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Arbuscular Mycorrhizal fungi species associated with rhizosphere of *Argania spinosa* (L.) Skeels in Morocco

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ABSTRACT

The identification and the evaluation of arbuscular mycorrhizal fungi in the level of the root mycorhization were performed respectively from spores isolated from soil samplings and roots preleaved from the argan tree rhizosphere of seven sites in the region of Essaouira, Agadir, Taroudant and Tiznit (southwest Morocco).

Analysis of the results revealed the presence in all samples of different characteristic of arbuscular endomycorrhizal structures. The frequency of mycorrhization was complete at Taroudant sites and Toufalazte (F = 100%) and between 50% and 33.33% in the Tamanar, Ait Melloul and Tiznit sites.

The mycorrhizal intensity is high at the site of Toufalazte (57%) and low in Ait Melloul sites and Tiznit (between 1.83% and 1.93). Furthermore, the arbuscular contents are higher at the site of Toufalazte (24.16%) and lowest in the site of Tiznit (0.082%). The vesicular contents ranged from 0.21% (Ait Melloul) and 34.14% (Toufalazte). The density of spores in the rhizosphere of Argania spinosa also varied between 188 spores / 100 g soil (Toufalazte), and 28 and 44 spores / 100g of soil (Tiznit and Ait Melloul).

The identification of isolated spores allowed to note the presence of 26 species of mycorrhizal fungi, divided into five genera (Glomus Scutellospora, Entrophospora, Pacispora, Gigaspora). Glomus etunicatum is the most abundant species, its frequency of occurrence reached 16.26%.

Key words: *Morocco, Argania spinosa, rhizosphere, arbuscular mycorrhizal fungi (AMF), diversity.*

INTRODUCTION

The argan tree, the only representative of the Sapotaceae family in North Africa, is an endemic tree species of southwestern Morocco). It extends from the sea in 1600- 1700 meters above sea level on the southern slopes of the western High Atlas and Anti-Atlas[5]. Isolated colonies of the argan tree are also found in the north and the Oriental areas[11,85]. The argan tree is also considered as the second forest essence of the country, with an area of 800 000 ha[67].

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The argan tree plays an important ecological role[59] by creating a favorable bioclimate for the development of a large number of plant species and participant protection against soil erosion, especially in rugged terrain[4,67,81]. It also plays an outstanding economic role[15,64,75] ensuring the livelihood of nearly 3 million residents[103]. Every part of the tree is useable and provides a source of income or food. The wood is used as fuel, the leaves and fruits as fodder for goats[33,64]. Argan oil, consumed almost exclusively in the production region, now widely exported to many countries (Europe, North America, Japan, etc.), as a luxury food product, valued for its nutritional and organoleptic qualities, or used in cosmetics[32,74].

In many areas of south west Morocco, the argan tree is the basis of traditional agroforestry systems[72], which allowed so far to address the needs of rural populations in the arid and semi-arid areas[73]. According to these authors, ecological imbalance essentially anthropogenic led to the continuous decline of 'arganeraies' whose disappearance could lead to the collapse of these agro-ecosystems with alarming consequences both in economic and social ecologically[72,103]. It is therefore urgent to safeguard the native species which are best adapted to their environment and rehabilitate areas degraded by reintroducing the argan forest plantations[32,36]. The efforts of the Moroccan forest services in reforestation based Argan face to the difficulty of resumption of produced nursery plants[14,38,29]. According to these authors, there are several possible reasons for the observed failures: the precipitation deficit, inadequate post planting irrigation, mismatched tillage, or quality of used plants. Improved seedlings production techniques at nurseries is a must and must be controlled[58]. The controlled mycorrhizal plants at nurseries[71], for example, could possibly significantly increase the success of transplantation and initial growth of trees[32].

The argan tree's ability to establish a symbiotic association with AM fungi[3,76]. Arbuscular mycorrhizae are found in over 70% of vascular plant species[40] and allow the extension of the absorption surface and the volume of soil explored, beyond the depletion region of the rhizosphere[98]. This type of mycorrhizae allows better improve the assimilation of nutrients especially P and N[44,104], especially in arid and semi-arid environments, improving the aggregation and stability of soil[87] and protecting against phytopathogenic[26,69,83,84,99]. The CMA also help plants grow in arid and semi arid regions through mitigation of water stress[8,12,30,46,47,88,89], improved physicochemical and biological properties of soils[23,87,92] and other environmental stress[12,13,60,61,62,79].

Mycorrhization of argan tree seedlings is therefore an interesting avenue to explore for the restoration of degraded areas[6]. To achieve this objective, it is necessary to give particular importance to the diversity of fungi endomycorhizations at the rhizosphere of the argan tree growing in different areas of south west Morocco. In this sense, the present work is a continuation of the work of Nouaïm[71], Nouaïm and Chaussod[76], Nouaïm *et al*[70,71]., Kenny *et al*[54,55]., Echairi *et al*[32]., El Mrabet *et al*[37]., on endomycorrhizae of *Argania spinosa*. Indeed, few comprehensive studies have been conducted in Morocco on the diversity of CMA associated with the argan tree.

MATERIALS AND METHODS

Sites of samplings

Samplings were realized in seven sites distributed in the Essaouira, Agadir, Tiznit and Taroudant regions, The soil samples were taken at the foot of five plants of argan tree per site (2 kg / tree) at a depth of 0-20 cm and a composite sample of soil was achieved for each site. Very fine roots more likely to be mycorrhized and easily observable under the microscope were taken at the same time with the soil.

Physico-chemical analysis of Soil

The main physico-chemical characteristics of soils were determined by conventional analysis performed by the laboratory analysis of soil ORMVAG of Kenitra.

Measuring the rate of mycorrhizal roots

The roots were prepared according to the method of Koske and Gemma[57]. They were first washed with water, finer were cut to a length of 1 cm and immersed in a 10% KOH solution and placed in an oven at 90 ° C for one hour in order to eliminate intracellular constituents. Subsequently, the roots were rinsed and transferred in a solution of H2O2 (hydrogen peroxide) for 20 min at 90 ° C until bleaching roots. Roots were then rinsed and stained with cresyl blue 0.05% by submersion[82], at 90 ° C for 15 min.

After a final rinse, thirty pieces of colored roots of 1 cm in length were randomly selected and mounted in groups of 10 to 15 segments in the glycerin between slide and coverslip. The remaining roots were stored in water or acid glycerol. The slides were observed under the microscope, each fragment is carefully checking its entire length, with magnification × 100 and 400 × in order to note the mycorrhizal structures: arbuscules, vesicles, intra- and intercellular hyphae, hyphae, extra-matrix and even endophytes.

