

Maazouzi S *et al.* (2023) **Notulae Scientia Biologicae** Volume 15, Issue 2, Article number 11405 DOI:10.15835/nsb15211405 **Research Article**



Mycorrhizal status of *Argania spinosa* (L.) Skeels in northeastern of Morocco

Soukaina MAAZOUZI^{1*}, Jalila AOUJDAD², Karima SELMAOUI¹, Mohamed CHLIYEH¹, Najoua MOUDEN³, Soukaina MSAIRI⁴, Salwa ELANTRY², Mustapha AZEROUAL⁵, Mohamed OUAJDI², Mohamed KARIMI², Amina OUAZZANI TOUHAMI¹, Allal DOUIRA¹

¹Laboratory of Plant, Animal and Agro-industry Productions, Team of Botany, Biotechnology and Plant Protection, Faculty of Sciences, Ibn Tofail University, Kenitra, Morocco; maazouzisoukaina0@gmail.com (*corresponding author); karima_selmaoui@yahoo.fr; mohamed_ch_bio@yahoo.fr; touhami01@hotmail.com; douiraallal@hotmail.com

²Forest Research Center, BP 763 Agdal Rabat, Ministry of Agriculture, Maritime Fisheries, Rural Development and Water and Forests, Morocco; jaoujdad@gmail.com; crfrabat@gmail.com; ouajdim@gmail.com; karimi.ef@gmail.com

³Laboratory of Molecular Chemistry, Materials and Environment, Multidisciplinary Faculty of Nador, University Mohammed Premier-Oujd, Oujda, Morocco; nadnajoua@gmail.com

⁴Laboratory of Phytobiotechnology, National Agency of Medicinal and Aromatic Plants (ANPMA), BP: 159, Taounate Principale, Taounate, Morocco; soukainamsairi@gmail.com

⁵Service des Études, de L'aménagement et de la Planification, Direction Régionale des Eaux et forêt de l'Orientale-Oujda, Département des Eaux et Forêt, Maroc, Morocco; zeroualmostafa@gmail.com

Abstract

The argan tree, an endemic species in Morocco, has been exhaustively studied in its southwestern range but neglected in its northeastern territory. Thus, the present study sets as objectives the identification of arbuscular mycorrhizal fungi and the evaluation of the argan tree's roots' mycorrhization level in two stations: one in the region of Béni Snassène (Berkane): Jebel Takermine with 7 prospected sites and the other at Jebel Aklim Alkabir with 2 sites. At the first station (Jebel Takermine), the mycorrhizal frequency of Argania spinosa roots varied between 64% and 100% with the root mycorrhizal intensity in the range of 30.77% and 66%. The arbuscular contents are higher at sites 2 (50.46%), 4 (50.33%), 7 (39.44%) and 1 (30.5%) against 18% and 20% at sites 6, 5 and 3. Argan trees from Jbel Aklim Alkbir, exhibited a high mycorrhization frequency and intensity ranging from 88% to 100% and between 39.4% and 73.4% respectively. Regarding arbuscular and vesicular rates, the highest values were associated to the roots of site 1 with 59.3% and 29.4% respectively compared to the lowest rates of 20% and 14% in those of site 2. Spore densities in the rhizosphere of the studied argan trees in the two stations were in the range of 78 and 697 spores/100 g of soil. The identification of isolated mycorrhizal spores revealed the presence of 28 species encompassing 7 genera: Acaulospora, Dentiscutata Claroideoglomus, Funneliformis, Glomus, Rhizophagus, Pacispora, and 5 Families: Glomeraceae (7 species), Acaulosporaceae (10 species), Pacisporaceae (2 species), Claroideoglomeraceae (2 species), Gigasporaceae (1 species).

Received: 05 Dec 2022. Received in revised form: 25 Jun 2023. Accepted: 26 Jun 2023. Published online: 29 Jun 2023. From Volume 13, Issue 1, 2021, Notulae Scientia Biologicae journal uses article numbers in place of the traditional method of continuous pagination through the volume. The journal will continue to appear quarterly, as before, with four annual numbers. *Keywords: Argania spinosa*; Béni Snassène; Jebel Takermine and Jebel Aklim Alkbir; mycorrhizal fungi; North-East of Morocco

Introduction

The argan tree (*Argania spinosa* L. Skeels), exclusively endemic to the dry lowlands of Southwest Morocco, is of particular interest due to its ecological role and its many nutritional and cosmetic uses (Charrouf *et al.*, 2009). It occupies an area of nearly 830,000 ha in southwest Morocco (M'Hirt *et al.*, 1998). Thus, Morocco is one of a few countries in North Africa to have a range of remarkable endemic biodiversity ecosystems Boudy (1950) and El Fasskaoui (2009).

The distribution area of argan trees in the northeastern sector of Morocco is much more complex and remains less studied (Charrouf *et al.*, 2009). Previous works of Emberger (1925), El Alaoui (1999) and Quézel *et al.* (1992) with those of Benabid (1985), Haloui (1991) and Reda Tazi *et al.* (2003) that addressed the study of this stand in the east, claimed that it has not yet been precisely determined. The prospecting investigations carried out by Faouzi *et al.* (2014) in the northeastern argan tree area of Morocco made it possible to highlight a synthetic map of the argan formations' geographical area indicating its current state at the level of the western Beni-Snassen piedmonts and for the first time, its presence at the level of the eastern Rif on the Bou-Areg plain (Bled Arimane and Ouelad Mohand in Kari and Arekmane).

The argan tree grows well in the Souss plain, on the southern slopes of the Western High Atlas and on the northern and southern slopes of the Western Anti-Atlas up to altitudes of 1300 to 1500 m (Msanda, 2005). Beyond this geographical location, two small areas of the argan tree are registered in the upper Grou valley southeast of Rabat and in the northwestern piedmont of the Beni-Snassen, close Oujda (El Mousadik and Petit, 1996). There are only some argan trees scattered in the oriental sector where its plantations do not currently exceed 150 ha (Dommergues and Mangenot, 1970). Therefore, this area's expansion is recommended. The expansion will be necessary to mobilise the local forestry sector, but also to encourage the planting of argan trees on private land. Much work has been undertaken to optimise the plants growth in harsh environments and to control certain soil components likely to contribute to the rehabilitation of these degraded ecosystems (Pearson, 1982; Strutlu, 1991). Among the telluric components in particular involved in biological processes are mycorrhizal fungi. They establish a symbiotic relationship with argan plants to improve the efficiency of water drawn by the roots and succeed in improving the efficiency of water uptake by their roots and subsequently improve their nutrition and growth (Dommergues and Mangenot, 1970; Gianinazzi Pearson, 1982; Strutlu, 1991).

