



DIVERSITY OF ENDOMYCORRHIZAL FUNGI ASSOCIATED WITH BARLEY (*HORDEUM VULGARE*) IN NORTH WEST OF MOROCCO

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Abstract

Surveys were carried out in April-May, 2017 in plots of barley located in different localities of northwest Morocco. Root and soil samples were taken under barley feet growing in these plots. The spores were isolated from the soil samples by wet sieving and the roots were stained with Cresyl blue. The observation of barley plants roots fragments from the sites of the localities studied made it possible to highlight the presence of several endomycorrhizal structures, with a frequency of root mycorrhization which varies between 73.33 and 100%. The mycorrhizal intensity of the roots is high at the level of locality Mogren B and low (3.13%) at the level of Souk El Arbaâ, respectively of 17.26 and 3.13%. The contents of the roots in arbuscules are higher (12.286%) at the level of the locality of Mogren B and low at the level of that of Souk El Arbaâ (1.596%). As for the vesicular contents, they vary from one site to another between 0% and 5.511%. The density of spores in the rhizosphere of barley plants in the studied localities varies between 13.33 (locality of Mogran A) and 35 spores / 100 g of soil (locality of Ouazzane). The preliminary morphological identification of these spores made it possible to note the presence of 90 different morphotypes divided into twelve genera: *Glomus* and *Acaulospora* (28 species for each); *Entrophospora*, *Funneliformis* and *Scutellospora* (5 species for each); *Claroideoglomus*, *Diversispora*, *Pacispora*, *Racocetra* and *Rhizophagus* (3 species for each); *Gigaspora* (2 species); *Sclerocystis* (one species). *Glomus versiforme*, *Glomus macrocarpum* and *Scutellospora nigra* is spread in the different studied sites with a dominance compared to *Glomus deserticola*, *Rhizophagus intraradices*, *Funneliformis mosseae*, *Racocetra castanea* which are recorded in sites and not in others whereas certain species are specific to a single site such as *Acaulospora alpina*, *Acaulospora denticulate* and *Acaulospora mellea*.

Key words: Barley, Muscular mycorrhizal arbuscular, diversity.

Introduction

Barley (*Hordeum vulgare*) is a cereal that belongs to the Poaceae family. It is cultivated in several regions of the world on a large type of soils and climates and occupies the fourth rank in terms of cereal production after rice, corn and wheat, with a production of 137 Millions (FAO STAT, 2014).

Barley is one of the most important species in Morocco and it is cultivated in a wide range of varied agro climatic conditions. It plays a vital role in both human and animal nutrition (Alaoui, 2003).

More than 95% of terrestrial plants can live in symbiosis with symbolic mushrooms (Smith and Read, 1997). Basidiomycetes, Ascomycetes and Glomeromycetes

are the only three groups of known fungi capable of contracting a symbiotic association with the roots of plants to form mycorrhizae (Garbaye, 2013).

The mycorrhizal symbiosis and in particular the arbuscular mycorrhizal symbiosis, which concerns more than 80% of terrestrial plants and almost all cultivated plants, allows both better growth and better resistance of plants to many biotic (Dalpé, 2005; Gianinazzi *et al.*, 2010; Pozo *et al.*, 2013) and abiotic stresses (Smith and Read, 2008; Debiane *et al.*, 2008, 2009; Campagnac *et al.*, 2010; Miransari *et al.*, 2010). The potential of arbuscular mycorrhizal fungi (MACs) as bio-control agents has been described for various root infections (Khaosaad *et al.*, 2007; Fiorilli *et al.*, 2011; Castellanos-Morales *et al.*, 2012) but very little in the protection against leaf diseases (Gallou *et al.*, 2011; Campos-Soriano *et al.*, 2011; Li *et al.*, 2013;

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Maffei *et al.*, 2014) and not at all in the case of biotrophic fungal pathogens.

In this work, the diversity of arbuscular mycorrhizal fungi (CMA) is highlighted in the rhizosphere of barley plants growing in different localities in northwest Morocco. In Morocco, in the literature, mycorrhization of barley has not yet been reported.

Materials and methods

Surveys and sampling

Surveys were conducted in different northern communities west of Morocco, two paths were followed: 1/ Kenitra, Sidi Yahia, Sidi Slimane, Sidi Kacem and Had Kourt and 2/ Kénitra, Souk Larbaa, Larache, Belksiri and Ouazzane. Six stations containing plots of barley were chosen in these localities. The collected soils are placed in white plastic bags bearing the indications relating to their origin (place and date of sampling) and brought back to the laboratory.

