# Mycorrhizal Status and Mycorrhizal Colonization Potential of Rhizospheric Soils Around Introduced and Natural Argan Trees in Northwest Morocco

S. Maazouzi, J. Aoujdad, K. Selmaoui, S. El Gabardi, M. Artib, S. Elantry, M. Ouajdi, M. Bellaka, B. Kerdouh, A. Ouazzani Touhami, R. Benkirane, and A. Douira

*Student researcher, Laboratory of Plant, Animal. and Agro-industrial Productions, Ibn Tofail University, Kénitra, Morocco; Researcher Doctor, Forest Research Center, Rabat, Morocco; Professor, Laboratory of Plant, Animal, and Agro-industrial Productions, Ibn Tofail University, Kénitra, Morocco; Student researcher, Laboratory of Plant, Animal, and Agro-industrial Productions, Ibn Tofail University, Kénitra, Morocco; Student researcher, Laboratory of Plant, Animal, and Agro-industrial Productions, Ibn Tofail University, Kénitra, Morocco; Engineer, Forest Research Center, Rabat, Morocco; Technician, Forest Research Center, Rabat, Morocco; Engineer, Forest Research Center, Rabat, Morocco; Technician, Forest Research Center, Rabat, Morocco; Professor, Laboratory of Plant, Animal, and Agro-industrial Productions, Ibn Tofail University, Kénitra, Morocco; Professor, Laboratory of Plant, Animal, and Agro-industrial Productions, Ibn Tofail University, Kénitra, Morocco.*

## Abstract

Soil and root samples were collected from the rhizosphere of planted and natural argan trees growing in the Bounaga and Smimou sites, respectively, in northwest Morocco. Frequency and intensity of mycorrhization and arbuscular content of the roots of argan trees varied between sites. Spore morphotypes on the two sites belonged to 14 species and 6 genera (*Acaulospora, Gigaspora, Glomus, Entrophospora, Scutellospora* and *Pacispora*). The number of infectious propagules of arbuscular mycorrhizal fungi (AMF) in the rhizospheric soils of argan trees at the two sites was estimated using the most probable number method (MPN). These results demonstrate that introduced argan trees form symbiotic AMF. Mycorrhizal inoculation of argan plants at the nursery stage may be beneficial, especially for harsh sites.

## Introduction

The argan tree (*Argania spinosa* [L.] Skeels) is a fruit tree endemic to southwest Morocco. This species is biologically, phytogenetically, ecologically, economically, and socially important for the country (Aït Hammouda et al. 2013). Alarmingly, this fruit tree is declining rapidly due to the arid climate, poor soils, and anthropozoogenic action of its distribution area (Bousselmame et al. 2002, Reda Tazi et al. 2003). The natural regeneration of argan forests is totally absent, due to over-exploitation and the fact that the natural environment no longer has satisfactory conditions for seed germination (El Aïch et al. 2007). Furthermore, germination in the nursery does not exceed 27 percent because of embryonic dormancy, poor seed viability, and pre- and post-emergent damping-off diseases (Bani-Aameur and Alouani 1999).

The Water and Forestry services have made many efforts to plant argan trees in Algeria, Egypt, and Tunisia (Baumer and Zeraïa 1999), as well as in different areas of Morocco. In Morocco, the first introduction of the argan tree outside its natural area was in the early 1930s when reforestation work was launched on the banks of the Oued Cherrate River, 38 km south of Rabat; argan trees are still present on this site. These efforts have met with varying success, including some failures (Harrouni et al. 1999) and some with only a few surviving trees.

The argan tree is a mycotrophic species capable of developing a symbiotic association with endomycorrhizae (AMF) (Nouaïm and Chaussod 1996), an association that improves plant nutrition (mainly phosphorus), especially in arid and semi-arid environments, improves soil aggregation and stability (Rillig and Mummey 2006), and protects plants against phytopathogens (Newsham et al. 1995). AMF

also helps plants in arid and semi-arid areas by reducing water stress (Honrubia 2009) and other environmental stresses (Martínez-García 2010) and improving physio-chemical and biological soil properties (Schmid et al. 2008). The production of good-quality plants is a necessary step to improve the survival and growth of plants in reforestation sites (Duryea 1985). Controlled mycorrhization of plants in nurseries (Nouaïm and Chaussod 1994), for example, could significantly increase growth (Ouallal et al. 2018, Sellal et al. 2017) and survival after outplanting (Echairi et al. 2008).

Sellal et al. (2016) described an indigenous endomycorrhizal complex encountered in 15 argan groves in southwest Morocco. The Water and Forest services have tried to introduce argan trees by planting them in these areas, but success has been mixed (Harrouni et al. 1999); the mycorrhizal status of introduced argan trees in these areas is unknown. Thus far, no research has been done on establishment of argan trees in northern Morocco. The aim of this work was to study AMF levels and colonization potential of the rhizospheric soils of introduced and natural argan trees in the northwest regions of Rhamna and Essaouira.

## Materials and Methods

### Study Sites

The study was carried out in two sites in northwest Morocco: Smimou (province of Essaouira; 31° 29′ 40″ N, 9° 28 57″ W) and Bounaga (Sidi Bou Othmane province of Rhamna; 31° 54′ 12″ N, 7° 56′ 32″ W). The Smimou site is located at an altitude of 665.5 m and has a dry climate with average annual precipitation of 251.1 mm, intense summer heat reaching 45 °C, and winter lows of 5 °C. The Bounaga site is located at an altitude of 450 m, has a warm Mediterranean climate with dry summers, and is characterized by an average annual rainfall of 250.9 mm, summer heat reaching 37 °C, and winter lows of 5 °C.