The objective of the present paper is to determine the biodiversity of the argan tree's endomycorrhizal fungi in the region of Beni Snassène (Berkane), where a few of this endemic species' saplings are encountered sparsely. This mycorrhizal biodiversity is seen for the first time in this area.

Materials and Methods

Sampling sites

Soil samples were collected from the rhizosphere of the argan trees located in the Beni Snassène region (Berkane) at two stations spaced 8.7 km apart: Jebel Takermine and Jebel Aklim Alkbir (Figure 1). In station 1, where the argan tree is generally well preserved, sampling was carried out at 7 sites of which four sites (1, 2, 3 and 4) are located on the mountain crest, two on the banks of the river (sites 5 and 6) and the last one (site 7) is located 14 m from up of the river (Oued); the argan trees of site 7, unlike those of the other sites, are stunted by grazing and present a dwarfed appearance. In station 2 where the argan tree is very degraded, sampling was

done at 2 sites. The first one was done from the rhizosphere of argan trees deprived of thorns while the second site is featured by rocky substrate.

Samples are collected at a rate of 1 kg of soil per tree, at a depth of 0 to 20 cm, and a composite soil sample is taken per site. Very fine roots, more likely to be mycorrhized and more easily observable under the microscope are collected at the same time as the soil (Figure 1).

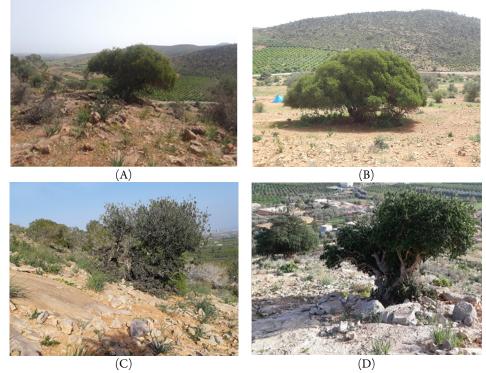


Figure 1. Scattered argan trees located at Jbel Takermine A and B (station 1), and at Jebel Aklim Alkbir C and D (station 2)

Root staining

The root samples taken from the argan trees' rhizosphere (at a depth of 20 cm), were removed from the substrate by washing them thoroughly with running water in a sieve, and only the finest roots were selected.

According to the thinning and staining technique of Philips and Haymann (1970), the roots were cut into segments of 1 cm length, then immersed in a solution of 10% KOH (potassium hydroxide) and placed in a water bath at 90 °C for 15 min. Then, the root segments were bleached by adding a few drops of H_2O_2 to the KOH solution. After 15 min, root fragments were rinsed then stained with cresyl blue, at 90 °C for 15 min. After the final rinse, thirty fragments were randomly selected and mounted, in groups of 10 to 15 segments, in glycerine between slides and coverslips.

Evaluation of mycorrhization rate

The evaluation of mycorrhization parameters was performed by observing thirty fragments of dyed roots of 1 cm length randomly selected (Trouvelot *et al.*, 1986; Amir and Renard, 2003) and mounted, in groups of 10 to 15 segments, in glycerine between slide and coverslip.

The slides were examined under a microscope with each fragment being thoroughly checked over its entire length, at magnifications settings of x 100 and x 400 to observe and to note mycorrhizal frequency and the mycorrhizal structures (arbuscules, hyphae, vesicles, external hyphae, intra and intercellular hyphae and

even the endophytes structures). Vesicular and arbuscular frequencies and the content of endomycorrhizal fungi inside the roots were measured assigning a mycorrhization index ranging from 0 to 5 (Derkowska *et al.*, 2008), 0: absence; 1: traces; 2: less than 10%; 3: 11-50%; 4: 51-90%; 5: more than 91%.

Frequency of mycorrhization (F) reflects the colonisation percentage of the root system:

 $F\% = 100 \times (N - n0)/N.$

Where:

N = total number of root fragments.

n0 = number of non-mycorrhizal root fragments.

Intensity of mycorrhization (IM) estimates the proportion of colonised cortex in the root system:

MI = (95n5 + 70n4 + 30n3 + 5n2 + n1)/N.

Where:

n = number of fragments with the index 0, 1 2, 3, 4, or 5 of colonisation

(According to the scale developed by Derkowska *et al.* (2008) as follows: n1 = trace; n2 = less than 10%;

n3 = 11 to 50 %; n4 = 51 to 90%; and n5 = more than 90%).

N = total number of root fragments.

Arbuscular content (A%) estimates the proportion of the root cortex containing arbuscules:

A = (100 mA3 + 50 mA2 + 10 mA1)/100.

mA = (95 n5A + 70 n4A + 30 n3A + 5 n2A + n1A)/N.

Where (using the n and N numbers determined above for MI)

A = abundance of arbuscules (A3: 51 to 100 %; A2: 11 to 50 %; A1: 1 to 10%).

nA denotes the number of root fragments for a given n and A (e. g., n4A3 is the number of fragments denoted 4 with A3).

Vesicle content (V %) estimates the proportion of the root cortex containing vesicles:

V = (100 mV3 + 50 mV2 + 10 mV1)/100.

mV = (95 n5V + 70 n4V + 30 n3V + 5 n2V + n1V)/N.

Where (using the n and N numbers determined above for MI)

V = abundance of vesicles (V3: 51 to 100 %; V2: 11 to 50 %; V1: 1 to 10 %).

nV denotes the number of root fragments for a given n and V (e.g., n4V3 is the number of fragments denoted 4 with V3).