Measuring the root mycorrhizal rate

The roots were prepared according to the method of Koské and Gemma, 1989). They were first washed with water; the finest were cut to a length of 1 cm then immersed in a 10% KOH solution and placed in the oven at 90°C for one hour to remove intracellular constituents. At the end of this period, the roots were rinsed and transferred to a solution of H₂O₂ (hydrogen peroxide) for 20 min at 90°C until the roots whiten. The roots were then rinsed, then stained with 0.05% cresyl blue by submersion (Philips and Hayman, 1970 modified) modified at 90°C for 15 min.

After a final rinsing, thirty fragments of colored roots 1 cm in length were chosen at random and mounted in groups of 10 to 15 segments in the glycerin between blade and coverslip (Kormanik and McGraw, 1982). The remaining roots were kept in water or in acidic glycerol. The slides were observed under a microscope, each fragment being carefully checked over its entire length, at magnifications of ×100 and ×400 to note the mycorrhizal structures: arbuscules, partitions of hyphae, vesicles, intra- and intercellular hyphae, extramatrix hyphae and even endophytes..

The frequency and the arbuscular and vesicle contents of endomycorrhizal fungi inside the root bark are measured by assigning a mycorrhization index ranging from 0 to 5 (Derkowska *et al.*, 2008):

0: absence; 1: traces; 2: less than 10%; 3: 11 to 50%; 4: 51 to 90%; 5: more than 91%

Mycorrhizal frequency

The mycorrhizal frequency (F%), reflects the

importance of infection of the root system of the host plant by mycorrhizal fungi :

$$F\% = 100 (N - N_0) / N$$

With N: number of fragments observed and N₀: number of non-mycorrhized fragments.

Mycorrhizal intensity

The intensity of mycorrhization (M%) expresses the portion of the colonized cortex in relation to the entire root system:

$$M\% = (95n_5 + 70n_4 + 30n_3 + 5n_2 + n_1) / N$$

In this formula n₅, n₄, n₃, n₂ and n₁ respectively denote the number of fragments noted 5, 4, 3, 2 and 1.

Arbuscular content (A%) of the mycorrhizal part

$$A\% = (100m_{A3} + 50m_{A2} + 10m_{A1}) / 100$$

Where m_{A3}, m_{A2}, m_{A1} are the % affected respectively of the notes A₃, A₂, A₁, with m_{A3} = (95 n₅ A₃ + 70 n₄ A₃ + 30 n₃ A₃ + 5 n₂ A₃ + n₁ A₃) / N. Similarly, for A₁, A₂. In this formula, n₅A₃ represents the number of fragments noted 5 with A₃; n₄A₃ the number of fragments noted 4 with A₃.

A₀: no trees; A₁: few 10% lowercase; A₂: moderately abundant arbuscules 50%; A₃: very abundant arbuscules: 100%.

Vesicular content (V%)

The vesicular content is calculated in the same way as that of the arbuscular content:

$$V\% = (100 m_{V3} + 50 m_{V2} + 10 m_{V1}) / 100$$

Where m_{V3}, m_{V2}, m_{V1} are the % affected respectively of the notes V₃, V₂, V₁ m_{V3} = (95 n₅ V₃ + 70 n₄ V₃ + 30 n₃ V₃ + 5 n₂ V₃ + n₁ V₃) / N. Similarly, for V₁, V₂,

In this formula, n₅V₃ represents the number of fragments noted 5 with V₃; n₄V₃ the number of fragments noted 4 with V₃;

V₀: no vesicles; V₁: few vesicles 10%; V₂: moderately abundant vesicles 50%; V₃: very abundant vesicles: 100%.

Spore extraction

The spores are extracted according to the wet sieving method described by Gerdemann and Nicolson, (1963). In a 1L beaker, 100 g of each composite soil sample is submerged in 0.5 L of running water and stirred for 1 min with a spatula. After 10 to 30 seconds of decantation, the supernatant is passed through four superimposed sieves with decreasing mesh (500, 200, 80 and 50 μm). This operation is repeated twice. The content retained by the 200, 80 and 50 μm sieves is distributed into two tubes and