### Soil and Root Sampling

Soil samples were collected in May 2017 at the base of five introduced argan trees (2 kg per tree) at the Bounaga site (figure 1) and five natural argan trees from the Smimou site (figure 2). Soil samples from each site were then composited. Additionally, samples of very fine roots, likely to be mycorrhizal and easily observable under the microscope, were collected from each tree at the same time as soil collection.

### AMF Spore Extraction and Evaluation of AMF in Soil Samples

AMF spores were extracted from the soil samples according to the wet sieving method described by Gerdemann and Nicolson (1963). In a 1 L beaker, 100 g of each composite soil sample was submerged in 0.5 L of running water and stirred for 1 minute with a spatula. After 10 to 30 seconds of decantation, the supernatant was passed through four superimposed sieves with decreasing mesh sizes (500, 200, 80, and 50 µm). This procedure was repeated twice.

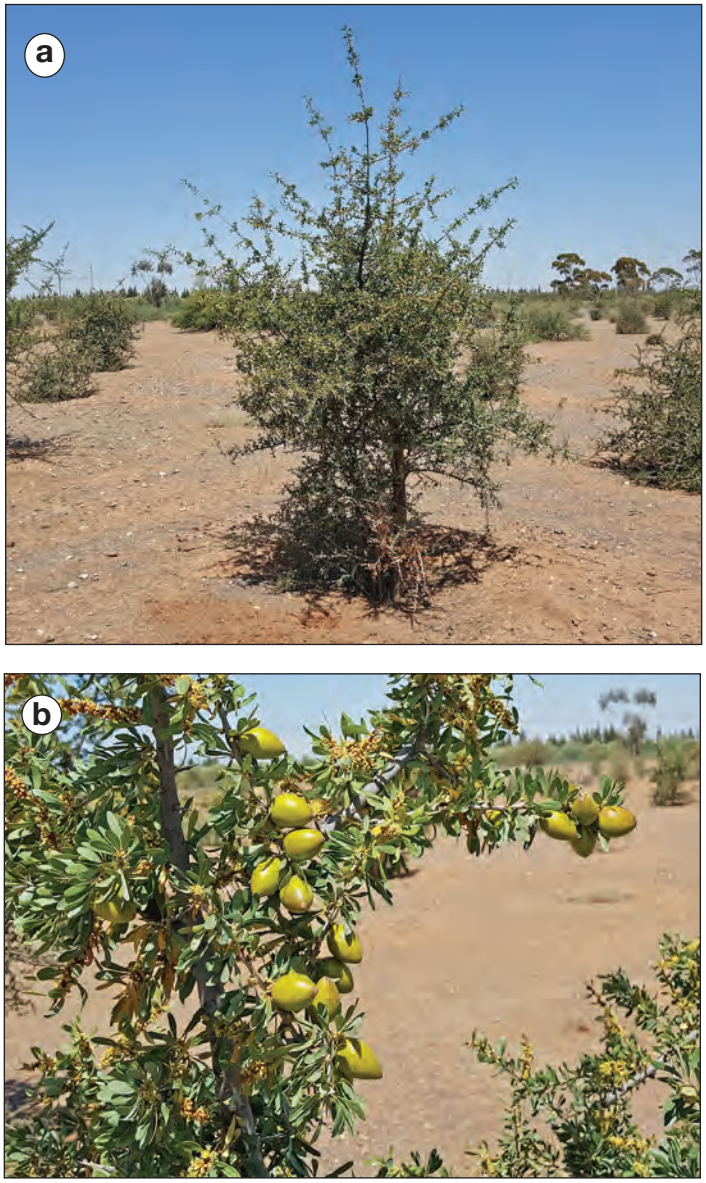


**Figure 1.** Soil and root samples were collected from argan trees introduced to the Bounaga site (Rhamna province) of northwestern Morocco. (Photos by M. Ouajdi 2017)

