that occur in nature (in about 80% of plant species) and mycorrhizal fungi are key components of natural ecosystems. They are considered as essential for ecosystem functioning [16, 28] because they play a fundamental role in soil fertility and in the maintenance of stability and biodiversity within plant communities [13]. The success of any reforestation programme depends on colonisation of the new woodland stands by mycorrhizae [18, 23].

Studies conducted so far in Morocco have been focused on parcelling out and phyto-ecological studies of *Tetraclinis* woodlands [11]. No surveys have been carried out on AM fungus colonisation in *T. articulata* woodlands. The abundance, diversity, distribution and functional role of fungal symbionts in these areas are therefore still unknown. The purpose of this study was to determine the mycorrhizal status of *T. articulata*, identify the morphotypes or species of AM fungi that occur in *Tetraclinis* areas, and assess the species abundance and frequency.

2. MATERIALS AND METHODS

2.1. Study sites

This study was conducted in seven *T. articulata* woodlands (Fig. 1). The geographical position and physical and chemical soil characteristics of each site are given in Table I. Medium to highly moderate semiarid conditions prevailed at sites 1, 2, 3, 4 and 5, and the substratum was schistose or siliceous with relatively sandy schist and pelite. Site 6 was an arboretum in a semiarid region with a moderate winter, superficial brown calcareous soil with banded encrusting. Site 7 was in the lower semiarid zone with predominantly sandy or silty soil.

2.2. Vegetation at the studied sites

Habitat information is as important as the taxonomic identity of fungi when selecting isolates for practical use [6]. *Tetraclinis articulata* is present in woodland stands in mixtures with other plant species. At site 1, the most common plant species were: *Pistacia atlantica*, *Rhamnus lycioides*, *Rhus pentaphylla*, *Coronilla viminalis* and *Asparagus altissimus*. At sites 2, 3, 4, and 5, we found: *Olea europea*, *Phillyrea media*, *Prasium majus*, *Arisarum vulgare*, *Cistus monspeliensis*, *Cistus salvifolius*, *Cistus albidus*, *Lavandula multifida*, *Lavandula Stoechas*, and *Asphodelus microcarpus*. At site 6, an old isolated plantation, the most common plants encountered were: *Chamaerops humilis*, *Zizyphus lotus*, *Arisarum vulgare*, *Asphodelus microcarpus* and *Urginea maritima*. At site 7, a young plantation, the main plant species identified were: *Zizyphus lotus*, *Lavandula Stoechas*, *Asphodelus microcarpus*, *Asphodelus tenuifolius*, *Hamada scoparia*, *Scolymus hispania*, *Urginea maritima*, *Carlina involucrata*, *Vicia sativa*, *Asparagus albus*, *Arisarum vulgare* and *Stipa retorta*.

2.3. Sample collection

At each site, we collected approximately 3 to 5 kg of soil around *T. articulata* roots in 10 different places. Soils were taken from the depth of 10 to 70 cm and homogenised to obtain a representative sample for the entire site. A 3 kg sub-sample of homogenised soil was taken to the laboratory for physico-chemical analyses and arbuscular mycorrhizal spore extraction. *T. articulata* fine roots were collected at the same time. The sampling was conducted from October to December in 2002 and 2003.

2.4. Root clearing and staining

One to 5 g of *T. articulata* fine roots were collected and maintained in a glycerol/ethanol/distilled water (GEE) solution [10]. We first screened for the possible presence of ectomycorrhizae under a stereomicroscope. Roots were then cleared in 10% KOH and stained with 0.05% trypan blue in lactophenol [22] to reveal fungal structures. Stained roots were cut into 1 cm fragments and crushed on slides in a drop of polyvinyl alcohol-lacto-glycerol (PVLG: 8.33 g polyvinyl alcohol, 50 mL lactic acid, 5 mL glycerine and 50 mL water) [17]. Five to 10 fragments were mounted on each slide with 10 replications. Each fragment was observed under a microscope (10× and 40× magnification) to estimate the extent of arbuscular mycorrhizal infection as described by Trouvelot et al. (1986) [27]. This procedure involved scoring the proportion of cortex colonized by the endomycorrhizal symbiont as follows: 0: no fungal infection, 1: trace of fungal infection, 2: less than 10% of fungal infection, 3: fungal infection ranging from 11 to 50%, 4: fungal infection ranging from 51 to 90% and 5: fungal infection over 90%. These scores were used to calculate:

– Mycorrhizal frequency (F%), which indicates the extent of fungal colonization: $F = 100(N - n_0)/N$.