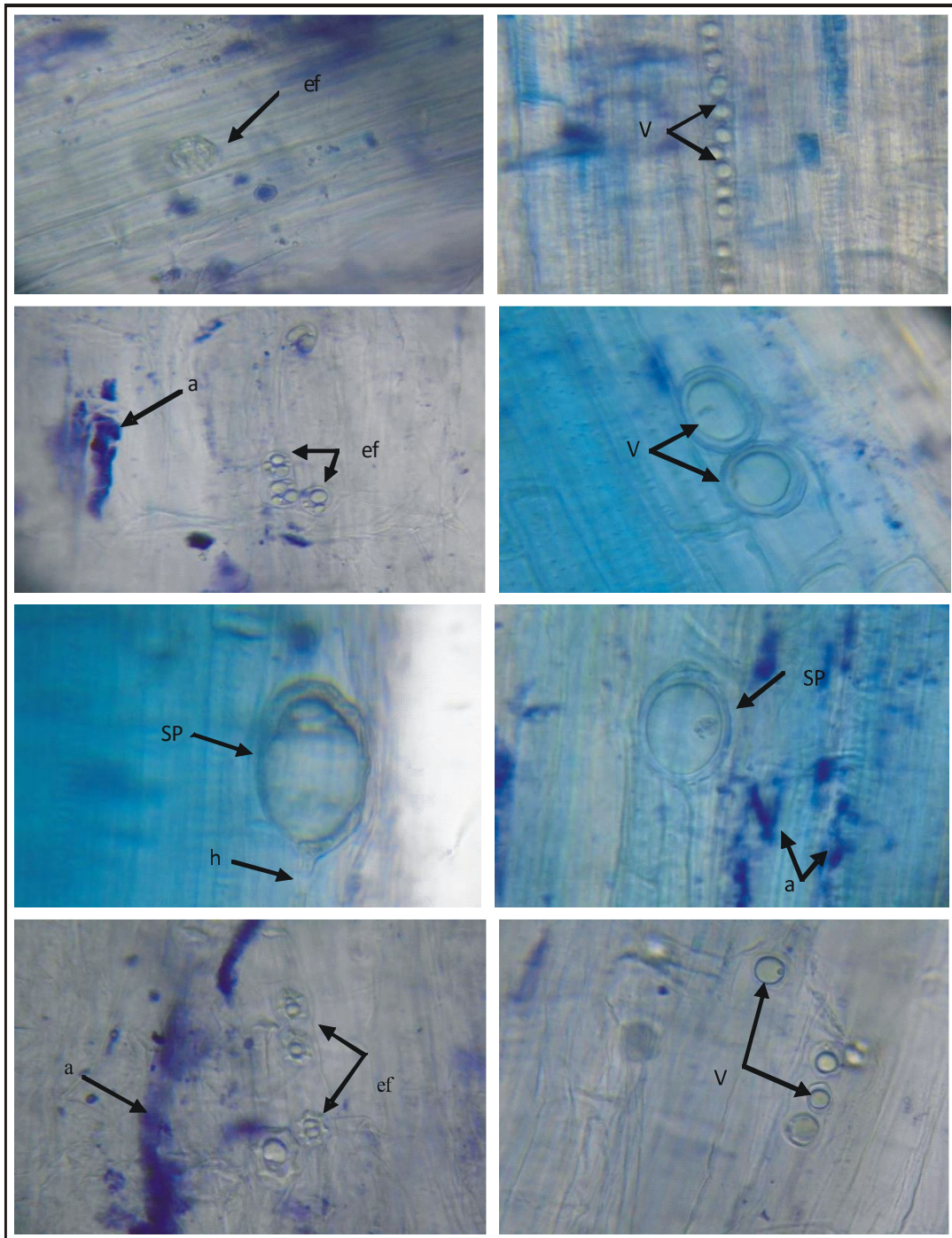


Fig. 1: Different endomycorrhizal structures of the roots of barley plants. (SP: spores; V: vesicles; ef:

centrifuged for 4 min at 9000 rpm. The supernatant is discarded and a viscosity gradient is therefore created by adding 20 ml of a 40% sucrose solution to each centrifuge tube (Walker *et al.*, 1982). The mixture is quickly stirred and the tube put back into the centrifuge for 1 min at 9000 rpm.

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 and then disinfected with an antibiotic

solution (Streptomycin). The spores are then collected with a little distilled water in an Erlenmeyer flask.

Specific richness and spores appearance frequency

The specific richness represents the total number of species observed by sampling site and the frequency of appearance of the species corresponds to the percentage of sites where each species is detected.

Results

Microscopic examination of the barley plants roots

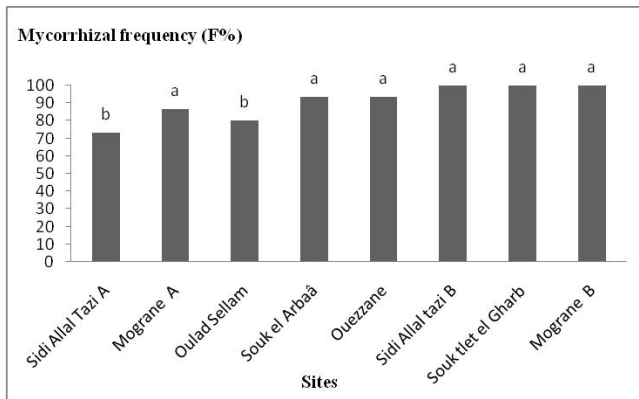


Fig. 2: Frequency of barley root mycorrhization at the studied sites.

fragments prepared by the technique of Philips and Hayman, (1970) and stained with cresyl blue, showed that all the roots were colonized by mycorrhizal fungi and that the cytological organization of these mycorrhizae was of the arbuscular type, with the presence of vesicles of different shapes (globular, oval and amorphous) and endomycorrhizogenic hyphae (Fig. 1).