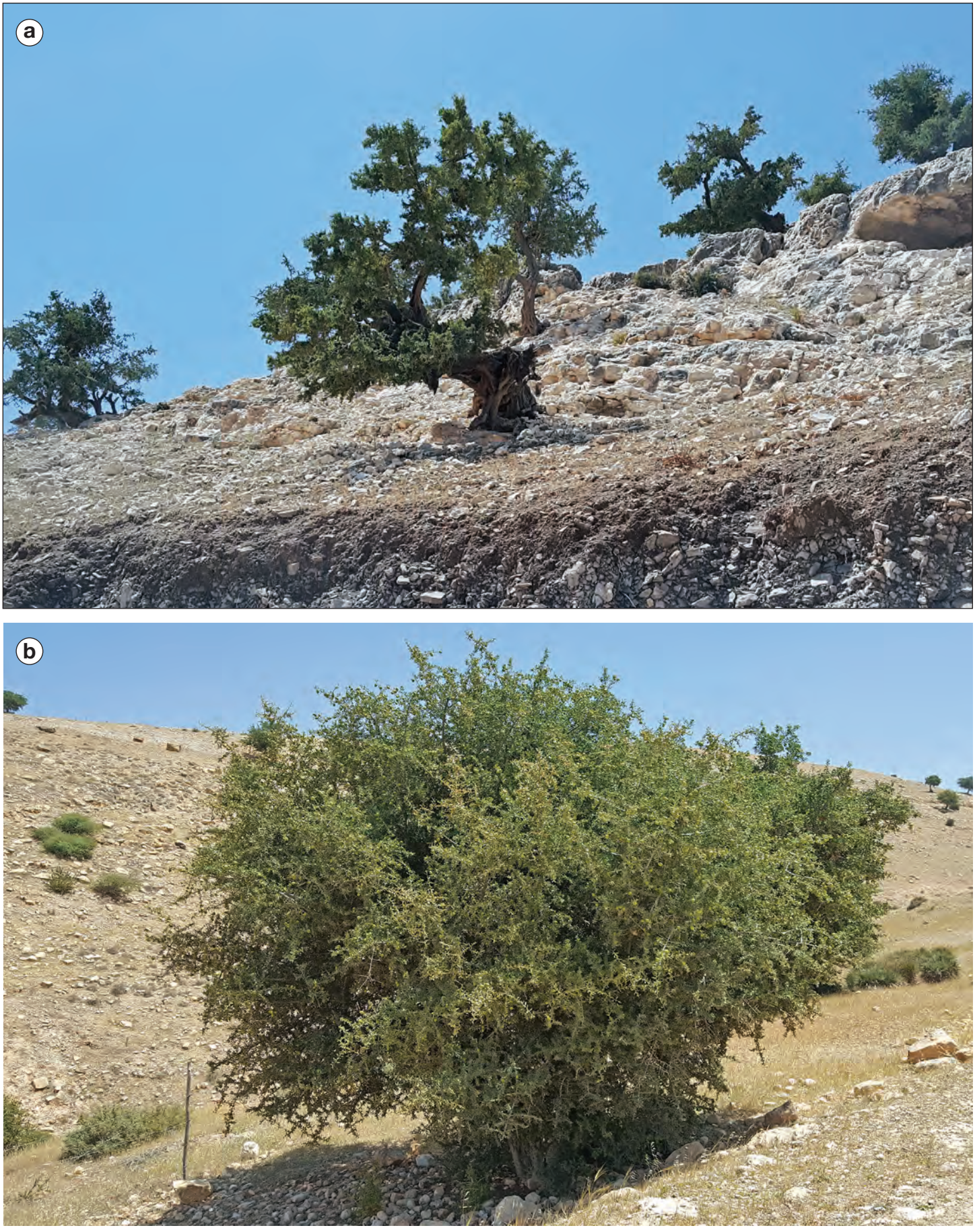


**Figure 2.** Soil and root samples were collected from argan trees introduced to the Smimou site (Essaouira province) of northwestern Morocco. (Photos by M. Ouajdi 2017)

**Table 1.** To estimate infectious propagules of AMF in the rhizospheric soils of argan trees at the two sites using the most probable number method (MPN) substrates were prepared with nine dilutions (n=3).

| Dilution | Proportion of non-sterile soil | Quantity of non-sterile soil (g) | Quantity of sterile soil (g) |
|---|---|---|---|
| 1 | 1\1 | 100.000 | 0.000 |
| 2 | 1\2 | 50.000 | 50.000 |
| 3 | 1\4 | 25.000 | 75.000 |
| 4 | 1\8 | 12.500 | 87.500 |
| 5 | 1\16 | 6.250 | 93.750 |
| 6 | 1\32 | 3.125 | 96.875 |
| 7 | 1\64 | 1.562 | 98.438 |
| 8 | 1\128 | 0.781 | 99.219 |
| 9 | 1\256 | 0.390 | 99.600 |
| Control | 0 | 0.000 | 100.000 |

The content retained by the 200, 80, and 50 µm sieves was distributed into two tubes and centrifuged for 4 min at 9000 rpm. The supernatant was discarded, and a viscosity gradient was created by adding 20 ml of a 40 percent sucrose solution to each centrifuge tube (Walker and Sanders 1982). The mixture was quickly stirred, and the tube returned to the centrifuge for 1 min at 9000 rpm. The supernatant was then poured over a 50 µm mesh screen. The substrate obtained was rinsed with distilled water to remove the sucrose, then disinfected with an antibiotic solution (Streptomycin). The spores were then recovered with a little distilled water in an Erlenmeyer flask. Species richness was determined by the total number of species observed from each sampling site. The spores were observed using an optical microscope and identified morphologically according to several criteria including spore color, shape, size, and surface ornamentation. Spore identification was performed according to descriptions provided by the International Collection of Arbuscular Vesicular Mycorrhizal Fungi (INVAM 2017).

The number of infectious propagules of AMF in the rhizospheric soils of argan trees at the two sites was estimated using the most probable number method (MPN) based on Declerck et al. (1999) method. Sorghum seedlings were used as a mycotrophic plant. This plant is highly sensitive to colonization by AMF and exhibits rapid root development (Utobo et al. 2011). A dilution factor of 2 was used with 9 dilutions (table 1). Three replicate pots of each dilution were prepared for 100 g soil sampled from each of the two sites. In addition, a control pot containing only sterile soil was included. A sorghum plant was transplanted and grown for 6 weeks in each pot. Sorghum seeds were disinfected with sodium hypochlorite at 10 percent concentration for 15 min and rinsed 3 times with distilled water before being germinated. A 6-day-old plant was transplanted into each pot. Pots were placed in a greenhouse and watered with distilled water as needed. After 6 weeks, plants were removed from pots and assessed for AMF colonization and MPN calculations were made using the formula from Fisher and Yates (1949):

$$\text{Log MPN} = (x.\log a) - k(y,S)$$

Where:

x = the average number of mycorrhizal plants (total divided by number of replications)

S= the number of dilution levels

a = the dilution factor

y = the average of nonmycorrhizae plants (S-x).

K is given by the tables of Fisher and Yates (1949) as a function of y and S.