The estimate of mycorrhization is made according to the method described by Trouvelot *et al*[106]. The proposed scoring system is based on the overall assessment of each 30 fragments[27]. The evaluated parameters are the mycorrhizal frequency and intensity, arbuscular and vesicular contents of endomycorrhizal inside the root bark[24,53,97].

Extraction of spores

The spores are removed by the wet sieving method described by Gerdemann and Nicolson[41]. In a 1L beaker, 100 g of each composite sample of soil is submerged in 0.5 L of tap water and stirred for 1 minute with a spatula. After 10 to 30 seconds of settling, the supernatant was passed through four superimposed decreasing mesh sieve (500, 200, 80 and 50 microns). This operation is repeated twice. The content retained by the sieve of 200, 80 and 50µm is divided into two tubes and centrifuged for 4 minutes at 9000 rev / min. The supernatant is discarded and a viscosity gradient is created by adding 20 ml of a 40% sucrose solution to each centrifuge tube[108]. The mixture was rapidly stirred and the tube returned again in the centrifuge for 1 min at 9000 rev / min.

Unlike the first centrifugation step, the supernatant is poured onto the sieve with a mesh of 50 microns, the resulting substrate was rinsed with distilled water to remove the sucrose, then disinfected with an antibiotic solution (Streptomycin). The spores are then recovered with a little distilled water in an Erlenmeyer flask, observed under a microscope (× 100) and classified according to their size and color[109].

The morphological identification of spores was carried out by consulting the work of Schenek and Perez[91], Morton and Benny[65] and international databases: Arbuscular mycorrhizal fungi (Glomeromycota) Endogone and Complexipes species Deposited in the Department of Plant Pathology, University of Agriculture in Szczecin, Poland[18,19,20] and International Culture Collection of (Vesicular) Arbuscular Mycorrhizal Fungi[66].

Species richness and frequency of spore's occurrence

Species richness is the total number of species observed by sampling site and species occurrence rate is the percentage of sites where each species is detected.

Statistic analysis

The statistical treatment of results focused on the analysis of variance with one classification criterion (ANOVA1).

RESULTS

The physico-chemical data (Table 1) conducted in the laboratory of Soil analysis of the Office of Agricultural Development of the Gharb (ORMVAG) show that rhizospheric soils of argan prospected areas are characterized by alkaline pH, (vary between 8.22 in Essaouira and 8.54 in Tamanar) and a conductivity that varies between 0.30 (Toufalazte) and 0.60 mmhos / cm (Tiznit). Nitrogen rates vary from one site to another, in the order of 60.8 ppm in the site Taghazoute and 214.4 ppm in Tiznit. The available phosphorus and organic matter vary respectively 14 and 66 ppm and 1.77 and 7.87%. The potassium content reaches 423 ppm in the soil of Essaouira and 1139 ppm in the site of Tiznit. Carbon rate varies between 1.03% and 4.57%.

Observation of root fragments of *Argania spinosa* (L.) Skeels, prepared by the technique of Philips and Hayman[82] (1970) and stained with Cresyl blue, allowed to highlight the presence of mycorrhizal structures. Thus, among the fragments of the roots of this species, fungal hyphae are internal and external, the vesicles are of regular or irregular shapes and sometimes arbuscules are present in the root cells. Endophytes are also present in the roots (Fig. 1 and 2).

Mycorrhizal frequency of *Argania spinosa* roots varies from one site to another. It is maximap at of Taroudant and Toufalazte sites (F = 100%), 96.66% at the sites of Elkhssass and Essaouira (96.66%), and between 50 and 33.33% in Tamanar sites Ait Melloul and Tiznit (Fig. 3).

The mycorrhizal intensity is high at the site of Toufalazte (57%) and low in Ait Melloul and Tiznit sites (1.83% and 1.93% respectively) (Fig. 3).

Furthermore, the arbuscular contents are high at the site of Toufalazte (24.16%). By cons, these levels are low in the site of Tiznit 0.082% (Fig 3). Vesicles contents also show variations from one site to another, they vary between 0.21% (Ait Melloul) and 34.14% (Toufalazte) (Fig. 4).

Regarding the estimation of the density of spores in the rhizosphere of *Argania spinosa* developing in the studied sites (Fig. 5), the average recorded varies from one site to another. It is raised in the site of Touflaazte (188 spores / 100 g soil, and relatively low in the level of Tiznit and Ait Melloul sites 28 and 44 spores / 100 g soil respectively.

Species richness varies from site to site, it is 14 species in the site of Toufalazte and 12 species in Taroudant and Elkhssass sites, followed by the site of Essaouira (10 species), the sites of Taghazoute and Tamanar (8 species). Ait Melloul and Tiznit sites have almost the same species richness, respectively 4 and 5 species (Fig. 6). A first identification was based on morphological criteria of spores, allowed to isolate 36 species of mycorrhizal fungi (Fig. 7 and 8). Seventeen species belong to the genus *Glomus* (*Glomus versiforme* **(**(P. Karsten) S.M. Berch), *Glomus macrocarpum* (Tul. & C. Tul.), *Glomus minutum* (Blaszk., Tadych & Madej) , *Glomus mosseae* **(** (Nicol. & Gerd.) Gerd. & Trappe), *Glomus aggregatum* (N.C. Schenck & G.S. Sm. emend. Koske) , *Glomus etunicatum* (W.N. Becker & Gerd.), *Glomus proliferum* (Dalpe & Declerck), *Glomus clarum* (Nicol. & Smith), *Glomus intraradices* (N.C. Schenck & G.S. Sm.), *Glomus claroideum* (N.C. Schenck & G.S. Sm.), *Glomus monosporum* (Gerd. & Trappe), *Glomus multicaule* (Gerd. & B.K. Bakshi), *Glomus aureum* (Oehl & Sieverd.), *Glomus diaphanum* (J.B. Morton & C. Walker),*Glomus* sp1,*Glomus* sp2,*Glomus* sp3), nine to the *Acaulospora* genus (*Acaulospora denticulata* (Sieverd. & S. Toro) , *Acaulospora gedanensis(* Blaszk.), *Acaulospora foveata* (Trappe & Janos), *Acaulospora alpina* (Oehl, Sykorova & Sieverd.), *Acaulospora rehmii* (Sieverd. & S. Toro), *Acaulospora minuta* (Oehl, Tchabi, Hount., Palenz., Sánchez-Castro & G.A. Silva), *Acaulospora sp1, Acaulospra sp2, Acaulospora sp3*), three others belong to the *Scutellospora* genera (*Scutellospora castanea* (C. Walker), *Scutellospora heterogama* ((T.H. Nicolson & Gerd.) C. Walker & F.E. Sanders), *Scutellospora nigra* (J.F. Redhead) C. Walker & F.E. Sanders), and *Entrophospora* (*Entrophospora kentinensis* (C.G. Wu & Y.S. Liu), *Entrophospora* sp.), and one belongs to *Pacispora* genus (*Pacispora robiginia* (Sieverd. & Oehl), *Gigaspora* (*Gigaspora decipiens* (I.R. Hall & L.K. Abbott)). *Glomus etunicatum* is the most abundant species, its frequency of occurrence reaches 16.26%, followed by *Acaulospora gedanensis* (10.52%) and *Glomus macrocarpum* (8.37%).