The rate of colonization of barley roots varies from site to site (Fig. 2). This colonization is total at the Sidi Allal Tazi B, Souk Tlet El Gharb and Mograne B sites (with F = 100%) and high at the Souk El Arbaâ, Ouezzane (with F = 93.33%), in Mograne A (F = 86.66%) and in Oulad Sellam (F = 80%) and average in Sidi Allal Tazi A (F = 73.33%).

The intensity of mycorrhization or the mycorrhizal intensity corresponds to the percentage of the mycorrhized root cortex, the highest value was observed in the roots of barley plants growing at the Mograne B site (17.26%) and the lowest in those of the plants. the Souk el Arbaâ (3.13%) and Sidi Allal Tazi A (4.03%) sites (Fig. 3).

The arbuscular and vesicle contents of barley roots are higher at the Mograne B site (A = 12.28% and V = 5.51%) and low in the roots of barley plants at the site of Souk El Arbaâ, with a percentage of 1.59 in arbuscules

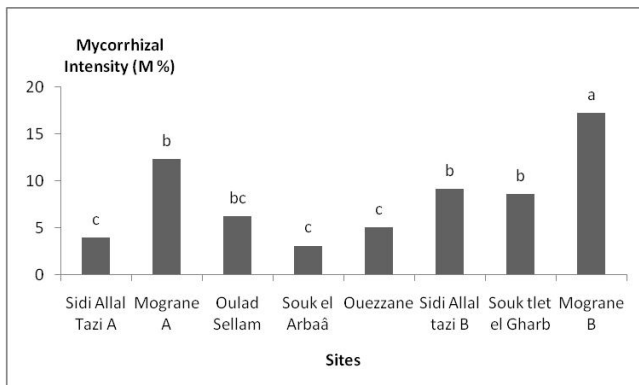


Fig. 3: Mycorrhizal intensity of barley root at the studied sites.

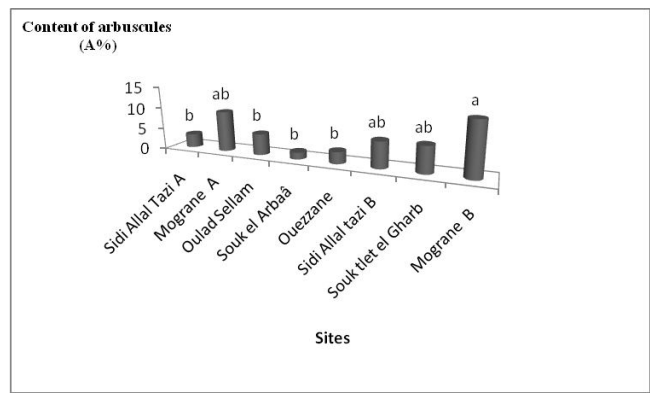


Fig. 4: Arbuscular contents of barley roots in the studied sites.

and 0.13% in vesicles. The roots of the barley plants at the Sidi Allal Tazi A and Oulad Sellam sites did not show vesicles (Fig. 4 and 5).

Diversity of spores of arbuscular mycorrhizal fungi (AD)

The number of endomycorrhizal fungal spores isolated from the rhizosphere of barley plants varies from site to site (Table 2). The number of spores recorded in the rhizosphere of barley plants from Mograne B sites (34 spore / 100g of soil), Ouazzane (35 spore / 100g of soil), Souk Tlet el Gharb (31 spore / 100g of soil) and Souk el Arbaâ (24 spores / 100g of soil) is higher. It is average at the level of the rhizosphere of the barley plants of the Sidi Allal Tazi site (20 spores / 100g of soil) and low of the Mograne A sites (13 spores / 100g of soil) and Oulad Sellam (14 spores / 100g of soil) (Fig. 6).

The preliminary morphological identification of the isolated spores made it possible to highlight the presence of 90 morphotypes in the rhizosphere of the barley plants of the sites studied (Table 1 and Fig. 6). All the morphotypes noted belong to 8 families: *Glomeraceae*, *Pacisporaceae*, *Acaulosporaceae*, *Diversisporaceae*, *Gigasporaceae*, *Claroideo-Glomeraceae*, *Entrophosporaceae* and *Archaeosporaceae*, to twelve genres: *Glomus* and *Acaulospora* (28 species); *Entrophospora*, *Funneliformis* and *Scutellospora* (5

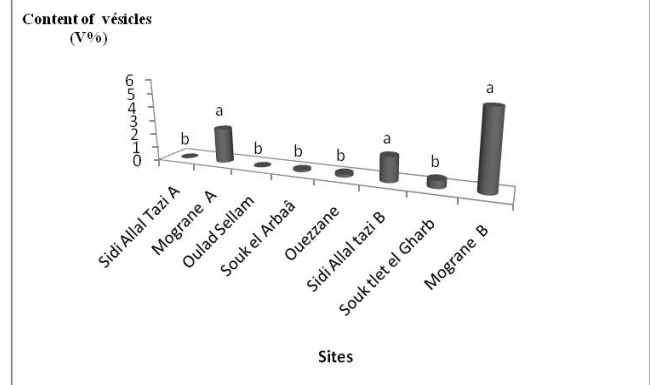


Fig. 5: Vesicular contents of barley root in the studied sites

Table 1: Identification of endomycorrhizae isolated from the barley rhizosphere of the different sites studied.