N is the total number of observed fragments and n_0 is the number of fragments without mycorrhizae.

– Mycorrhizal intensity (M%): $M = (95n_5 + 70n_4 + 30n_3 + 5n_2 + n_1)/N$.

n_5 , n_4 , n_3 , n_2 and n_1 are, respectively, the number of fragments scored 5, 4, 3, 2 and 1.

2.5. Extraction and counting of AM fungus spores

The Gerdemann and Nicolson (1963) [12] method was used to extract Glomalean spores from the soil. One hundred grams of dry soil was wet sieved on 500 to 50 μm mesh sieves and centrifuged in a water sucrose solution (50% w/v) for 10 min at 1500 rpm. Spores were counted under a stereomicroscope and grouped according to their mor-

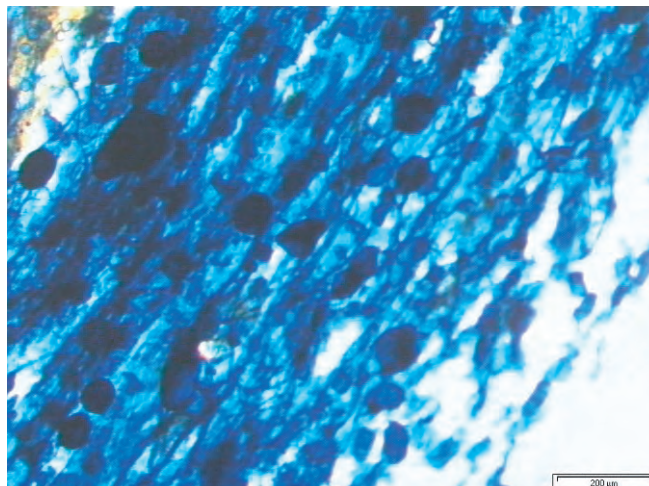


Figure 2. *T. articulata* root colonisation by AM fungi (10× magnification).

phological characteristics. The richness and relative abundance of each fungal type were calculated per 100 g of dry soil.

2.6. Spore identification

Morphological characters: spore size and colour were assessed in water under a stereomicroscope (Olympus SZ H10 research stereomicroscope) and photographed (an average of 20 spores). Spore wall structures and other specific attributes were observed under a microscope (connected to a computer with digital image analysis software) on permanent slides prepared according to Azcon-Aguilar et al. (2003) [3].

Spore identification was mainly based on morphological features, e.g. colour, size, wall structure and hyphal attachment [14, 21]. Morphotypes were classified to the genus level and, when possible, to the species level.

3. RESULTS

3.1. Natural mycorrhizae of *T. articulata*

The cytological organisation of mycorrhizae was the same in all samples. Microscopic observations of stained roots showed that *T. articulata* formed abundant endomycorrhizae (Fig. 2), but no ectomycorrhizae were detected. In some cases, the frequency and intensity of mycorrhizal infection reached 100% and 57%, respectively (Tab. II). Different endomycorrhizal structures were observed, including hyphal coils that seemed to ramify straight along the root cortex (Fig. 3) and oval vesicles were present between the cortex cells. “Paris-type” arbuscules were noted.