Table 1: Physico-chemical proprieties of the sampling soils

Localities	pH	Electrical conductivity (mmhos/cm) (1/5)	Total limestone (%)	Organic matter (%)	Carbon (%)	Ammoniacal nitrogen (ppm)	Nitrate nitrogen (ppm	Mineral nitrogen (ppm)	Assimilable phosphore (ppm)	Assimilable potassium (ppm)
Essaouira	8.22	0.29	3	7.87	4.57	21.2	45.9	67.1	62	423
Taghazoute	8.38	0.39	36.40	4.26	2.47	16.2	44.6	60.8	38	452
Ait melloul	8.37	0.21	0.80	1.80	1.05	23.4	98.0	121.4	20	834
Tamanar	8.54	0.17	11.50	3.28	1.90	25.2	53.3	78.5	14	470
Toufalazte	8.40	0.30	11.40	4.72	2.74	27.7	141.4	169.1	29	1127
Elkodya El bayda (Taroudant)	8.36	0.17	2.00	1.77	1.03	34.9	48.4	83.3	20	517
Tiznit	8.47	0.60	23.70	4.92	2.85	23.4	191.0	214.4	66	1139
Elkhssass	7.65	0.28	3.00	5.68	3.30	35.64	57.04	92.7	32	1556

Fig. 1: Mycorrhizal roots of argan tree showing vesicules (v), endophytes (e), hyphae extra-and intra-radicular (he and hi) and spores of MA (s) (G × 400)

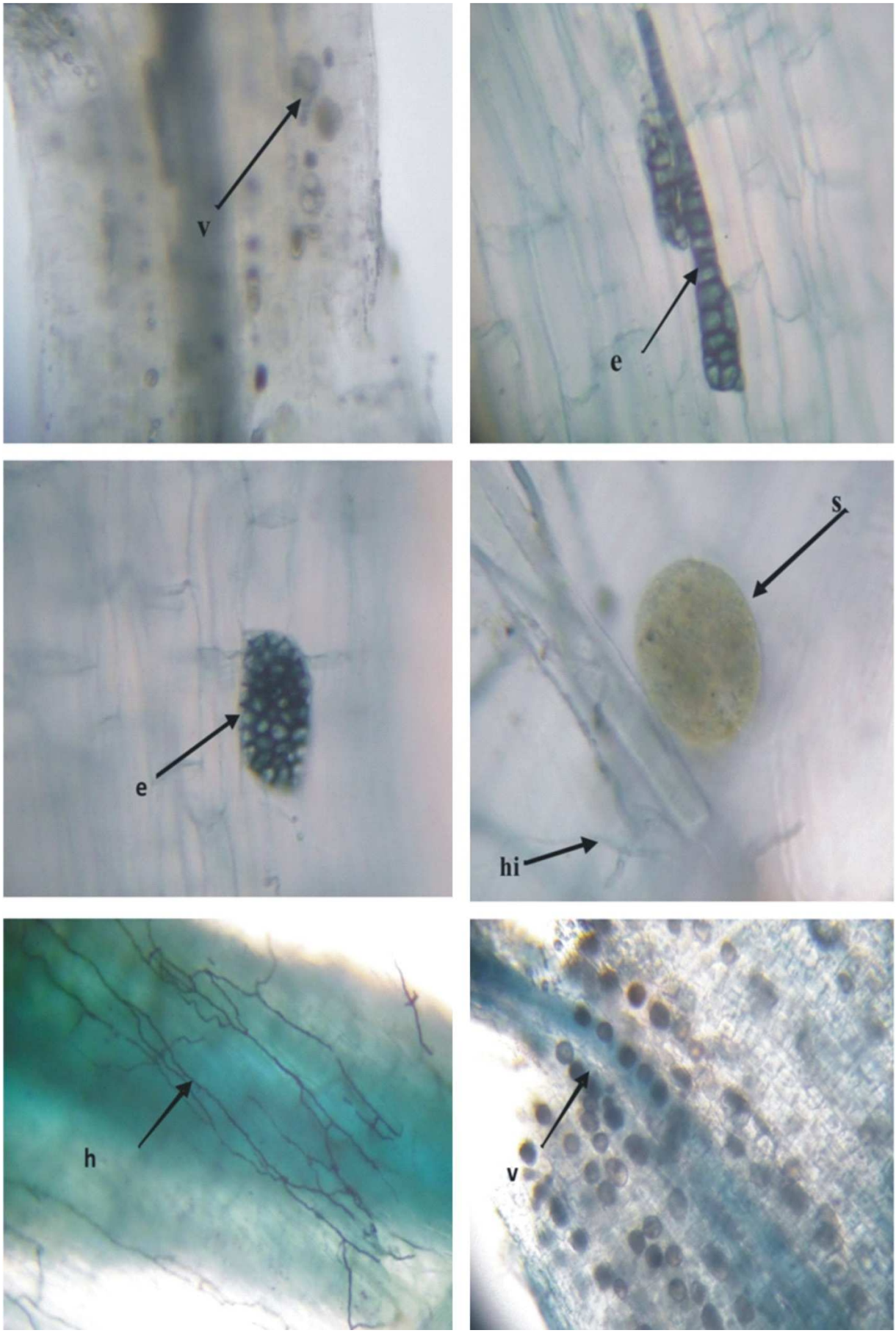


Fig. 2: Structures of mycorhizae in the level of the *Argania spinosa* roots. Arbuscules (a); endophytes (e) ; internal hyphae (ih) ; external hyphae (he) ; vesicules (v) (M ×10) and (M ×400)