No.	Name	Form	Color	Spore's shape (µm)	Hyphas length (µm)	Spore's Surface
1	<i>Funneliformis mosseae</i>	Globular	Clear yellow	166.5	-	Smooth
2	<i>Rhizophagus clarus</i>	Oval	Dark yellow	199.8	-	Granular
3	<i>Glomus. macrocarpum</i>	Globular	Dark yellow	73.26	13.32	Smooth
4	<i>Glomus</i> sp1	Globular	Light yellow	109.89	36.63	Smooth
5	<i>Glomus</i> sp2	Globular	Dark yellow	76.59	-	Smooth
6	<i>Glomus versiform</i>	Globular	Brown yellow	66.6	16.65	Granular
7	<i>Glomus deserticola</i>	Globular	Brown	116.55	-	Smooth
8	<i>Scutellospora nigra</i>	Globular	Black	33.3	-	Smooth
9	<i>Glomus verruculosum</i>	Subglobular	Black	83.25	-	Smooth
10	<i>Claroideoglomus walkeri</i>	Globular	orange	66.6	-	Smooth
11	<i>Acaulospora. dilatata</i>	Globular	yellow	119.88	-	Granular
12	<i>Acaulospora</i> sp1	Oval	Clear yellow	83.25	-	Smooth
13	<i>Diversispora</i> sp.	Oval	white	106.56	33.3	Smooth
14	<i>Rhizophagus intraradices</i>	Subglobular	Brown	99.9	-	Smooth
15	<i>Racocetra gregaria</i>	Oval	Brown yellow	99.9	-	Smooth
16	<i>Claroideoglomus lamellosum</i>	Globular	Light yellow	53.28	-	Granular
17	<i>Racocetra. castanea</i>	Subglobular	Dark brown	149.85	-	Granular
18	<i>Archaeospora trappei</i>	Oval	white	69.93	-	Granular
19	<i>Glomus. microcarpum</i>	Globular	Brown yellow	69.93	-	Smooth
20	<i>Glomus</i> sp3	Subglobular	Dark brown	86.58	-	Smooth
21	<i>Acaulospora. gedanensis</i>	Globular	Light yellow	99.9	-	Smooth
22	<i>Acaulospora</i> sp2	Globular	Yellow	89.91	-	Granular
23	<i>Pacispora</i> sp1	Subglobular	White yellow	119.88	-	Granular
24	<i>Glomus glomerulatum</i>	Subglobular	Light yellow	49.95	26.64	Smooth
25	<i>Rhizophagus. fasciculatus</i>	Globular	Brown yellow	179.82	49.95	Smooth
26	<i>Acaulospora. kentinensis</i>	Oval	Light yellow	66.6	13.32	Smooth
27	<i>Glomus ambisporum</i>	Globular	Brown yellow	133.2	-	Smooth
28	<i>Glomus</i> sp4	Globular	White yellow	66.6	-	Granular
29	<i>Scutellospora heterogama</i>	Globular	Brown yellow	116.55	-	Smooth
30	<i>Funneliformis. geosporum</i>	Globular	Orange	53.28	26.64	Smooth
31	<i>Septoglomus constrictum</i>	Globular	Dark brown	213.12	-	Smooth
32	<i>Rhizophagus manihotis</i>	Globular	Brown yellow	99.9	-	Smooth
33	<i>Claroideoglomus etunicatum</i>	Ellipsoid	Dark yellow	39.96	-	granular
34	<i>Scutellospora aurigloba</i>	Ellipsoid	Orange	199.8	-	Smooth
35	<i>Diversispora aurantium</i>	Globular	Brown yellow	99.9	-	Smooth
36	<i>Glomus lacteum</i>	Oval	Yellow	116.55	-	Smooth
37	<i>Acaulospora foveata</i>	Globular	Orange	133.2	-	Granular
38	<i>Acaulospora longula</i>	Ellipsoid	Brown yellow	99.9	166.5	Smooth
39	<i>Racocetra minuta</i>	Subglobular	Orange	133.2	-	Smooth
40	<i>Glomus brohultii</i>	Globular	Orange	66.6	-	Smooth
41	<i>Acaulospora reducta</i>	Globular	Clear yellow	99.9	-	Granular
42	<i>Pacispora</i> sp2	Globular	Light yellow	66.6	-	Granular
43	<i>Pacispora</i> sp3	Globular	Yellow	56.61	-	Granular
44	<i>Acaulospora</i> sp(2)3	Globular	Dark brown	116.55	-	Smooth
45	<i>Glomus macrocarpum</i>	Subglobular	Clear yellow	76.59	-	Smooth
46	<i>Glomus intraradices</i>	Ellipsoid	Light yellow	166.5	-	Granular
47	<i>Acaulospra</i> sp4	Globular	Clear yellow	143.19	33.3	Granular
48	<i>Glomus</i> sp5	Globular	White yellow	133.2	399.6	Smooth