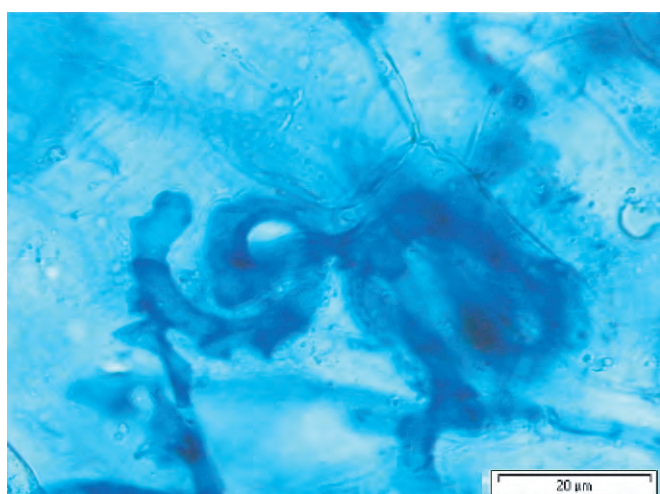
3.2. Diversity of AMF spores

The number of spore morphotypes detected at each site, according to shape, colour and size, ranged from 3 at Oued Cherrat to 6 at Oued Beht and Sidi Jaber (Tab. III). Most of the morphotypes were common to all sites, and few were specific. All spores belonged to the Glomineae order represented by the

Tableau II. Number of spores, mycorrhizal frequency and intensity of *T. articulata* at the studied sites.

Sites	Spore number in 100 g dry soil	F (%)	M (%)
1	>400	100	57
2	135	100	34
3	300	100	34
4	150	93	27
5	>400	100	34
6	210	95	51
7	>400	86	42

F: Mycorrhizal frequency; M: mycorrhizal Intensity.

**Figure 3.** Hyphal coil of AM fungi in root cells of *T. articulata* (100×).

Glomaceae and Acaulosporaceae families. The most representative spore morphotypes of these families were divided in two groups: the first one included small (90 μm) hyaline, white to dark-yellow spores (Fig. 4), and the second corresponded to large (295 μm) light orange (Fig. 5) to dark orange-brown spores (Fig. 6).

A detailed analysis of the morphological characteristics of this spore community revealed the presence of two genera:

- *Glomus*: characterised by a generally multi-layered wall that blended with the wall of subtending hyphae (Fig. 7). Species in this genus were the most abundant, sometimes accounting for up to 80% of all spores counted. Five distinct species were observed: *G. constrictum*, *G. agregatum*, *Glomus* sp. 1, *Glomus* sp. 2 and *Glomus* sp. 3.

- *Acaulospora*: characterised by spores that became sessile after detachment from a sporiferous saccule (Fig. 8). This genus was represented by only one species: *Acaulospora* sp.

3.3. Relative abundance of common AMF species

The number of spores per 100 g of dry soil was above 400 at sites 1, 5 and 7, between 210 and 300 at sites 3 and 6, and

Tableau III. Diversity and abundance of AM fungal spores in *Tetraclinis* woodlands.

Site number	Colour and Reference	Size (μm)	Relative abundance (%)
1	Dark orange (OBr1)	184 ± 30	18.7
	Light white (Obj)	70 ± 5	51.3
	Green yellow (Obv)	67 ± 5	30.0
2	Light Orange (Ocr)	117 ± 18	38.2
	White to yellow (Ocj)	87 ± 13	54.0
	Shrunken spores	–	7.8
3	Dark red (KRr)	180 ± 10	45.3
	Faint yellow (KRj)	78 ± 12	49.1
	Shrunken spores	–	5.6
4	Light to dark orange (MGr)	152 ± 7	31.0
	Faint yellow (MGj)	90 ± 6	62.0
	Shrunken spores	–	7.0
5	Dark orange (BSr1)	236 ± 31	18.9
	Light orange (BSr2)	192 ± 11	14.2
	Yellow (BSj1)	57 ± 15	24.8
	Whitish yellow (BSj2)	39 ± 10	32.0
	Black (BSn)	221 ± 4	10.3
6	Dark yellow (KTj1)	250 ± 17	10.5
	Light yellow (KTj2)	44 ± 2	42.2
	Crimson red (KTj2)	279 ± 45	24.8
	Shrunken spores	–	22.5
7	Yellow (SJj1)	96 ± 7	65.0
	Dark yellow (SJj2)	183 ± 7	12.4
	Dark orange (SJr)	136 ± 2	21.2
	Shrunken spores	–	1.4

Tableau IV. distribution of AMF species at the 7 studied sites.

Species	Sites	1	2	3	4	5	6	7
<i>Glomus constrictum</i>								
<i>Glomus agregatum</i>								
<i>Glomus</i> sp. 1								
<i>Glomus</i> sp. 2								
<i>Glomus</i> sp. 3								
<i>Acaulospora</i> sp.								

between 135 and 150 at sites 2 and 4 (Tab. III). The species distribution within the two genera is presented in Table IV.