## Evaluation of AMF Root Colonization

Fine roots collected from each argan tree, as well as roots collected from sorghum plants grown in rhizospheric soils, were prepared according to the

method of Koské and Gemma (1989). They were first washed with water, cut in 1- to 2-cm lengths, immersed in a 10 percent KOH solution, then placed in an oven at 90 °C for 1 hour to remove intracellular constituents. The roots were then rinsed and transferred to a hydrogen peroxide solution (a few drops of hydrogen peroxide in 100 ml of distilled water) for 20 min at 90 °C until they whitened. The roots were then stained by submersion in 0.05 percent brilliant cresyl blue (modified from Philips and Hayman 1970) at 90 °C for 15 min.

After a final rinsing, 30 colored argan root fragments from both Smimou and Bounaga sites were randomly chosen and mounted in groups of 10 to 15 in glycerin between blade and cover slip (Kormanik and McGraw 1982). The remaining roots were kept in water or glycerol acid. Under a microscope, each fragment was carefully examined over its entire length, at magnifications of 100x and 400x to observe and record any mycorrhizal structures: arbuscules, partitions of hyphae, vesicles, intra- and intercellular hyphae, extramatric hyphae, and endophytes.

The presence of AMF arbuscules and vesicles were assigned a mycorrhization index (Derkowska et al. 2008): 0=absent; 1=trace; 2=less than 10 percent; 3=11 to 50 percent; 4=51 to 90 percent; 5=more than 91 percent.

Mycorrhization frequency (MF), estimates the proportion of the host plant's fine roots colonized by AMF.

$$MF = 100 (N - N0) / N$$

Where:

N = total number of mycorrhizal root fragments observed

N0 = number of non-mycorrhizalroot fragments

Mycorrhization intensity (MI) estimates the overall concentration of AMF colonization in the entire fine rootsystem:

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

Where:

n5, n4, n3, n2, and n1 indicate the number of fragments denoted 5, 4, 3, 2, and 1 on the mycorrhization index, respectively.

Arbuscule abundance (A) is calculated as follows:

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

Where:

A1: 1 to 10 percent, A2: 11 to 50 percent, A3: 51 to 100 percent

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

Vesicle abundance (V) is calculated in the same way as that of the arbuscular abundance.

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

### Statistical Analyses

The statistical processing of the data focused on the analysis of variance with a single classification criterion (ANOVA1). IBM SPSS 21.0 software was used for these statistical analyses. Each site was analyzed separately. Although the two sites could not be compared due to variations in environment and lack of both natural and planted trees at each site, observational similarities and differences are noted.

## Results and Discussion

### AMF Spore Extraction

The concentration of spores in the rhizosphere of introduced argan trees was approximately 22 spores per 100 g of soil and include 11 morphotypes, the most dominant of which are *Acaulospora gedanensis* and *Claroideoglomus etunicatum*. On the Smimou site, rhizosphere spores around natural argan trees averaged 45 spores per 100 g soil and include 6 species, with an abundance of *Acaulospora bireticulata*, *Dentiscutata nigra*, and *Gigaspora margarita*. The two sites have two species in common: *Endogone versiformis* and *Rhizophagus intraradices* (figure 3 and table 2). The duration of mycorrhization depends on the host, the infectious power of the mycorrhizogenic fungus, and the growing medium (Plenchette and Fardeau 1988). In the Ait-Baha region, Elmaati et al. (2015) noted 1127.66 spores per 100 g soil indicating that spore density in the northwestern Rhamna region studied is low compared with that of southern Morocco.