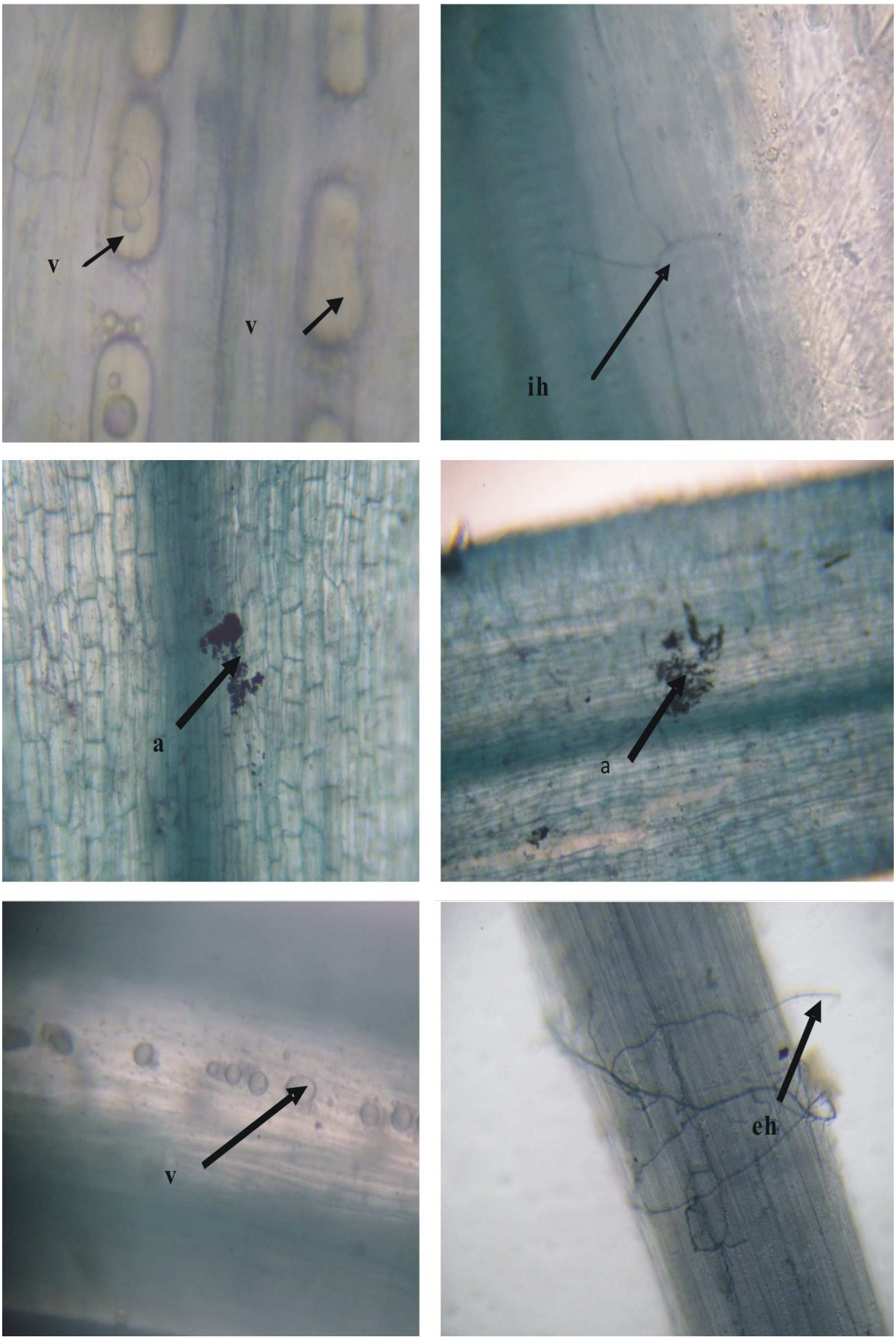


Fig. 3: Mycorrhizal frequency and intensity of *Argania spinosa* roots in the study sites

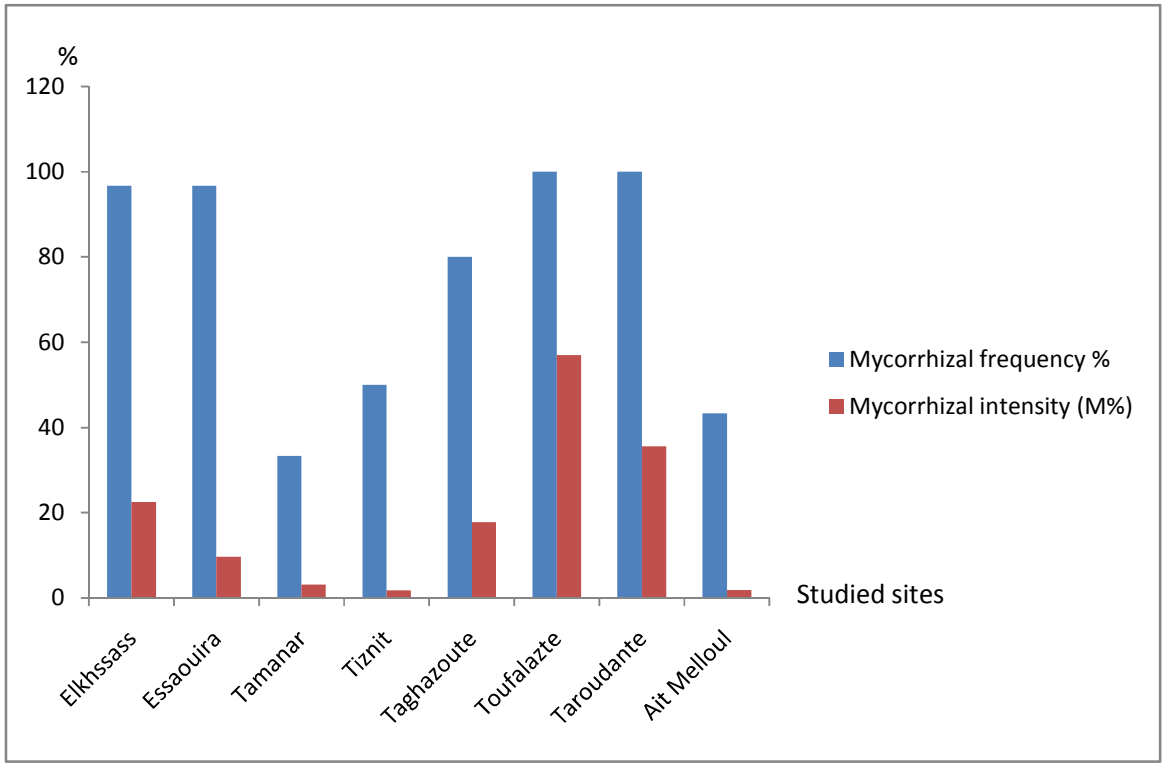


Fig. 4: Arbuscular and vesicular contents (%) in the roots of *Argania spinosa* in the studied sites