4. DISCUSSION

Microscopic analysis of *T. articulata* roots revealed a generally high presence of AM fungi and mycorrhizal colonisation levels in all root samples, reflecting the mycotrophic nature of the tree species. It is known that *T. articulata* is naturally infected by arbuscular mycorrhizal (AM) fungi [8]. Diaz and Honrubia (1993) [9] experimentally found that mycorrhizal infection was clearly visible in 2 month-old *T. articulata* seedlings. Between the 2nd and 7th month, the percentage of infection increased from 20 to 70%. The authors suggested that *T. articulata* could be considered as a mycorrhizae-dependent

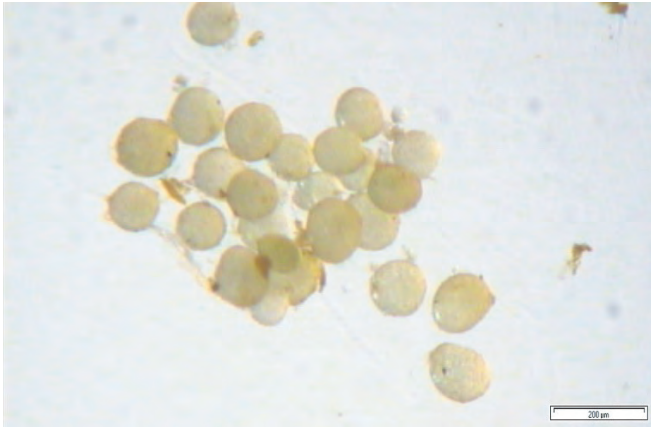


Figure 4. Spores of *Acaulospora* sp. In mixture with *Glomus* sp. mounted in PVLG (40×).

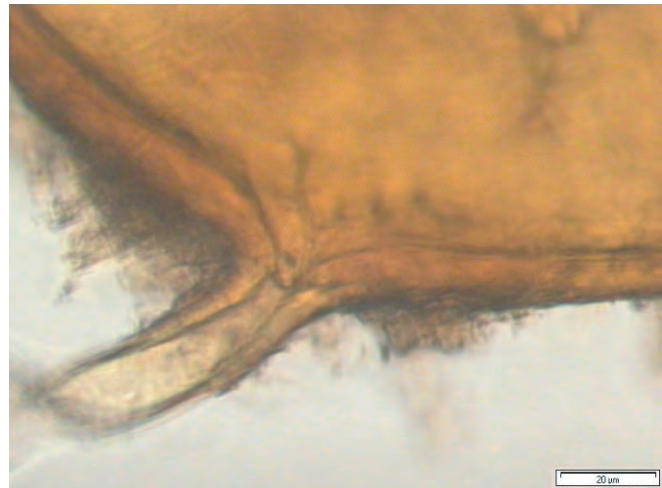


Figure 7. Subterranean hyphae and walls of *Glomus* sp. mounted in PVLG (10×).

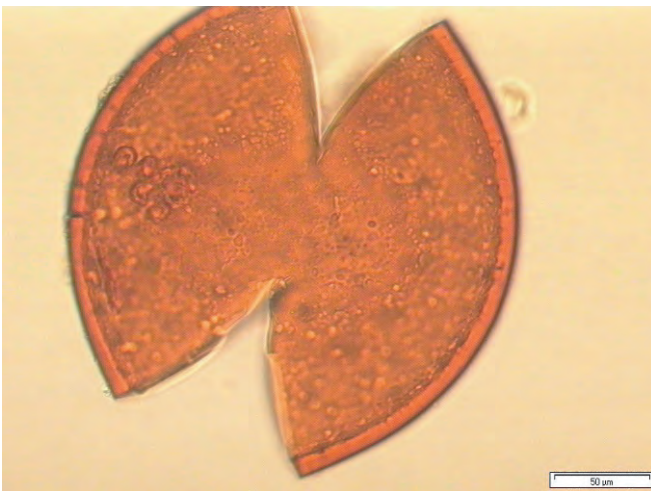


Figure 5. Light orange spore of *Glomus* sp. mounted in PVLG + Melzer (40×).