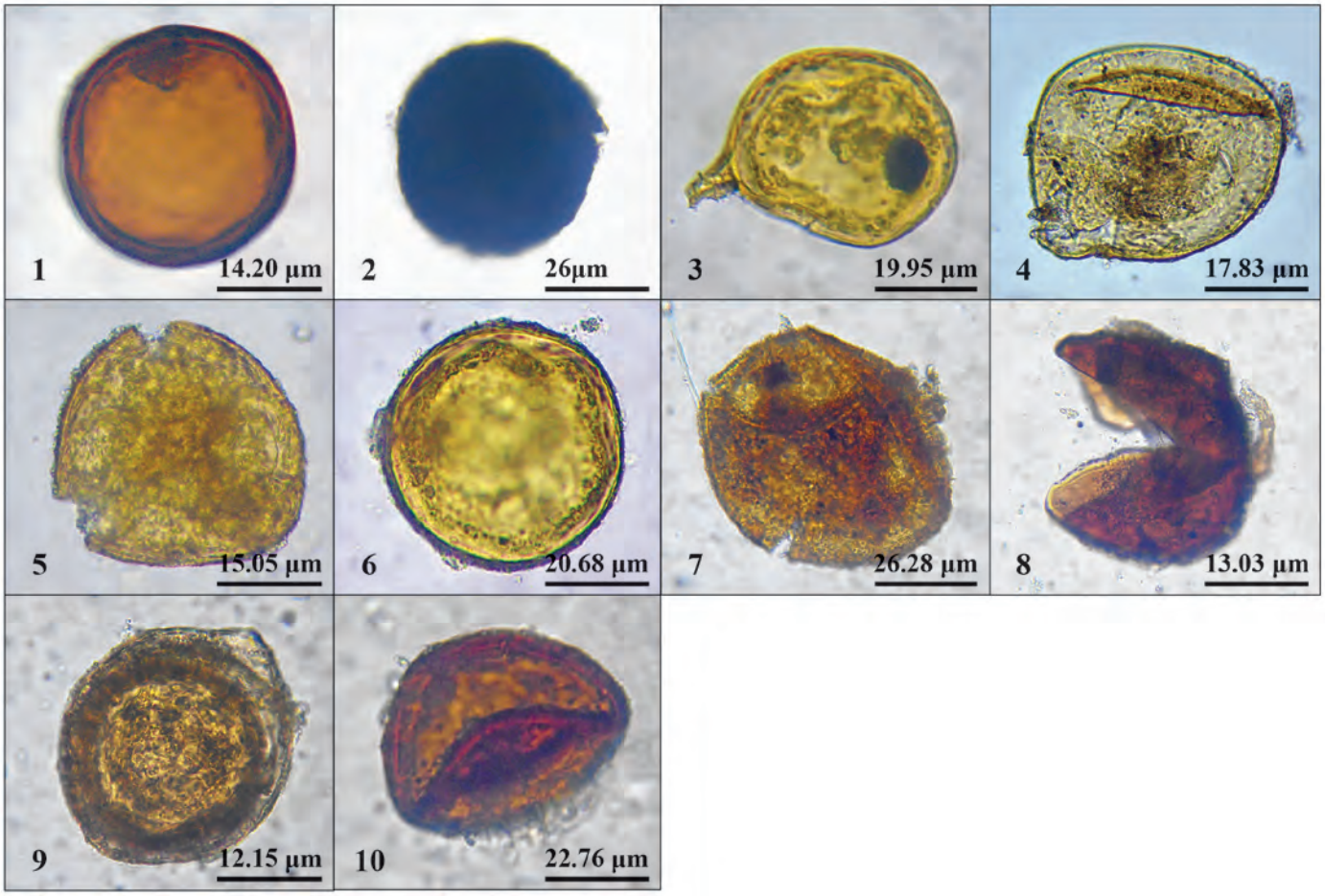


**Figure 3.** Some morphotypes of endomycorrhizal fungi isolated from the rhizosphere of argan trees on two sites in northwest Morocco (see also table 2). (Photos by S. Maazouzi 2018)

**Table 2.** Morphological characteristics of some species of endomycorrhizal fungi isolated from the rhizosphere of argan trees (see also figure 3).

| Number | Name | Form | Color | Average height | Wall surface | Hypha length | Number of walls |
|---|---|---|---|---|---|---|---|
| 1 | *Claroideoglomus etunicatum* | Globular | Orange | 83.3 | smooth | - | 2 |
| 2 | *Dentiscutata nigra* | Globular | Brown | 99.9 | granular | - | 2 |
| 3 | *Rhizophagus fasciculatus* | Oval | Yellow | 84.5 | granular | 17.4 | 2 |
| 4 | *Glomus intraradices* | Globular | Yellow | 99.9 | granular | - | 2 |
| 5 | *Gigaspora margarita* | Globular | Yellow | 89.6 | granular | - | 2 |
| 6 | *Funneliformis geosporum* | Globular | Yellow | 86.8 | smooth | - | 2 |
| 7 | *Glomus intraradices* | Oval | Light brown | 109.6 | granular | - | 2 |
| 8 | *Funneliformis verruculosum* | Oval | Dark brown | 67.9 | granular | 10 | 2 |
| 9 | *Glomus aggregatum* | Globular | Light brown | 68.9 | granular | - | 2 |
| 10 | *Endogone macrocarpa* | Dark brown | Dark brown | 133.2 | granular | - | 2 |

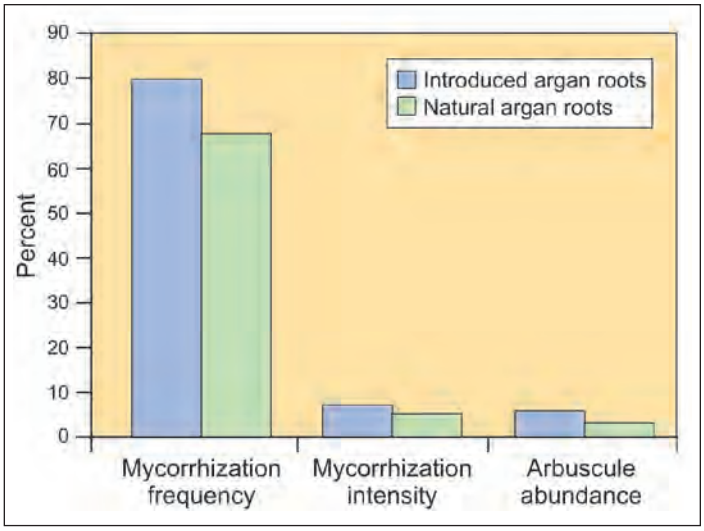


**Figure 4.** Mycorrhizae frequency (MF), Intensity (MI), and arbuscular content (A) of argan tree roots for introduced trees (Bounaga site) and natural trees (Smimou site).

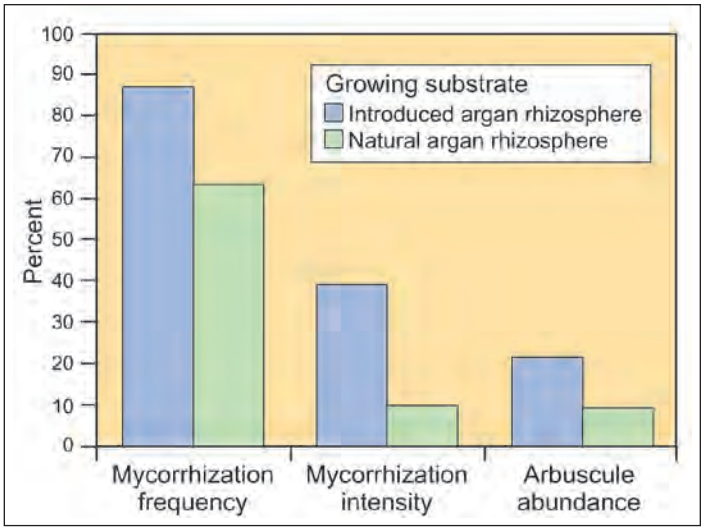


**Figure 6.** Frequency (MF), intensity of mycorrhization (MI), and arbuscular contents (A) of sorghum roots grown in rhizoshperic soils of argan trees.