Table 1 Continue ...

Continue Table 1 ...

49	<i>Glomus</i> sp6	Globular	Brown yellow	99.9	166.5	Smooth
50	<i>Ambispora fecundispora</i>	Globular	Orange	166.5	133.2	Granular
51	<i>Rhizophagus fasciculatus</i>	Globular	Clear yellow	99.9	-	Granular
52	<i>Acaulospora alpina</i>	Globular	Brown yellow	143.19	123.21	Granular
53	<i>Acaulospora mellea</i>	Globular	Brown yellow	99.9	-	Smooth
54	<i>Acaulospora</i> sp5	Globular	orange	103.23	-	Granular
55	<i>Glomus. Monosporum</i>	Oval	Clear yellow	106.56	-	Granular
56	<i>Glomus</i> sp7	Globular	Brown yellow	59.94	49.95	Smooth
57	<i>Acaulospora laevis</i>	Globular	White yellow	113.22	-	Smooth
58	<i>Glomus brohultii</i>	Globular	Brown yellow	63.27	39.96	Smooth
59	<i>Scutellospora gilmorei</i>	Globular	Light yellow	66.6	-	Smooth
60	<i>Acaulospora spinosa</i>	Globular	Yellow	66.6	-	Smooth
61	<i>Acaulospora colombiana</i>	Subglobular	orange	173.16	-	Smooth
62	<i>Sclerocystis coremioides</i>	Oval	Brown yellow	76.59	53.28	Smooth
63	<i>Funneliformis constrictum</i>	Globular	Brown	66.6	-	Smooth
64	<i>Rhizophagus diaphanus</i>	Subglobular	White yellow	69.93	-	Granular
65	<i>Acaulospora delicata</i>	Oval	White yellow	133.2	-	Granular
66	<i>Gigaspora gigantea</i>	Globular	Light yellow	76.59	-	Granular
67	<i>Pacispora dominikii</i>	Globular	White yellow	109.89	-	Granular
68	<i>Acaulospora reducta</i>	Ellipsoid	White yellow	89.91	-	Granular
69	<i>Glomus</i> sp8	Globular	Yellow	86.58	-	Granular
70	<i>Glomus claroideum</i>	Globular	Yellow	76.59	-	Smooth
71	<i>Funneliformis caledonium</i>	Oval	Dark brown	69.93	-	Smooth
72	<i>Acaulospora</i> sp6	Subglobular	Light yellow	93.24	-	Smooth
73	<i>Acaulospora scrobiculata</i>	Oval	Light yellow	66.6	-	Smooth
74	<i>Acaulospora excavata</i>	Globular	Clear yellow	89.91	-	Granular
75	<i>Entrophospora. infrequens</i>	Globular	brown yellow	153.18	83.25	Granular
76	<i>Gigaspora margarita</i>	Globular	Yellow brown	99.9	39.96	Granular
77	<i>Glomus</i> sp9	Globular	Black brown	76.59	16.65	Smooth
78	<i>Acaulospora</i> sp7	Globular	Yellow brown	119.88	-	Smooth
79	<i>Glomus</i> sp10	Oval	White yellow	83.25	16.65	Smooth
80	<i>Acaulospora capsicula</i>	Oval	White yellow	73.26	-	Smooth
81	<i>Acaulospora</i> sp8	Globular	Light yellow	99.9	-	Granular
82	<i>Glomus</i> sp11	Oval	Yellow	66.6	-	Granular
83	<i>Acaulospora</i> sp9	Globular	White yellow	86.58	-	Granular
84	<i>Glomus. aggregatum</i>	Globular	Dark brown	49.95	-	smooth
85	<i>Glomus</i> sp12	Ellipsoid	Brown yellow	66.6	-	Smooth
86	<i>Diversispora spurca</i>	Oval	Brown yellow	83.25	-	Smooth
87	<i>Racocetra coralloidea</i>	Globular	Brown yellow	73.26	-	Granular
88	<i>Acaulospor</i> sp10	Globular	Brown yellow	126.54	-	Granular
89	<i>Glomus. macrocarpum</i>	Globular	Brown yellow	49.95	33.3	smooth
90	<i>Glomus intaradices</i>	Oval	White yellow	66.6	53.28	smooth

species); *Claroideoglomus*, *Diversispora*, *Pacispora*, *Racocetra* and *Rhizophagus* (3 species); *Gigaspora* (2 species); *Sclerocystis* (one species) and to three orders (Glomerales, Diversisporales, Archaeosporales).