Spores' extraction

The spores were extracted by the wet sieving method described by Gerdemann and Nicolson (1963). In a beaker of 1L, 100 g of each soil sample was submerged in 0.5 L of tap water and it was stirred with a spatula for 1 minute. After 10 to 30 seconds of decantation, the supernatant was passed through four superposed sieves with a decreasing mesh size (500, 200, 80, and 50 microns). This operation was repeated twice. The content picked up by each mesh screen (200, 80 and 50 microns respectively) was transferred to centrifuge tubes and centrifuged at 2000 RPM for 5 min. The supernatant was discarded and a viscosity gradient was created by adding 4 ml of a solution of 40% sucrose in each centrifuge tube. The mixture was quickly stirred and the tube was handed back into the centrifuge for 1 min at 2000 RPM for 1 min, then a third centrifugation at 3000 RPM for 1 min was made. Unlike the first centrifugation process, the supernatant was poured into the sieve mesh of 50 microns; the substrate was rinsed with distilled water to remove the sucrose, and then disinfected with an antibiotic solution (streptomycin). The spores were then recovered with distilled water in an Erlenmeyer flask.

After extraction, the endomycorrhizal spores are counted and identified based on morphological characteristics. An estimation of the number of spores in the soil is made by counting the spores in 100 g of soil and extrapolating to the total volume (100 ml).

Richness and appearance frequency

Species richness is the total number of the observed species per site and the occurrence frequency of species corresponds to the percentage of sites where each species is detected.

Statistical analysis

The statistical treatment of results focused on the analysis of variance to a single criterion of classification (ANOVA).

Results and Discussion

The results of the present study showed that the argan trees examined in the different forests were colonised by AMF. However, the AMF colonisation status varied significantly depending on sampling point. Roots collected at the level of the rhizospheric soil of argan trees in two studied stations revealed the presence of different mycorrhizal structures (hyphae, vesicles and arbuscules) and endophytes (Figure 2).

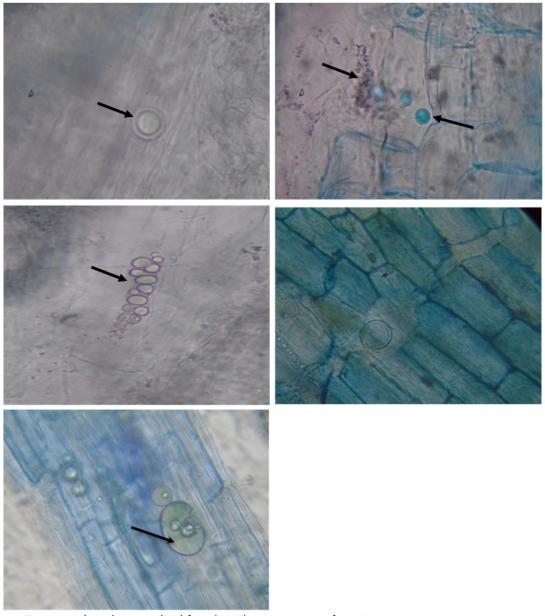


Figure 2. Arbuscular mycorrhizal fungi (AMF) growing in roots from *Argania spinosa* AMF within *Argania spinosa* roots produce arbuscules (a), numerous small round structures called vesicles (v), spore (s) and endophytes (en) (G. ×400).

The interaction extent of roots was marked by mycorrhizal frequencies and intensity in the range of 64% - 100% and 31%-66% respectively in the prospected sites of the first station (Figure 3).

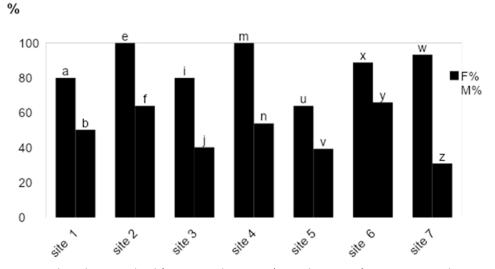


Figure 3. Arbuscular mycorrhizal fungi root colonisation (F%: colonisation frequency; M%: colonisation intensity) of *Argania spinosa* across different sites within Jebel Takrmine Different letters on top of bars indicate significant differences according to Turkey test (p < .05)

As for arbuscular contents, they were higher at sites 2, 4, 7 and 1 with 50.46%, 50.33%, 39.44% and 30.5% respectively, while they were between 18% and 20% at sites 6, 5 and 3. Similarly, the vesicular contents varied from one site to another, with values ranging from 9.33% to 50.33% (Figure 4).

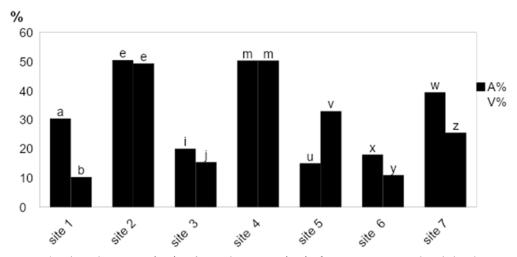


Figure 4. Arbuscular content (A%) and vesicular content (V%) of Argan tree roots within JbelTarkrmine Different letters on top of the bars indicate significant differences according to Turkey test (p < .05)

In the second station, the infection degree expressed as mycorrhization frequency (or the roots colonisation level) matching to mycorrhizal intensity were high, varying between 100%- 88% and from 73.4% to 39.4% respectively (Figure 5).

The arbuscular and vesicular contents varied with the highest values of 59.3% and 29.4% were recorded respectively in site 1 while the lowest contents in the order of 20% (arbuscules) et 14% (vesicles) were registered in site 2 (Figure 5).