## AMF Root Colonization

The argan trees introduced into the Bounaga site seem to have adapted to the ecological conditions of the region, as confirmed by the AMF diversity and frequency found in the rhizosphere around sampled trees (figure 4). In other studies, mycorrhization frequencies were 100 percent for natural argan trees from the Taroudante and Toufalazte sites (Sellal et al. 2016).

Sorghum roots grown in rhizospheric soils collected around natural and introduced argan trees were mycorrhizal with characteristic AMF structures (figure 5). MF, MI, and A tended to be higher in sorghum roots growing in the rhizospheric soils of the introduced argan trees, compared with those grown in the soil of natural argan trees (figure 6). The number of spores isolated from the rhizosphere of sorghum plants also varied by dilution factor (table 3). The substrate from the soils of natural argan trees (1/1 dilution) included 6 different morphotypes: *Dentiscutata nigra* (7 spores), *Rhizophagus intraradices* (5 spores), *Endogone versiformis* (8 spores), *Glomus aggregatum* (3 spores),

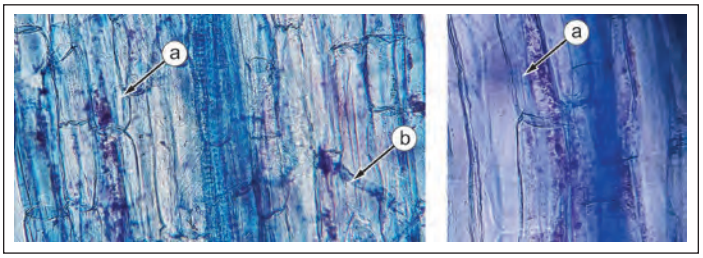


**Figure 5.** Different structures of arbuscular mycorrhizal fungi observed in the roots of sorghum plants: (a) arbuscules and (b) extracellular hyphae (× 400). (Photos by S. Maazouzi 2018)

*Endogone macrocarpa* (2 spores), *Funneliformis verruculosum* (3 spores). In the substrate of sorghum plants from introduced argan trees at the same dilution, 5 morphotypes were found: *Dentiscutata nigra* (6 spores), *Glomus* sp. (4 spores), *Gigaspora* sp. (3 spores), *Pacispora* sp. (2 spores), *Endogone versiformis* (5 spores).

## MPN Soil Propagules

The MPN of the rhizospheric soil of introduced argan trees was 7.14 propagules per 100 g soil and that of the rhizospheric soil of natural argan trees was 1.78 propagules per 100 g of soil. Other studies have reported varying propagule concentrations in rhizospheric soil around other species, including 100 propagules per g around olive trees (*Olea europaea* L.) (Mekahalia 2013), 360 propagules per g around onion (*Allium cepa* L.) (Sow et al. 2008), and 353 propagules per 100 g around palmier (*Phoenix dactylifera* L.) (Meddich et al. 2015). According to Requena et al. (1996), the number of propagules encountered in a soil type depends on the diversity of plant species and on the region's dominant ecological factors (Sanon et al. 2006). Increasing plant cover also causes a decrease in the number of infectious propagules (Richter et al. 2002).

According to Adelman and Morton (1986), MPN is a very interesting technique for estimating the mycorrhizogenic potential of a given soil, and the experimental conditions must reflect the conditions on the

**Table 3.** The number of spores of arbuscular mycorrhizal fungi varied by site and dilution.

| Dilutions | Natural argan trees | Introduced argan trees |
|---|---|---|
| 1/1 | 28 | 20 |
| 1/2 | 15 | 10 |
| 1/4 | 11 | 8 |
| 1/8 | 9 | 5 |
| 1/16 | 6 | 4 |
| 1/32 | 3 | 2 |
| 1/64 | 2 | 1 |
| 1/128 | 0 | 0 |
| 1/256 | 0 | 0 |

ground. Thus, the higher the substrate dilution, the greater number of spores present. According to Neffar (2012), the MPN is variable during the year and the number of propagules depends on plant species diversity (Sanon 2006). The same result was noted by El Gabardi et al. (2019a, 2019b, 2019c), who found that phosphate washing sludges colonized by different plant species had a large number of spores of endomycorrhizal fungi and a number of infectious propagules estimated by PIM and MPN techniques.

In Morocco, the use of AMF on argan plants in the nursery may become common practice. Mycorrhizal plants produced in nurseries tend to have very developed root systems and are therefore able to tolerate drought conditions after planting (Nouaïm and Chaussod 1997). According to Sellal et al. (2017) and Ouallal et al. (2018), argan plants inoculated with AMF are more vigorous and can adapt to different soil and climatic conditions once replanted.

## Conclusion

The results of the present study showed a diversity of endomycorrhizal fungal species in the rhizosphere of argan trees introduced into the Rhamna region. This diversity is significant compared to that encountered in the rhizosphere of natural argan trees at the Smimou site. These results demonstrate that introduced argan trees form functional and beneficial symbiotic associations with endomycorrhizae over time. Mycorrhization of argan plants at the nursery stage is likely to increase plant resistance to the harsh conditions they may encounter after being outplanted to the field.