The obtained results showed that the species *Glomus versiforme*, *Glomus macrocarpum* and *Scutellospora nigra* are widespread in the different studied sites (Table 2). *Glomus deserticola*, *Rhizophagus intraradices*,

Funneliformis mosseae, *Racocetra castanea* have been observed at some sites and not at others. Other species have been noted at the level of a single site, case of the Souk el Arbaâ site: *Acaulospora alpina*, *Acaulospora denticulata*, *Acaulospora mellea*, *Acaulospora* sp3, *Acaulospora* sp4, *Acaulospora* sp5, *Glomus brohultii*, *Ambispora fecundispora*, *Glomus* sp6, *Glomus* sp7, *Pacispora* sp2, *Pacispora* sp3. The species *Acaulospora* sp1, *Acaulospora* sp2,

Table 2: Species of AM fungi present in the different study sites.

Mycorrhizal species	Number of spores per 100g of soil							
	Mogran A	Oulad Sellam	Sidi Allal Tazi A	Souk El Arbaâ	Sidi Allal Tazi B	Ouezzane	Mogran B	Souk Tlet El-Gharb
<i>Acaulospora alpina</i>	-	-	-	1	-	-	-	-
<i>Acaulospora capsicula</i>	-	-	-	-	-	-	-	1
<i>Acaulospora colombiana</i>	-	-	-	-	-	1	-	-
<i>Acaulospora delicata</i>	-	-	-	-	-	1	-	-
<i>Acaulospora dilatata</i>	3	-	-	-	-	1	-	-
<i>Acaulospora elegans</i>	-	-	-	-	-	1	-	-
<i>Acaulospora excavata</i>	-	-	-	-	-	-	1	-
<i>Acaulospora gedanensis</i>	-	2	-	-	-	1	-	-
<i>Acaulospora leavis</i>	-	-	-	-	-	1	-	1
<i>Acaulospora foveata</i>	-	-	2	-	-	-	-	-
<i>Acaulospora longula</i>	-	-	1	-	-	-	-	-
<i>Acaulospora reducta</i>	-	-	-	1	-	2	-	1
<i>Acaulospora denticulata</i>	-	-	-	1	-	-	-	-
<i>Acaulospora mellea</i>	-	-	-	1	-	-	-	-
<i>Acaulospora scrobiculata</i>	-	-	-	-	-	-	1	1
<i>Acaulospora spinosa</i>	-	-	-	-	-	1	-	-
<i>Acaulospora kentinensis</i>	1	-	1	-	-	-	-	1
<i>Acaulospora</i> sp1	1	-	-	-	-	-	-	-
<i>Acaulospora</i> sp2	1	-	-	-	-	-	-	-
<i>Acaulospora</i> sp3	-	-	-	2	-	-	-	-
<i>Acaulospora</i> sp4	-	-	-	1	-	-	-	-
<i>Acaulospora</i> sp5	-	-	-	1	-	-	-	-
<i>Acaulospora</i> sp6	-	-	-	-	-	1	-	-
<i>Acaulospora</i> sp7	-	-	-	-	-	-	1	-
<i>Acaulospora</i> sp8	-	-	-	-	-	-	-	1
<i>Acaulospora</i> sp9	-	-	-	-	-	-	-	1
<i>Acaulospora</i> sp10	-	-	-	-	-	-	-	1
<i>Archaeospora trappei</i>	1	-	-	-	-	-	-	-
<i>Claroideoglosum lamellosum</i>	1	-	-	-	-	1	-	-
<i>Claroideoglosum etunicatum</i>	-	-	5	-	-	-	-	-
<i>Claroideoglosum walkeri</i>	1	-	-	-	-	-	-	-
<i>Diversispora spurca</i>	-	-	-	-	-	-	-	1
<i>Diversispora aurantium</i>	-	-	1	-	-	-	-	-
<i>Diversispora</i> sp	1	-	-	-	-	-	-	-
<i>Entrophospora. infrequens</i>	-	-	-	-	-	-	2	-
<i>Funneliformis caledonium</i>	-	-	-	-	-	1	-	-
<i>Funneliformis constrictum</i>	1	-	1	-	-	1	-	-
<i>Funneliformis geosporum</i>	-	-	1	-	-	-	-	-
<i>Funneliformis mosseae</i>	1	-	-	-	-	1	1	-
<i>Funneliformis verruculosum</i>	1	-	-	-	-	-	-	-
<i>Gigaspora gigantea</i>	-	-	-	-	-	2	-	-
<i>Gigaspora margarita</i>	-	-	-	-	-	-	1	-
<i>Glomus. aggregatum</i>	-	-	-	-	-	-	3	-
<i>Glomus ambisporum</i>	-	-	5	-	-	-	-	-
<i>Glomus brohultii</i>	-	-	-	1	-	1	-	-
<i>Glomus claroideum</i>	-	-	-	-	-	1	-	1
<i>Glomus clarum</i>	1	-	-	-	-	-	-	3

Table 2 Continue ...