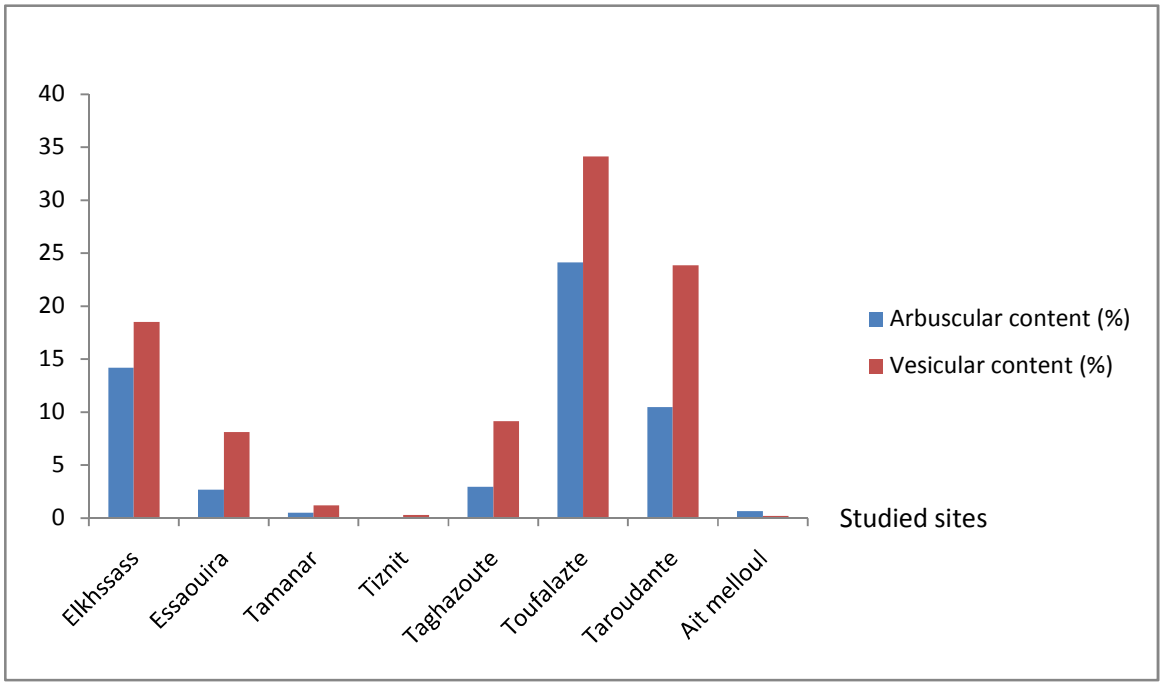


Fig. 5: Spores number of endomycorrhizal species in the studied sites

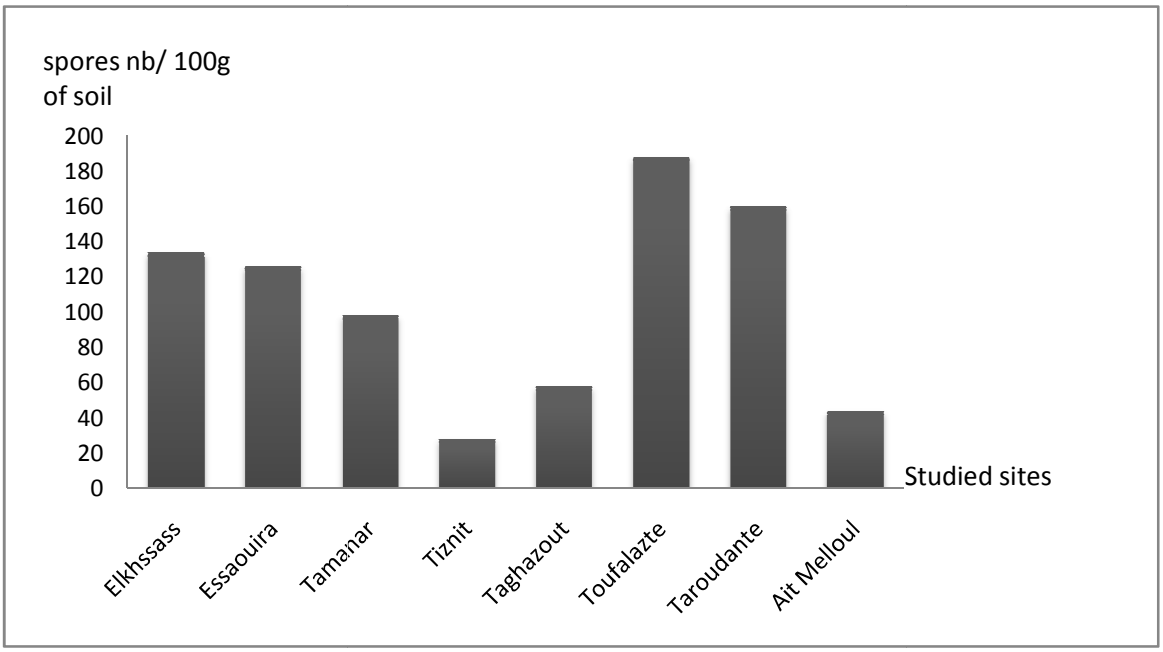


Fig. 6: Specific richness of the endomycorrhizal species in the rhizosphere of *Argania spinosa* in the studied sites