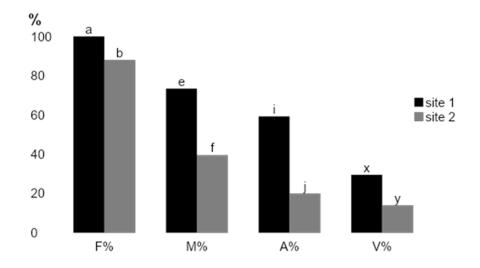
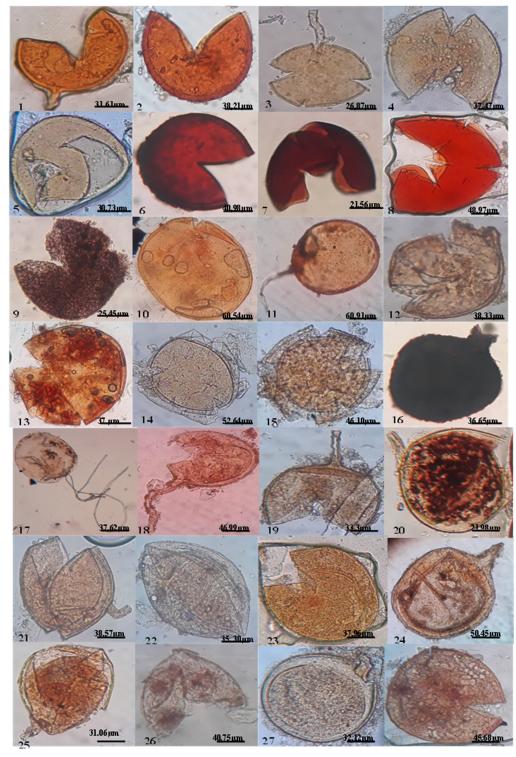


Figure 5. Frequency (F %), Intensity of mycorrhization (M %), arbuscular content (A %) and vesicular contents (V %) of roots of argan tree in Jebel Aklim Alkbir

Likewise, Sellal *et al.* (2016), have detected a community of arbuscular mycorrhizal fungi in relation to argan trees sampled in two sites located in southern area where the frequency and intensity of mycorrhization of Argan trees reached 100% in the Toufalazt and Taroudant sites, nearly 97% in the sites of Elkhssass and Essaouira. Hence, the presence of diverse endomycorrhizal structures indicated that AMF had established effective symbiotic relationships with this tree species allowing us to classify the argan tree as a mycotrophic species.

Regardless of argan tree variety (thorny or spineless), a seasonal variation of arbuscular and vesicular rates during winter and spring seasons was reported by El Adib (2015). Indeed, many studies have shown that mycorrhizal symbioses with vesicles and arbuscules improve the growth of young plants (Nouaim and Chaussod, 1996; Giri *et al.*, 2003; Citernesi *et al.*, 1998; Atkinson *et al.*, 2002) and induce morphological and physiological transformations that allow them to tolerate their environment's conditions (Porcel *et al.*, 2006). Nicolson (1960) found that the intensity of colonisation is related to variation in soil organic matter content. According to Wang *et al.* (2019), AMF colonisation intensity in forest ecosystems was significantly and negatively correlated with organic matter content. AMF hyphae improve the water and mineral nutrition of the host plant by increasing the volume of soil prospected, and by mobilising phosphorus from complex soil compounds (Munns *et al.*, 1981; Malaisse, 1979). The species richness of the argan trees' AMF differed widely among stations. The mean number of AMF spores ranged between 78 to 697 spores/100 g of soil.

A total of 17 AMF species were identified in the rhizosphere soils of argan trees within the first station (Funneliformis geosporum, Endogone versiformis, Acaulospora scrobiculata, Glomus macrocarpum, Acaulospora tuberculata, Acaulosporacolombiana, Acaulospora denticulata, Glomus aureum, Acaulospora sp4, Pacispora sp1, Entrophospora infrequens, Acaulospora morrowiae, Dentiscutata nigra, Rhizophagus intraradices, Funneliformis mosseae, Claroideoglomus claroideum, Pacispora franciscana) whereas those of the second station were in the number of 16 species (Claroideoglomus andunicatum, Glomus macrocarpum, Acaulospora tuberculata, Acaulospora colombiana, Acaulospora denticulata, Acaulospora rehmii, Acaulospora morrowiae, Dentiscutata nigra, Acaulospora sp1, Rhizophagus intraradices, Acaulospora capsicula, Funneliformis mosseae, Claroideoglomus claroideum, Pacispora franciscana, Glomus sp, Acaulospora capsiculata) (Figure 6, Table 1). According to the classification of Oehl and Sieverding (2011), these AMF belonged to 7 genera Claroideoglomus, Funneliformis, Glomus, Rhizophagus, Pacispora, Acaulospora, Dentiscutataand five



families: Glomeraceae (7 species), Acaulosporaceae (10 species), Pacisporaceae (2 species), Claroideoglomeraceae (2 species), Gigasporaceae (1 species).

Figure 6. Fungal species of endomycorrhizae isolated from the rhizosphere of argan tree studied in Jebel Takermine and Jbel Aklim Alkbir

| Number | Name | Shape | Color | Spore surface's | Average size of spores (µm) |
|--------|----------------------------|-----------|-----------------------|--------------------|-----------------------------------|
| 1 | Claroideoglomus etunicatum | Globular | Orange | Granular | 99.9 |
| 2 | Funneliformis geosporum | Globular | Orange-brown | Granular | 116.55 |
| 3 | Endogone versiformis | Globular | Yellow | smooth | 76.59 |
| 4 | Endogone versiformis | Globular | | Granular | 116.55 |
| 5 | Acaulospora scrobiculata | Globular | Pale yellow | Granular | 99.9 |
| 6 | Glomus macrocarpum | Globular | Orange -brown | Granular | 133.2 |
| 7 | Acaulospora tuberculata | Globular | Brown red darkened | smooth | 66.6 |
| 8 | Acaulospora colombiana | Globular | Orange | smooth | 149.85 |
| 9 | Acaulospora denticulata | Globular | Brown | Granular | 83.25 |
| 10 | Acaulospora rehmii | Oval | Orange brown | Granular | 199.8 |
| 11 | Glomus aureum | Globular | Yellow brown | Granular | 149.85 |
| 12 | Acaulospora sp4 | Oval | brown | Granular | 116.55 |
| 13 | Pacispora sp1 | Oval | Orange- brown | Granular | 116.55 |
| 14 | Entrophospora infrequens | Oval | Orange clear | Granular | 133.2 |
| 15 | Acaulospora morrowiae | Globular | Yellow- brown | Granular | 149.85 |
| 16 | Dentiscuta tanigra | Globular | Black | smooth | 116.55 |
| 17 | Acaulospora sp 1 | Globular | Brown | Granular | 66.6 |
| 18 | Rhizophagus intraradices | Globular | Yellow-brown | Granular | 116.55 |
| 19 | Rhizophagus intraradices | Globular | Yellow-brown | Granular | 99.9 |
| 20 | Acaulospora capsicula | Globular | Yellow-brown | Granular | 66.6 |
| 21 | Rhizophagus intraradices | Ellipsoid | Yellow-brown | Granular | 99.9 |
| 22 | Funneliformis mosseae | Ellipsoid | Yellow brown | Granular | 99.9 |
| 23 | Funneliformis mosseae | Globular | Orange-brown | Granular | 116,55 |
| 24 | Rhizophagus intraradices | Globular | Orange-brown | Granular | 149,85 |
| 25 | Claroideoglomus claroideum | Ellipsoid | Orange-brown | Granular | 83,25 |
| 26 | Pacispora franciscana | Oval | Yellow-white | Granular | 116.55 |
| 27 | <i>Glomus</i> sp | Globular | Yellow-white | Granular | 99.9 |
| 28 | Acaulospora scrobiculata, | Globular | Brown | Granular | 149.85 |