## Acknowledgments

This study was conducted under the project Utilisation des biotechnologies microbiennes pour la réhabilitation des écosystèmes sylvo-pastoraux dégradés et l'amélioration des rendements des cultures, funded by Hassan II Academy of Sciences and Technology, Morocco.


## REFERENCES


Adelman, M.J.; Morton, J.B. 1986. Infectivity of vesicular-arbuscular mycorrhizal fungi: Influence of host-soil diluents combinations on MPN estimates and percentage colonization. Soil Biology and Biochemistry. 18: 77–83.

Aït Hammouda, T.; Bendou, S.; Abdoun, F. 2013. L'arganeraie algérienne, caractéristiques écologiques et structurales. Actes du 2ème Congrès International de l'Arganier: 72–75.

Bani-Aameur, F.; Alouani, M.1999. Viabilité et dormance des semences d'arganier (*Arganiaspinosa* (L.) Skeels). EcologiaMediterranea. 25(1): 75–86.

Baumer, M.; Zeraïa, L. 1999. La plus continentale des stations de l'Arganier en Afrique du Nord, Rev. For. Fr.3 : 446.

Bousselmame, F.; Kenny, L.; Achouri, M. 20002. Effet des mycorhizes à vésicules et arbuscules sur la croissance et la nutrition de l'arganier (*Argania spinosa* L.). Revue Marocaine des Sciences Agronomiques et Vétérinaires. 22(4): 193–198.

Declerck, S.; Plenchette, C.; Risede, J.M.; Strullu, D.G.; Delvaux, B. 1999. Estimation of the population density of arbuscular mycorrhizal fungi in soils used for intensive banana cultivation in Martinique. Fruits. 54: 3–9.

Derkowska, E.; SasPaszt, L.; Sumorok, B.; Szwonek, E.; Gluszek, S. 2008. The influence of mycorrhization and organic mulches on mycorrhizal frequency in apple and strawberry roots. Journal of Fruit and Ornamental Plant Research. 16: 227–242.

Duryea, M.L., ed. 1985. Proceedings: Evaluating seedling quality: principles, procedures, and predictive abilities of major tests. Corvallis, OR: Oregon State University, Forest Research Laboratory. 143 p.

Echairi, A.; Nouaïm, R.; Chaussod, R. 2008. Intérêt de la mycorhization contrôlée pour la production de plants d'arganier (*Argania spinosa*) en conditions de pépinière. Sécheresse. 19 (4): 277–281.

El Aïch, A.; Bourbouze, A.; Morand-Fehr, P. 2007. Le système d'élevage caprin dans l'arganeraie. In: Kenny, L., ed. Atlas de l'arganier et de l'arganeraie. Rabat: Institut Agronomique et Vétérinaire Hassan II.: 179–190.

El Gabardi, S.; Chliyeh, M.; Selmaoui, K.; Ouazzani Touhami, A.; El Modafar, C.; Filali Maltouf, A.; Elabed, S.; Ibnsouda Koraichi, S.; Amir, S.; Moukhli, A.; Benkirane, R.; Douira A. 2019a. Diversity of endomycorrhizal fungi isolated from soil sites adjacent to Khouribga phosphate mines (Morocco). Interciencia. 44(4): 60–83.

El Gabardi, S.; Chliyeh, M.; Selmaoui, K.; Ouazzani Touhami, A.; El Modafar, C.; Filali Maltouf, A.; Elabed, S.; Ibnsouda Koraichi, S.; Amir, S.; Moukhli, A.; Benkirane R.; Douira A. 2019b. Study of the endomycorrhizogenic potential of phosphate laundered sludge. Wulfenia. I26(5): 38–57.

El Gabardi, S.; Chliyeh, M.; Mouden, N.; Ouazzani Touhami, A.; El Modafar, C.; Filali Maltouf, A.; Elabed, S.; Ibnsouda Koraichi, S; Amir, S.; Moukhli, A.; Benkirane, R.; Douira, A. 2019c. Determination of the endomycorrhizogenic potential of phosphate laundered sludge by using the mycorrhizal infectious method (PIM). Plant Cell Biotechnology and Molecular Biology. 20(11&12): 501–510.

Elmaati, 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*. American Journal of Innovative Research and Applied Sciences. 1(7): 235–243.

Fisher, R.A; Yates, F. 1949. Statistical tables for biological, agricultural and medical research. 112 p.

Gerdemann, J.W.; Nicolson, T.H. 1963. Spores of mycorrhizal endogone species extracted from soil by wet sieving and decanting. Transactions of the British Mycological Society. 46(2): 235–244.

Harrouni, M.; El Kherrak, H.; Mokhtari, M.; El Yazidi, A.; Abdellah, K.1999. Multiplication de l'Arganier (*Argania spinosa* (L.) Skeels) par bouturage. Proceedings of the International conference on biodiversity and natural resources preservation. Ifrane, Morocco: Al Akhawayn University.

Honrubia, M. 2009. The mycorrhizae: a plant fungus relation that has existed for more than 400 million years. Anales del Jardín Botánico de Madrid. 66: 133–144

INVAM. 2017. Species descriptions from reference cultures. International Culture Collection of (Vesicular) Arbuscular Mycorrhizal Fungi. Morgantown: West Virginia University, Davis College of Agriculture, Natural Resources and Design. http://fungi.invam.wvu.edu/the-fungi/species-descriptions.html.

Kormanik, P.P.; McGraw, A.C. 1982. Quantification of vesicular-arbuscular mycorrhizae in plant roots. In: Sheed, N.C., ed. Methods and principles of mycorrhizal research. St. Paul, MN: American Phytopathological Society:37–45.