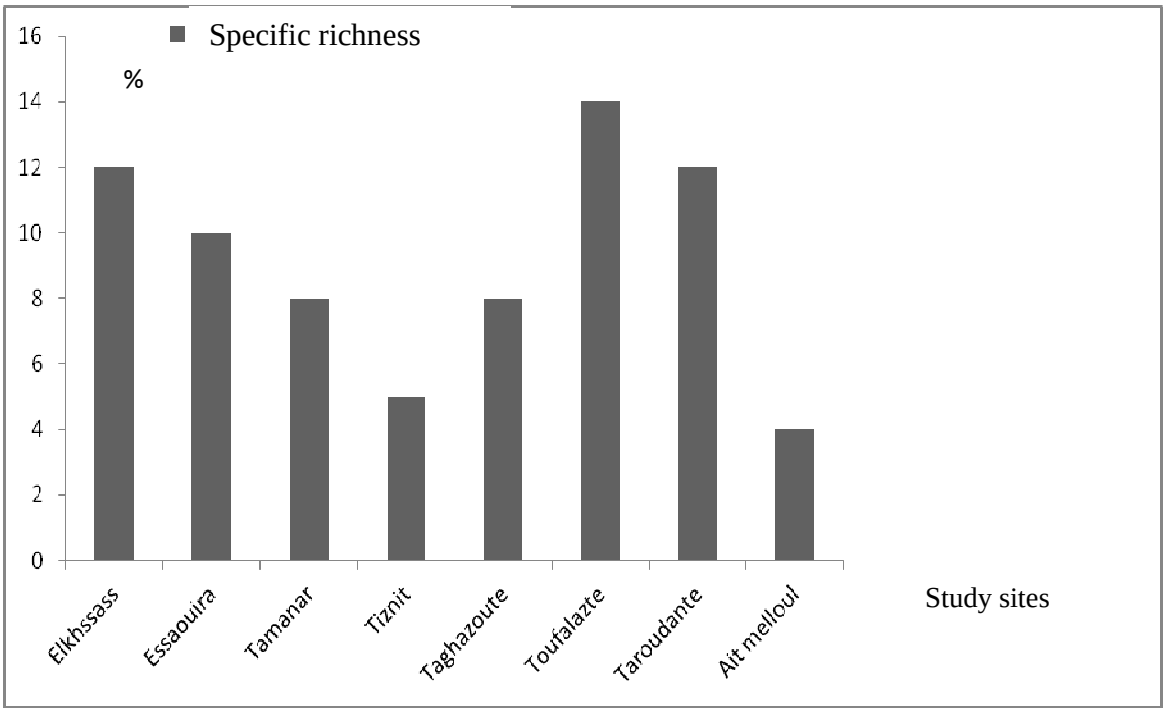


Fig. 7: Appearance frequency of the isolated mycorrhizal species from each site

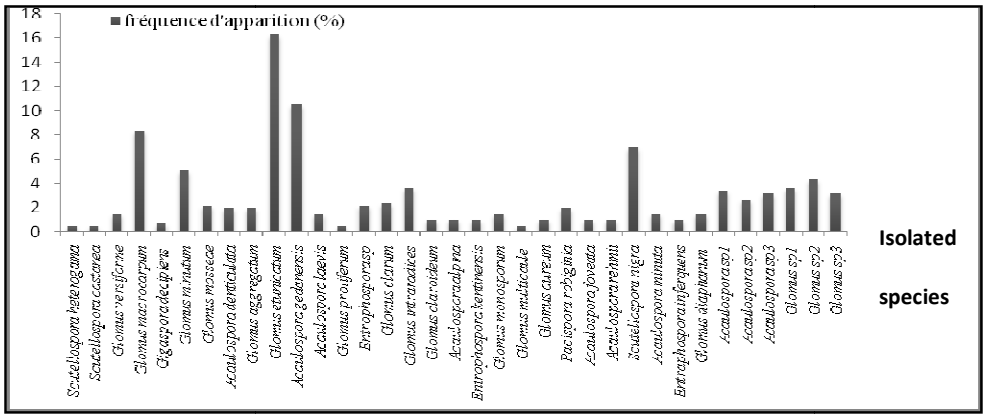
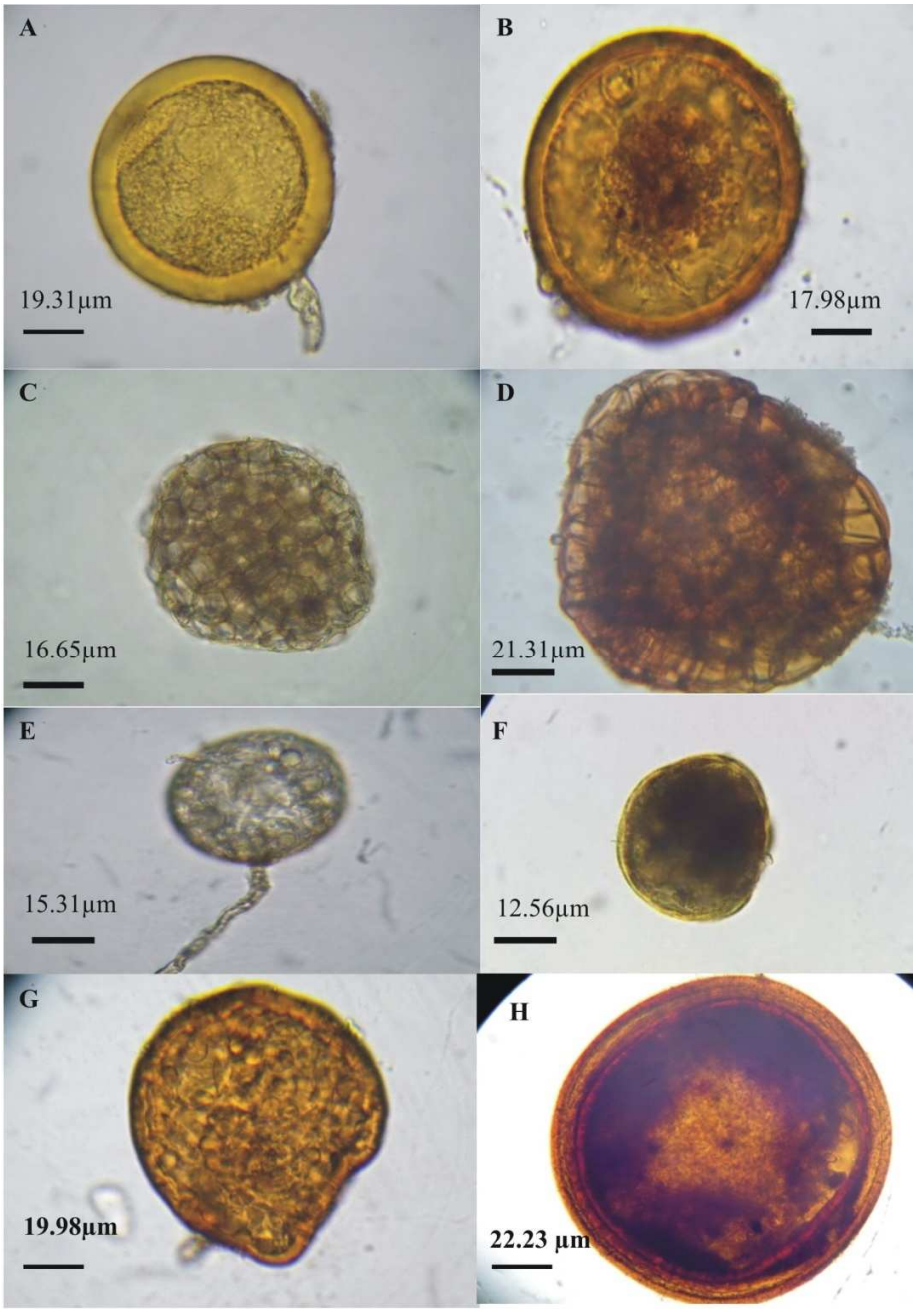


Fig. 8: Some endomycorrhizal species isolated from the rhizosphere of *Argania spinosa*



Glomus macrocarpum (A) ; *Glomus versiforme* (B) ; *Acaulospora* sp1(C) ; *Acaulospora denticulate* (D) ; *Glomus intraradices* (E) ; *Glomus* sp (F) ; *Glomus versiforme* (G) ; *Acaulospora laevis* (H).