Table 1. Identification of mycorrhizal fungi isolated from the argan tree rhizosphere in the differentstudied stations

Interestingly, based on the results related to AMF community composition of argan forests in the south's areas (Sellal *et al.*, 2017) share a number of species to that highlighted here viz, *Claroideoglomus etunicatum*, *Endogone versiformis, Glomus macrocarpum, Acaulospora denticulata, Acaulospora rehmii, Glomus aureum*,

Entrophospora infrequens, Dentiscutata nigra, Acaulospora sp1, Rhizophagus intraradices, Funneliformis mosseae, Glomus sp.

At the same time, our assessment of the species' richness revealed some differences amongst the studied sites and stations. This variation has also marked the investigated sites of southern Morocco (Sellal *et al.*, 2017; 2021). A similar trend was observed for the number of spores in a 100 g soil; thereby, the highest spore density (585 spores/100 g soil) was related to site 4 of the first station followed by 518 spores/100 g soil, 204 spores/100 g soil, 201 spores/100 g soil, 154 spores/100 g soil, 101 spores/100 g soil, and 78 spores/100 g soil, detected respectively in sites 2, 5,7, 1,6 and 3. *Dentiscutata nigra* and *Endogone versiformis* were the most common species with the respective frequency of occurrence of 32.13% and 15.77%. In comparison, spores' number/ 100 g of soil was of 697 spores/100 g soil recorded in site 1 of the second station greater than that of site 2 in the order of 169 spores/100 g soil with a dominance of the genus *Dentiscutata nigra* (32.13%) and *Acaulospora denticulata* (14.77%) (Figure 7).

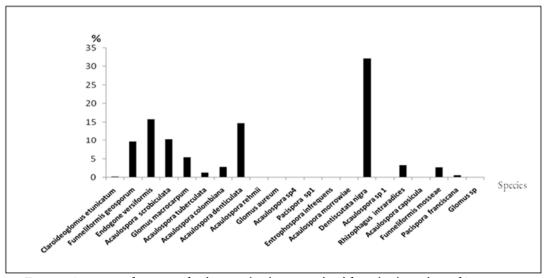


Figure 7. Appearance frequency of endomycorrhizal species isolated from the rhizosphere of Argan trees

These two species were more prevalent than *Glomus etunicatum* (16.26%), *Acaulospora gedanensis* (10.52%) or *Glomus macrocarpum* and much more represented in the AMF community structure in southern area (Sellal *et al.*, 2017). Regarding our findings about the number of spores extracted from the soil of argan trees, they seem to be lower than those cited by El Maati *et al.* (2015) who found 1127.66 spores/100 g soil and Nouaim *et al.* (1991) work in the subsoils of *Argania spinosa* in south-west Morocco reporting a number of 900-2080 spores / 100 g soil. Weak densities in the range of 84 and 160 spores /100 g of soil related to the carob rhizosphere, were also reported in research results of El Asri *et al.* (2014) in five provinces covering east to south-west Morocco (Taroudant, Khenifra, Azilal, Beni Mellal and Nador).

Moreover, it is known that the same fungus can colonise many plant species. Conversely, a plant can be colonised by several species of AM fungi, sometimes at the same time (Steinkellner *et al.*, 2007).

The relative low diversity and species richness is probably linked to the state of argan tree stands. According to Faouzi *et al.* (2014). The distribution of *Argania spinosa* at the piedmont of the western Beni-Snassen in the eastern Rif is presented as a matorral degraded by zone.

In fact, mycorrhizal associations may result in profound modifications of root structure and functioning for efficient acquisition and use of water by plants (Harley and Smith, 1983). Multiple factors influence the dynamics and structure of the AM fungal community such as soil texture, particle distribution, size, porosity,

water retention capacity, cation exchange capacity, organic matter content, pH, macronutrients and micronutrients (Mohammad *et al.*, 2003; Chaudhary *et al.*, 2008). Additionally, the number of AMF propagules estimates is linked to experimental conditions as temperature and harvesting time, which may affect root and propagule growth (Wilson and Trinick, 1982). In this context, Mohammad *et al.* (2003) claimed that spore numbers are negatively correlated with soil phosphorus levels. Indeed, the good functioning of mycorrhization is noted under conditions of P-limitation especially. It was also demonstrated that the host plant presents greater mycorrhizal potential and soil infectivity where there is a deficiency of soil nutrients (Meddich *et al.*, 2017).

Many plant species associated with endomycorrhizae exhibited a high tolerance to drought stress such as acacias (Maazouzi *et al.*, 2020; Diem *et al.*, 1981), Berberian thuja (Díaz and Honrubia 1993; El Khaddari *et al.*, 2020), Argan (Nouaïme *et al.*, 1991), Oleaster, Carob, Date Palm. This is due to the relevant role of vesicular and arbuscular endomycorrhizal allocation the plant to acquire mineral elements, especially those that are poorly mobile in the soil, such as phosphorus, copper and zinc (Strullu, 1991; Harrison, 1999). It is worth noting that the availability of water is the main environmental factor that limits forest production. Moreover, soil phosphorus availability is a limiting factor in the establishment of mycorrhizal symbiosis (Chen *et al.*, 2007). The first mechanism by which symbiosis promotes the water regulation of trees is through its effect on mineral nutrition. If a fungus is particularly effective in phosphorus supply or potassium, it will indirectly contribute to the plant to better manage water (Bondonga *et al.*, 2011). Nevertheless, (Wang *et al.*, 1993) have also demonstrated the effect of pH level on the development of endomycorrhizae (Wang *et al.*, 1993). An acidic pH level can limit the endomycorrhizal colonisation in the roots and may even inhibit it completely if it becomes too acidic (<5) (Abbott and Robson, 1985).