Koské, R.; Gemma, J.; Carreiro M. 1989. Seasonal dynamics of selected species of V-A mycorrhizal fungi in a sand dune. Mycological Research. 92(3): 317–321.

Martínez-García, L.B. 2010. Micorrizasarbusculares en ecosistemasemiáridos. Respuesta a factores de estrésambiental. Almería: Universidad de Almería. Doctoral thesis.

Meddich, A.; Hafidi, M.; Ait El Mokhtar, M.; Boumezzough, M. 2016. Characterization of physicochemical parameters and mycorrhizal potential of salt soils of North-east date palm grove of Marrakesh. Journal of Materials and Environmental Science., 6 (9): 2469–2475.

Mekahlia, M.N.; Beddiar, A.; Chenchouni, H. 2013. Mycorrhizal dependency in the olive tree (*Olea europaea*) across a xeric climatic gradient. Advances in Environmental Biology. 7(9): 2166–2174.

Neffar, S. 2012. Étude de l'effet de l'âge des plantations de figuier de Barbarie (*Opuntia ficus-indica* L. Miller) sur la variation des ressources naturelles (sol et végétation) des steppes algériennes de l'Est. Cas de Souk Ahras et Tébessa. Annaba, Algeria: University of Annaba. 236 p. PhD. Thesis

Newsham, K.K.; Fitter, A.H.; Watterson ,A.R. 1995. Arbuscularmycorrhiza protects an annual grass from root pathogenic fungi in the field. Journal of Ecology. 83: 991–1000

Nouaïm, R.; Chaussod, R. 1994. Mycorrhizal dependency of micro propagated argan tree (*Argania spinosa*). 2. Mineral nutrition. Agroforestry Systems. 27(1): 67–77.

Nouaïm, R.; Chaussod, R. 1996. Rôle des mycorhizes dans l'alimentation hydrique desplantes, notamment des ligneux en zones arides. Options Méditerranéennes. 20: 9–26.

Nouaïm, R.; Chaussod, R. 1997. Effet de la mycorhization contrôlée sur la croissance de l'arganier (*Argania spinosa*) après sa transplantation en sol non désinfecté. Al Awamia. 96: 65–76

Ouallal, I.; Rochdi, A.; Ouajdi, M.; Hamamouch, M.; El Yacoubi, H.; Abbas, A. 2018. Effects of native arbuscular mycorrhizae inoculation on the growth of *Argania spinosa* L. seedlings. Tree Planters' Notes. 61(1): 35–44.

Philips, J.M.; Hayman, D.S. 1970. Improved procedures for clearing root and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Transactions of the British Mycological Society. 55: 158–161.

Plenchette,C.; Fardeau, J.C. 1988. Effet du pouvoir fixateur du sol sur le prélèvement de phosphore par les racines et les mycorhizes. Comptes Rendus de l'Académie des Sciences, Paris. 306: 201–206.

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). Note de recherche, Science et changements planétaires/Sécheresse.14: 23–27.

Requena, N.; Jeffries, P.; Barea, J.M.,1996. Assessment of natural mycorrhizal potential in a desertified semiarid ecosystem, Applied and Environmental Microbiology. 62: 842–847.

Richter, B.S; Tiller, R.L.; Stutz, J.C. 2002. Assessment of arbuscular mycorrhizal fungal propagules and colonization from abandoned agricultural fields and semiarid grasslands in riparian floodplains. Applied Soil Ecology. 20: 227–238.

Rillig, M.; Mummey, D.L. 2006. Mycorrhizas and soil structure. New Phytologist. 171, 41–53. http://dx.doi.org/10.1111/j.1469-8137.2006.01750.x.

Sow, H.A.; Diop, T.A.; Ndiaye, F.. Manga, A.G.B; Diallo, A. 2008. Influence de la mycorhization arbusculaire sur la culture intensive de l'oignon (*Allium ceps* L.) au Sénégal. Journal Des Sciences. 8(1): 1–6.

Sanon, A.; Martin, P.; Thioulouse, J.; Plenchette, C.; Spichiger, R.; Lepage, M.; Duponnois, R. 2006. Displacement of an herbaceous plant species community by mycorrhizal and non-mycorrhizal *Gmelina arborea*, an exotic tree, grown in a microcosm experiment. Mycorrhiza.16: 125–132.

Schmid, T.; Meyer, J.; Oehl, F.2008. Integration of mycorrhizal inoculum in high alpine revegetation. Mycorrhiza works. In: Feldmann, F.; Kapulnik, Y.; Baar, J., eds. Proceedings of the International Symposium Mycorrhiza for Plant Vitality and the Joint Meeting of Working Groups 1-4 of COST Action 870 Braunschweig: Deutsche Phytomedizinische Gesellschaft.2 (5): 85–97.

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. Indian Journal of Pure & Applied Biosciences. 4 (1): 82–99.

Sellal, Z.; Ouazzani Touhami, A.; Mouden, N.; Ouarraqi, El M.; Selmaoui, K.; Dahmani, J.; Benkirane, R.; El Modafar, C.; Douira A. 2017. Effect of an endomycorrhizal inoculum on the growth of argan tree. International Journal of Environment, Agriculture and Biotechnology. 2(2): 928–939.

Utobo, E.B; Ogbodo, E.N; Nwogbaga, A.C. 2011. Techniques for extraction and quantification of arbuscular mycorrhizal fungi. Libyan Agriculture Research Center Journal International. 68–78.

Walker, C.; Sanders, F.E. 1982. Associated with subterranean clover: dynamics of colonization, sporulation and soluble carbohydrates. New Phytologist. 124: 215–219.