DISCUSSION AND CONCLUSION

The surveys in the studied sites have shown that in all study sites, the roots of the argan tree are carriers of endomycorrhizal structures: vesicules, arbuscules, internal and external hyphae. The presence of these characteristic structures of endomycorrhizae classify the Argan tree as a mycotrophic species[55,75,77].

Estimation parameters of the root colonization degree change from one site to another. Thus, at values of mycorrhization frequency (F) and root colonization rate or mycorrhizal intensity (M), the highest can be up to 100% (Toufalazt and Taroudant) for F and 57% (Toufalazt) for M, whereas they are relatively weak at the Tamanar and Ait Melloul sites. Furthermore, the highest arbuscular and vesicular contents were also registred at the site of Toufalazat 24.16% and 14% respectively.

The variability of the mycorrhization frequency from one site to another may be explained by differences in the physicochemical properties of the substrates. The highest mycorrhizal frequencies are noted at the Toufalazt and Taroudant sites with low levels of available phosphorus, 20 and 29 ppm respectively. In this sense, the literature indicates that the sites where the species are highly endomycorrhizal could therefore have a phosphorus and nitrogen deficiency, the plant research mycorrhizae to solve this nutrient. These results are also consistent with those reported by Harley and Smith[45], Vivekanandan and Fixen[107], Kachkouch *et al*[52,53]., Sghir *et al*[93,94]., those state that mycorhization frequencies are high in low levels of total phosphorus in soils. But this relation doesn't appear to be valid in other sites. Indeed, mycorrhizal frequencies lower than 50% were observed at the Tiznit site which has a substrate with a phosphorus content of 66 ppm, the highest content of the prospected sites.

It was also noted a negative correlation between the intensity of mycorrhizal root cortex and available phosphorus concentration in soil[21,93]. The highest mycorrhizal intensity (57%) was observed in the roots of argan developing in the Toufalazt site, with a phosphorus content of 20 ppm. The mycorrhizal intensities of roots are 10% and 1.93% at the Essaouira and Tiznit, richer in phosphorus (respectively 62 and 66 ppm). At the Carob tree, no reaction was observed between high levels of mycorrhizal intensity and the low amount of phosphorus in the soil[34].

In some sites, mycorhization frequencies are in phase with the spores densities. The richest sites in spores (the number exceeds 140 spores per 100 g of soil), are those where mycorhization frequencies are highest: Taroudant and Toufalazte sites. The specific richness was noted at these two sites, is 14 and 12 species respectively. Similarly, the site of Tiznit, where the roots of argan trees have shown the frequency of the lowest mycorhization frequency, is less rich in spores (28 spores) and species richness (5 species). However, at some sites, Tamanar case, the number of spores (98 spores) and species richness (8 species) is average compared to other surveyed sites, but the frequency of mycorrhization is low (33.33%).

Some authors[68,108] have reported that there is no relationship between the number of spores and intensity of mycorrhiza. By cons, other authors, found a suitable correlation in often controlled conditions among the population of spores and infection of roots[50]. In certain sites, for example, case of Taroudant and Toufalazte, the mycorrhizal intensities are correlated with spore densities. These sites are more rich in spores (the number exceeds 140 spores per 100 g of soil) and roots mycorhization of argan have high intensities. The richness at these two sites, 14 and 12 species respectively, is also the most important. Similarly, the site of Tiznit where the roots of argan showed a mycorrhizal intensity of 1.8 %, is less rich in spores (28 spores) and species richness (5 species).

However, at the site of Tamanar, for example, the number of spores (98 spores) and species richness (8 species) is average compared to other surveyed sites, but the intensity of mycorrhizal roots is lower. Kachkouch *et al*[53]., noted a large number of spores (1208) in a site where the roots of the olive are less infected (5.06%). The lowest number of spores (104) was registered in another site where roots are infected with 12.4%. According to Jasper *et al*[49]., the weak relationship between the endomycorrhizae formation and the quantity of the isolated spores is due to the fact that some propagules would be dormant. In all cases, according to Diagne and Ingleby[28], it is risky to bring the infectious activity of AMF of a given soil at the number of spores present in this soil. Sporulation may depend on the species AMF, edaphic characteristics of the soil and climatic conditions.

The works of Johnson *et al*[51]., found positive correlations between increasing organic matter (including some elements such as carbon and nitrogen) and endomycorrhizal species diversity, especially Glomales. The site of Essaouira which presented the highest organic content (7.87%) contains 10 species. But, the opposite effect was observed in Taroudant site. This site is rich in endomycorrhizal species (14 species), but the substrate is less rich in organic matter and organic carbon (1.77% and 1.03). Work and print these observations and provide that the combination of a low organic matter content (carbon and nitrogen) is relatively abundant and a greater diversity of Glomales.

The highest density of spores (188 spores / 100 g soil) was observed in the site of Toufalazt. This number is still low compared to that reported by El Maati *et al*[35].,: 1127.66 spores / 100 g soil at the rhizosphere of *Argania spinosa* in Ait Baha. Nouaim[75] reported values ranging from 900-2080 spores per 100 g of soil collected in the basements *Argania spinosa* in southwestern Morocco. At the rhizosphere of Carob tree the Ourika Valley[80] and Oudmine[35], this number is respectively 2100 and 2014 spores per 100 g of soil. El Asri *et al*[34]., noted a variable density of spores, varies between 84 and 160 spores / 100 g soil, in the rhizosphere of Carob tree developing in five provinces (Taroudant, Khenifra, Azilal, Beni Mellal and Nador), spread from the East to South West of Morocco. In rhizosphere of palm tree in Tafilalt[21] or Tafilalt and Zagora[94], the number of spores has varied respectively between 1900 and 295 and between 132 and 80 spores per 100 g soil.

In general, the number of spores listed in the studied sites is more or less identical to that found in the rhizosphere of certain plant species growing in different regions of Morocco, case of *Cupressus atlantica*[78], *Tetraclinis articulata*[1] and *Anthyllis cytisoides* , *Stipa tenacissima, Retama sphaerocarpa*[86]. *Quercus rotundifolia- Tetraclinis articulata*[10]. *Populus alba*[101], *Juncus maritimum*[100], *Lycium europaeum*[105], *Olea europaea* spp. *Oleaster*[93] and *Olea europaea*[52,53].

The registered differences may be due to the physico-chemical and microbiological properties of soils of arid and semi-arid areas[7,48,51], fluctuations in microclimate[25,56], vegetation cover[16] the sampling season[21,42], the spore formation process, and the deterioration of germination[96].