Conclusions

The argan tree's rhizosphere in northeastern Morocco has exhibited a significant specific richness of mycorrhizal fungi representing 28 taxa. The mycorrhizal community develops variable spores' densities across the site considered which remain relatively lesser in respect to those of argan trees in the southwest of the Kingdom.

We suspect that this difference is due to the irrational exploitation of unevenly distributed Argania *spinosa* in these two regions and abiotic factors such topography, soil properties which may influence the AMF community structure.

Authors' Contributions

SM methodology and writing—original draft; JA, KS, MC, NM interpretation and validation; SM review and editing; S E, MA, MO, MK investigation and sampling; AOT supervision and AD Conceptualization and design of experiments. All authors read and approved the final manuscript

Ethical approval (for researches involving animals or humans)

Not applicable.

Acknowledgements

This research received no specific grant from any funding agency in the public, commercial, or not-forprofit sectors.

Conflict of Interests

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

References

- Abbott LK, Robson AD (1985). The effect of soil pH on the formation of VA mycorrhizas by two species of *Glomus*. Soil Research 23(2):253-261. *https://doi.org/10.1071/SR9850253*
- Amir H, Renard A (2003). Etude microbiologique générale de quelques sols de forêts sclérophylles de Nouvelle-Calédonies: Statuts des mycorhizes à arbuscules [General microbiological study of some sclerophyllous forest soils in New Caledonia: Status of arbuscular mycorrhizae]. International Journal of Pure & Applied Bioscience 22.
- Atkinson D, Baddeley JA, Goicoechea N, Green J, Sanchez-Diaz M and Watson CA (2002). Arbuscular mycorrhizal fungi in low input agriculture. In: Gianinazzi S (Ed). Mycorrhizal Technology in Agriculture 211-222. https://doi.org/10.1007/978-3-0348-8117-3_17
- Benabid A (1985). Les écosystèmes forestiers préforestiers et presteppiques: du Maroc diversité, répartition biogéographique et problèmes posés par leur aménagement. Département botaniqueécologie forestières [The preforest and pre-steppe forest ecosystems of Morocco: diversity, biogeographical distribution and problems posed by their management]. Forêt Méditerranéenne 7(1):53-64.
- Bondonga MH, Baboy L, Louis KJ (2011). Quantification de la symbiose mycorhizienne des essences de la forêt claire (miomboV) du Katanga: application au reboisement. Cas de *Pteocarpus angolensis, P. tinctoruis, Uapaka kirkiana* et *U. pilosa* [Quantification of mycorrhizal symbiosis of woodland species (miomboV) in Katanga: application to reforestation. Case of *Pteocarpus angolensis, P. tinctoruis, Uapaka kirkiana* and *U. pilosa*]. Engineering dissertation, Lubumbashi RDC University.
- Boudy P (1950). Monographie et traitement de l'arganier [Monograph and treatment of the argan tree]. Paris, France, Éd. Larose, tome II, fascicule I 382-416. *https://www.congresarganier.com/pdf/Cia2011/actes-du-1er-congres-d-arganier-2011.pdf*
- Chaudhary M, Lau N, Johnson N (2008). Macroecology of microbes-biogeography of the Glomeromycota. Varma A (Ed). Mycorrhiza 529-561. *https://doi.org/10.1007/978-3-540-78826-3_26*
- Chen BD, Zhu YG, Duan J, Xiao XY, Smith SE (2007). Effects of the arbuscular mycorrhizal fungus *Glomus mosseae* on growth and metal uptake by four plant species in copper mine tailings. Environmental Pollution 147:374-380. https://doi.org/10.1016/j.envpol.2006.04.027
- Citernesi AS, Vitagliano C, Giovannetti M (1998). Plant growth root system morphology of *Olea europaea* L. Rooted cuttings as influenced by arbuscular mycorrhizas. Journal of Horticultural Science and Biotechnology 73(5):647-654. https://doi.org/10.1080/14620316.1998.11511028
- Díaz G, Honrubia M (1993). Arbuscular mycorrhizae on *Tetraclinis articulata* (Cupressaceae): development of mycorrhizal colonization and effect of fertilization and inoculation. Agronomie 13:267-274. https://doi.org/10.1051/agro:19930403
- Diem HG, Gueye l, Gianinazzi-Pearson V, Fortin JA, Dommergues YR (1981). Ecology of VA mycorrhizae in the tropics: Ecology of VA mycorrhizae in the tropics: The semi-arid zone of Senegal. Acta Oecologica 53-62.
- Dommergues Y, Mangenot F (1970). Ecologie microbienne du sol [Microbial soil ecology]. Masson (Paris), pp 796.
- El Adib S, Slim S, BenJeddi F (2015). Etude de la dynamique de la colonisation mycorhizienne de deux variétés d'arganier en Tunisie [Study of the dynamics of mycorrhizal colonization of two varieties of argan tree in Tunisia]. Journal of New Sciences, Agriculture and Biotechnologies 17(3):603-614.