Figure 8. Spore of *Acaulospora* sp. mounted in PVLG (40×).

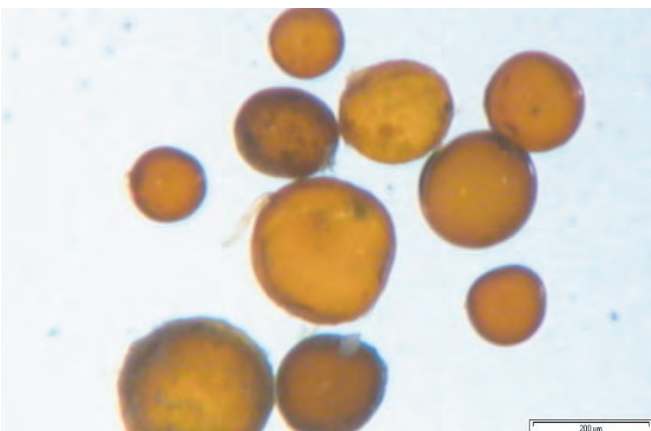


Figure 6. Brown spores of *Glomus* sp. mounted in PVLG (10×).

plant. Our observations reported here are in accordance with this suggestion.

A “Paris-type” mycorrhizal structure was found in all samples investigated. This structure has been reported in previous gymnosperm AM studies [24], including the Cupressaceae family [25], which is characterised by an absence of intercellular hyphae. The fungus develops symplastically, spreads directly from cell to cell within the root cortex, and forms many intracellular hyphal coils from which arbuscules are formed as intercalary structures.

Studying the presence and abundance of mycorrhizal symbionts in *T. articulata* woodlands was an important step in assessing the diversity and richness of the AM fungal community in this area [1]. We thus focused on identifying AM fungi in soils and roots according to the morphological characteristics of the fungi. The number of spores recovered from the soil samples was relatively high, especially at three sites. This is a

characteristic of semiarid soils [23, 26], but in some cases this number is lower, probably due to soil degradation. Indeed, degraded areas often exhibit low densities of indigenous mycorrhizal propagules [23].

In our investigations, the morphological characteristics of the spores only indicated the presence of AM fungi belonging to the Glomineae order, which is represented by two groups of spores belonging to the Glomaceae and Acaulosporaceae families. These observations were confirmed by the frequent presence of vesicles in all samples.

The higher frequency of oval and elongated vesicles compared to irregular and lobed vesicles highlighted the dominance and diversity of *Glomus* species over *Acaulospora* species. This result confirms our previous observations [1]. In forest nurseries in the southeastern part of the Iberian Peninsula, *T. articulata* seedlings are usually mycorrhized with *Sclerocystis sinuosa* Gerdeman and Bakshi, *Glomus diaphanum* Morton and Walker, or *Glomus mosseae* Nicolson and Gerdman [8], but the most effective species observed by these authors was *Glomus fasciculatum* Gerdemann and Trappe. emend. Walker and Koske. This community composition pattern could be due to the type of woodland. Differences in characters could be explained by the presence of fungal ecotypes in soil samples obtained from the areas of study [7, 15], because tree species can also differentially alter fertility and other physical and chemical characteristics of soils, which in turn can affect the AM community structure. The diversity of AM fungi present in the rhizosphere of *T. articulata* (six AM fungal spore morphotypes were consistently detected) indicated degradation of this area and corroborated previous surveys on AM fungus species richness in degraded arid and semiarid environments [3, 26].

In conclusion, our results showed a high relative abundance of spores in some cases, which should be preserved and utilised in such ecosystems by including them in nursery plant production programs. Indeed, mycorrhizal inoculation technologies can partially overcome problems of dieback of *T. articulata* seedlings after transplanting, as observed by Morte and Honrubia (1996) [20], who found that the survival of mycorrhizal *Tetraclinis* plants was superior (60%) to that of control plants (40%). Further investigations are also required to identify *Glomus* sp. and *Acaulospora* sp. at the species level in order to determine the symbiotic performance of AM fungi with *Tetraclinis articulata*.

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