Enumeration of the spores of mycorrhizal fungi showed a predominance of *Glomus* (13 species). This dominance has also been reported in various African ecosystems[9,29,31,52]. In Morocco, for example, this dominance was observed in the rhizosphere of the olive tree[24,52,53], of oleaster[93], palm tree[21,94], *Ceratonia siliqua*[34,102], *Populus alba* and *Juncus maritimum*[100,101]. According to Beaver *et al*[17]., the dominance of the Glomus genus is due to its ability to produce more spores in a shorter time than other genres such as *Gigaspora* and *Scutellospora.* This abundance is also due to its adaptation to drought and soil salinity[18,43].

The fungal species associated with the roots of argan trees belonging to endomycorrhizae are very varied. In soils of southern Morocco of argan tree, different species of endomycorrhizal fungi were highlighted. Preliminary identification (based solely on spores morphological criteria), led to the isolation of 35 mycorrhizal fungal species *Glomus versiforme* (P. Karsten) S.M. Berch), *Glomus macrocarpum* (Tul. & C. Tul.) , *Glomus minutum* (Blaszk., Tadych & Madej) , *Glomus mosseae* ((Nicol. & Gerd.) Gerd. & Trappe), *Glomus aggregatum* (N.C. Schenck & G.S. Sm. emend. Koske) , *Glomus etunicatum* (W.N. Becker & Gerd.), *Glomus proliferum* (Dalpe & Declerck), *Glomus clarum* (Nicol. & Smith), *Glomus intraradices* (N.C. Schenck & G.S. Sm.), *Glomus claroideum* (N.C. Schenck & G.S. Sm.), *Glomus monosporum* (Gerd. & Trappe), *Glomus multicaule* (Gerd. & B.K. Bakshi), *Glomus aureum* (Oehl & Sieverd.), *Glomus diaphanum* (J.B. Morton & C. Walker), *Glomus* sp1, *Glomus* sp2, *Glomus* sp3, *Acaulospora denticulata* (Sieverd. & S. Toro) , *Acaulospora gedanensis* (Blaszk.), *Acaulospora foveata* (Trappe & Janos), *Acaulospora alpina* (Oehl, Sykorova & Sieverd.), *Acaulospora rehmii* (Sieverd. & S. Toro), *Acaulospora minuta* (Oehl, Tchabi, Hount., Palenz., Sánchez-Castro & G.A. Silva), *Acaulospora* sp1, *Acaulospra* sp2, *Acaulospora* sp3, *Scutellospora castanea* (C. Walker), *Scutellospora heterogama* ((T.H. Nicolson & Gerd.) C. Walker & F.E. Sanders), *Scutellospora nigra* (J.F. Redhead) C. Walker & F.E. Sanders, et *Entrophospora* (*Entrophospora kentinensis* (C.G. Wu & Y.S. Liu), *Entrophospora sp*), *Pacispora robiginia* (Sieverd. & Oehl), *Gigaspora decipiens* I.R. Hall & L.K. Abbott, divided into 6 genera (*Glomus, Scutellospora, Entrophospora, Pacispora, Gigaspora, Acaulospora*). *Glomus etunicatum* is the most abundant species, its frequency of occurrence reaches 16.26%, followed by

Acaulospora gedanensis (10.52%) and *Glomus macrocaparum* (8.37%). Kenny *et al*[55]. (2009) noted the presence at 9 argan sites (Aghroud, Teferdine, Essaouira, Bouzemour, Tamaït, Barrage Abdelmoumen, Ikherdiden, Douar Ighaline and the Reserve of IAV in Agadir) a great diversity of endomycorrhizal species. Only certain species were determined: *Glomus aggregatum*, *G. constrictum*, *Gigaspora margarita*, *Entrophospora infrequens* and other unidentified species (three species of the genus *Scutellospora* and another genus *Acaulospora*). El Maati *et al*[35]., reported at five argan sites (Tizi, Tartatin, Tirhmi, Ait Baha, Tiguert and Alma) the presence of *Glomus fasciculatum* and other unspecified species belonging to the genera *Glomus* (4 *Glomus* sp.) And *Gigaspora* (*Gigaspora* sp.). Morpho-anatomical analysis of spores isolated from argan tree in 12 sites in the regions between Marrakech, Essaouira and Agadir enabled to note the presence of several AMF genera belonging to *Glomus* genera *Gigaspora, Scutellospora and Acaulospor*a[90]. In Algeria, three morphotypes, unidentified, were reported in the rhizosphre of old argan trees of Stidia (Wilaya de Mostaganem)[2].

The identification of the isolated mycorrhizal species from organ rhizosphere was performed on the basis of the morphological characteristics of the mycorrhizal spores. This identification is very difficult and takes time and patience to bring the diversity of morphotypes spores that may be encountered in the rhizosphere of the argan tree. Sanguin *et al*[90]., used phenotypic and molecular approaches to reveal the diversity of fungi associated endomycorrhizal argan. The results revealed a much wider variety of symbionts and also noted a certain level of specificity in symbiotic association Argan / Glomeromycota. A study of mycorrhizal diversity, based mainly on molecular identification of mycorrhizal species, is to be undertaken, including the use of new sequencing technologies.

The high mycorrhizal dependency of argan and possible use as a basis for endomycorrhizae inoculation to improve its growth have been demonstrated[32,35,75]. This controlled endomycorhization could be a biotechnological tool of choice to enhance the production of the argan tree, however its implementation in nursery is still very limited due to the lack of interest given to this biotechnology. Production of well mycorrhizal plants at the exit of the nursery is an essential condition for the success of a plantation. According to Ouahmane *et al*[79]., and Johnson *et al*[51]., the effectiveness and sustainability of controlled mycorrhization operations are dependent on edaphic origin of the inoculum, preferences based on indigenous native species, and interaction with the genetic diversity of the host plant. In this sense, in the USA, the inoculation of Citrus grown in nurseries with MVA fungi has become a common practice[63].

Inocula based on endomycorrhizae that will be used to produce vigorous argan plants in nursery should be mastered, it is important to understand the mechanisms involved in producing these beneficial inoculum. The inoculum that will be designed to inoculate the plants should be able to stimulate growth and installation of plants in different soil and climatic zones but also increase the availability of nutrients for these plants in a poor substrate. Monitoring of development of mycorrhizal plants (length of the aerial part, number of branches, development of root mass, determination of mycorrhizal parameters) after transplantation and the evolution of the inoculum in the field (multiplication and endomycorrhizal species adaptation) versus time in the rhizosphere of plants is desirable. The effects of the interaction plant / mycorrhizal take time to emphasize.

Reinforcing the argan mycorrhizal plants can be achieved by the use of other inoculants based on filamentous fungi bio-fertilizers, bio-stimulators and bioprotectant. Positive results were observed on tomato plants[24], date palm tree[95], and carob tree inoculated with endomycorrhizae and *Trichoderma*.

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