- El Alaoui N (1999). Paysages, usages et voyages d'*Argania spinosa* (L.) Skeels (IXe-Xe siècles) [Landscapes, uses and travels of *Argania spinosa* (L.) Skeels (IX-X centuries)]. Journal d'Agriculture Traditionnelle et de Botanique Appliquée 41(2):45-79. *https://doi.org/10.3406/jatba.1999.3711*
- El Asri A, Talbi Z, Chliyhe M, Sghir F, Touati J, Ouazzani Touhami A, Benkirane R, Douira A (2014). Arbuscular mycorrhizal fungi associated with rhizosphere of carob tree (*Ceratonia siliqua* L.) in Morocco. International Journal of Pure and Applies Bioscience 2(3):286-297.
- El Fasskaoui B (2009). Fonctions, défis et enjeux de la gestion et du développement durables dans la Réserve de Biosphère de l'Arganeraie (Maroc) [Functions, challenges and issues of sustainable management and development in the Arganeraie Biosphere Reserve (Morocco)]. Études Caribéennes 12. https://doi.org/10.4000/etudescaribeennes.3711
- El Khaddari A, El Gabardi S, OuazzaniTouhami A, Aoujdad J, Ouajdi M, El Antry S, Douira A, Dahmani J (2019). Diversity of endomycorrhizal fungi in the thuya rhizosphere, Sefrou region (Middle Eastern Atlas, Morocco). Plant Cell Biotechnology and Molecular Biology 20(23-24):1143-1159.
- El Maati Y, Msanda F, El Mousadik A, El Hamdaoui A, El Mrabet S, Ouahmane L (2015). Contribution to the characterization of mycorrhizae in the south west of Morocco and their effect on growth parameters of Argania spinosa. The American Journal of Innovative Research and Applied Sciences 1(7):235-243. https://doi.org/10.19182/bft2017.334.a31490.
- El Mousadik A, Petit R (1996). High level of genetic differentiation for allelic richness among populations of the argan tree [*Argania spinosa* (L.) Skeels] endemic to Morocco. Theoretical and Applied Genetics 92:832-839. https://doi.org/10.1007/BF00221895
- Emberger L (1925). Les limites naturelles climatiques de l'arganier [The natural climatic limits of the argan tree]. Bulletin de la Société Botanique de France 5(3):84-97. *https://doi.org/10.1080/00378941.1925.10832788*
- Gerdemann, JW, Nicolson TH (1963). Spores d'espèces mycorhiziennes d'Endogone extraites du sol par tamisage humide et décantation [Spores of Endogone mycorrhizal species extracted from soil by wet sieving and decantation]. Transactions of the British Mycological Society 46:235-244. http://dx.doi.org/10.1016/S0007-1536(63)80079-0
- Gianinazzi-Pearson V (1982). Importance des mycorhizes dans la nutrition et la physiologie des plantes. In: Colloque: Les Mycorhizes: Biologie et Utilisation (No. 13). INRA Editions. [Importance of mycorrhizae in plant nutrition and physiology. In Symposium: Mycorrhizae: Biology and Use (No. 13). INRA Editions].
- Giri B, Kapoor R, Mukerji KG (2003). Influence of arbuscular mycorrhizal fungi and salinity on growth, biomass and mineral nutrition of *Acacia auriculiformis*. Biology and Fertility of Soils 38:176-180. https://doi.org/10.1007/s00374-003-0636-z.
- Haloui B (1991). La végétation du Maroc oriental. Phytoécologie phytomasse minéralomasse et productivité des principaux écosystèmes forestiers. Thèse de Doctorat, Université Mohammed 1er Oujda, pp 180. [The vegetation of eastern Morocco. Phytoecology Phytomass Mineralomass and productivity of the main forest ecosystems. Doctoral thesis, Mohammed 1st Oujda University, 180].
- Harley JL, Smith SE (1983). Mycorrhizal symbiosis. Academic Press London, pp 483.
- Harrison MJ (1999). Biotrophic interfaces and nutrient transport in plant/ fungal symbioses. Journal of Experimental Botany 50:1013-1022.
- Kormanik PP, McGraw AC (1982). Quantification of vesicular-arbuscular mycorrhizae in plant roots. In: Schenk NC (Ed). Methods and Principles of Mycorrhizal Research. APS Press, Minneapolis.
- M'hirit O, Benzyane M, Benchekroun F, Elyousfi SM, Bendaanoun M (1998). L'arganier: une espèce fruitière-forestière à usage s multiples [The argan tree: a multipurpose fruit-forest species.]. Sprimont, Belgique, éditons Mardaga, pp 151.
- Maazouzi S, Aoujdad J, Selmaoui K, Gabardi S, Artib M, Elantry S, ... Et Douira A (2020). Évaluation Du Statut Mycorrhizal Des Acacias Dans Le Rhamna-Sidi Bouathman Et Les Régions Haha Au Maroc. [Evaluation of the Mycorrhizal Status of Acacias in the Rhamna-Sidi Bouathman and Haha Regions of Morocco]. Biotechnologie Des Cellules Végétales Et Biologie Moléculaire 21 (1-2):1-18.
- Malaisse F (1979). Contribution à l'étude de l'écosystème forêt claire (Miombo) [Contribution to the study of the clear forest ecosystem (Miombo)]. Note 8. Le projet Miombo. Annales Université Abidjan, Ecologie 6:227-250. https://doi.org/10.2307/3601202
- Meddich A, Ait El Mokhtar M, Wahbi S, Boumezzough A (2017). Évaluation des potentialités mycorhizogènes en lien avec les paramètres physico-chimiques des sols de palmeraies du Maroc (Marrakech et Tafilalet). [Evaluation of

mycorrhizal potential in relation to the physico-chemical parameters of the soils of palm groves in Morocco (Marrakech and Tafilalet)]. Cahiers Agricultures 26(4):45012. *https://doi.org/10.1051/cagri/2017044*.

- Msanda F, El Aboudi A, Peltier JP (2005). Biodiversité et biogéographie de l'arganeraie marocaine [Biodiversity and biogeography of the Moroccan argan plantation]. Cahiers Agricultures 14:357-364.
- Munns DN, Hohenber JS, Richetti TL, Lauter DJ (1981). Soil acidity tolerance of symbiotic and nitrogen fertilized soybeans. Agronomy Journal 73(3):407-410. https://doi.org/10.2134/agronj1981.00021962007300030006x
- Nicolson TH (1960). Mycorrhiza in the Gramineae: III. *Glomus fasciculatus* as the endophyte of pioneer grasses in a maritime sand dune. Transactions of the British Mycological Society 72(2):261-268. https://doi.org/10.1016/S0007-1536(79)80041-8
- Nouaim R, Chaussod R (1996). Rôle des mycorhizes dans l'alimentation hydrique et minérale des plantes, notamment des ligneux de zones arides; La mycorhization dès les plantes forestières milieu aride et semi-aride et la lutte contre la désertification dans le bassin méditerranéen, Saragosse, CIHEAM [Role of mycorrhizae in the water and mineral supply of plants, in particular ligneous plants in arid zones; Mycorrhization of forest plants in arid and semi-arid environments and the fight against desertification in the Mediterranean basin, Zaragoza, CIHEAM]. Cahiers Options Méditerranéennes 20:9-26.
- Nouaïm R, Chaussod R, El Aboudi A, Schnabel C et Peltier JP (1991). L'Arganier, essai de synthèse des connaissances sur cet arbre. Physiologie des arbres et arbustes en zones arides et semi-aride [The Argan tree, essay summarizing knowledge about this tree. Physiology of trees and shrubs in arid and semi-arid zones]. Groupe Paris pp 373-388.
- Oehl F, Sieverding E, Palenzuela J, Ineichen K (2011). Advances in Glomeromycota taxonomy and classification. IMA Fungus 2(2):191-199. *https://doi.org/10.5598/imafungus.2011.02.02.10*
- Philips JM, Hayhomme DS (1970). Amélioré procédures pour clairière roots et coloration parasite et champignons mycorhiziens arbusculaires vésiculaires pour une évaluation rapide de l'infection. [Improve procedures for clearing roots and staining parasite and vesicular arbuscular mycorrhizal fungi for rapid evaluation of infection]. Transactions of the British Mycological Society 55:158-161. http://dx.doi.org/10.1016/S0007-1536(70)80110-3
- Porcel R, Aroca R, Azcón R, Ruiz-Lozano JM, (2006). PIP aquaporin expression génique dans la glycine mycorhizienne arbusculaire. [PIP aquaporin gene expression in arbuscular mycorrhizal glycine]. Molecular Biology 60:389-404. https://doi.org/10.1007/s11103-005-4210-y
- Quezel, P, Barbero M, Benabid A, Rivas-Martínez S (1992). Contribution a l'étude des groupements forestiers et pré forestiers du Maroc oriental. [Contribution to the study of forest and pre-forest groupings in eastern Morocco]. http://hdl.handle.net/10366/73770
- Reda Tazi M, Berrichi A, Benyounes Haloui B (2013). Esquisse cartographique de l'aire de l'arganier *Argania spinosa* (L.) écharpes au Maroc nord-oriental. Bulletin de l'Institut Scientifique, Rabat, Section Sciences de la Vie 25:53-55.
- Reda Tazi M, Berrichi A, Haloui B (2003). Effet du polyéthylène glycol sur la germination et la croissance *in vitro* de l'arganier (*Argania spinosa* L. Skeels) des Beni-Snassen (Maroc oriental). [Effect of polyethylene glycol on the in vitro germination and growth of the argan tree (*Argania spinosa* L. Skeels) from Beni-Snassen (eastern Morocco)]. Science et Changements planétaires/Sécheresse 14(1):23-27.
- Sellal Z, Ouazzani Touhami A, Chliyeh M, Dahmani J, Benkirane R, Douira A (2016). Arbuscular mycorrhizal fungi species associated with rhizosphere of *Argania spinosa (L.)* Skeels in Morocco. International Journal of Pure and Applied Bioscience 4(1):82-99. http://dx.doi.org/10.18782/2320-7051.2201.
- Sellal Z, Ouazzani touhami A, Mouden N, Ouarraqi M, Selmaoui K, Dahmani J, Benkirane R, El Modafar, Douira A (2017). Effect of an endomycorrhizal inoculum on the growth of arganier. International Journal of Environment, Agriculture and Biotechnology 2. https://doi.org/10.22161 / ijeab / 2.2.47
- Steinkellner S, Mammarler R, Vierheilig H (2005). Germination des microconidies du pathogène de la tomate Fusarium oxysporum en présence d'exsudats racinaires. [Germination of microconidia of the tomato pathogen Fusarium oxysporum in the presence of root exudates]. Journal of Plants Interaction 23-30. https://doi.org/10.1080/17429140500134334.
- Strullu DG, Plenchette C (1991). Les mycorhizes en horticulture. [Mycorrhizae in horticulture]. PHM Revue Horticole 352:50-55.
- Trouvelot A, Kough JL et Gianinazzi V (1986). Mesure de taux de mycorhisation VA d'un système radiculaire. Recherché de méthodes d'estimation uneyant une signification fonctionnelle. Dans physiologique et génétique aspects de

mycorhize. [Measurement of VA mycorrhization rate of a root system. Search for estimation methods with functional significance. In physiological and genetic aspects of mycorrhiza]. INRA, Paris, pp 217-221.

- Wang GM, Stribley DP, Tinker PB, Walker C (1993). Effects of pH on arbuscular mycorrhiza I. Field observations on the long-term liming experiments at Rothamsted and Woburn. New Phytologist 124:465-472. https://doi.org/10.1111/j.1469-8137.1993.tb03837.x
- Wilson JM, Trinick MJ (1983). Factors affecting the estimation of numbers of infective propagules of vesicular arbuscular mycorrhizal fungi by the most probable number method. Soil Research 21:73-81. https://doi.org/10.1071/sr9830073



The journal offers free, immediate, and unrestricted access to peer-reviewed research and scholarly work. Users are allowed to read, download, copy, distribute, print, search, or link to the full texts of the articles, or use them for any other lawful purpose, without asking prior permission from the publisher or the author.



License - Articles published in *Notulae Scientia Biologicae* are Open-Access, distributed under the terms and conditions of the Creative Commons Attribution (CC BY 4.0) License.

© Articles by the authors; Licensee SMTCT, Cluj-Napoca, Romania. The journal allows the author(s) to hold the copyright/to retain publishing rights without restriction.

Notes:

- Material disclaimer: The authors are fully responsible for their work and they hold sole responsibility for the articles published in the journal.
- Maps and affiliations: The publisher stay neutral with regard to jurisdictional claims in published maps and institutional affiliations.
- <u>Responsibilities</u>: The editors, editorial board and publisher do not assume any responsibility for the article's contents and for the authors' views expressed in their contributions. The statements and opinions published represent the views of the authors or persons to whom they are credited. Publication of research information does not constitute a recommendation or endorsement of products involved.