Continue Table 2 ...

<i>Glomus deserticola</i>	4	-	1	3	1	1	1	-
<i>Glomus fasciculatum</i>	-	-	3	1	-	2	-	1
<i>G.fecundisporum</i>	-	-	-	1	-	-	-	-
<i>Glomus glomerulatum</i>	-	-	2	-	-	-	-	-
<i>Glomus intraradices</i>	1	-	2	1	-	-	-	-
<i>Glomus lacteum</i>	-	-	1	-	-	-	-	-
<i>Glomus.macrocarpum</i>	7	5	12	1	1	-	-	-
<i>Glomus.Monosporum</i>	-	-	-	-	1	-	-	-
<i>Glomus scintillans</i>	-	-	-	-	-	1	-	-
<i>Glomus versiforme</i>	5	10	11	2	6	5	9	-
<i>Glomus sp1</i>	1	-	-	-	-	-	-	-
<i>Glomus sp2</i>	1	-	-	-	-	-	-	-
<i>Glomus sp3</i>	-	1	-	-	-	-	-	-
<i>Glomus sp4</i>	-	-	1	-	-	-	-	-
<i>Glomus sp5</i>	-	-	1	-	-	-	-	-
<i>Glomus sp6</i>	-	-	-	1	-	-	-	-
<i>Glomus sp7</i>	-	-	-	1	-	-	-	-
<i>Glomus sp8</i>	-	-	-	-	1	-	-	-
<i>Glomus sp9</i>	-	-	-	-	-	1	-	-
<i>Glomus sp10</i>	-	-	-	-	-	-	-	1
<i>Glomus sp11</i>	-	-	-	-	-	-	-	1
<i>Glomus sp12</i>	-	-	-	-	-	-	1	-
<i>Glomus sp13</i>	-	-	-	-	-	-	-	1
<i>Pacispora sp1</i>	-	-	-	-	-	-	-	1
<i>Pacispora sp2</i>	-	-	-	1	-	-	-	-
<i>Pacispora sp3</i>	-	-	-	1	-	-	-	-
<i>Racocetra gregaria</i>	1	-	-	-	-	-	-	-
<i>Racocetra castanea</i>	1	-	-	-	-	-	1	2
<i>Racocetra minuta</i>	-	-	1	-	-	-	-	-
<i>Rhizophagus diaphanus</i>	-	-	-	-	-	1	-	-
<i>Rhizophagus fasciculatus</i>	-	-	1	-	-	-	-	-
<i>Rhizophagus manihotis</i>	-	-	1	-	-	-	-	-
<i>Sclerocystis coremioides</i>	-	-	-	-	-	1	-	-
<i>Scutellospora aurigloba</i>	-	-	1	-	-	-	-	-
<i>Scutellospora coralloidea</i>	-	-	-	-	-	-	-	1
<i>Scutellospora gilmorei</i>	-	-	-	-	1	-	-	-
<i>Scutellospora heterogama</i>	-	-	1	-	-	-	-	1
<i>Scutellospora. nigra</i>	5	25	1	2	9	5	12	9
Total	13	14	19	24	20	35	34	31

Archaeospora trappei, *Diversispora* sp., *Funneliformis verruculosum*, *Claroideoglomus walkeri*, *Glomus* sp1, *Glomus* sp2, *Racocetra gregaria* were observed in the rhizosphere of barley plants from the Mogran A site.

The species noted in the rhizosphere of the Ouazzane site are *Acaulospora colombiana*, *Acaulospora delicata*, *Acaulospora spinosa*, *Acaulospora* sp6, *Funneliformis caledonium*, *Gigaspora gigantea*, *Glomus* sp9, *Glomus scintillans*, *Rhizophagus diaphanus*, *Sclerocystis coremioides*. The species

observed at the Sidi Allal Tazi A site are *Acaulospora foveata*, *Acaulospora longula*, *Glomus ambisporum*, *Diversispora aurantium*, *Claroideoglomus etunicatum*, *Funneliformis geosporum*, *Glomus glomerulatum*, *Glomus lacteum*, *Rhizophagus manihotis*, *Glomus* sp4, *Glomus* sp5, *Scutellospora aurigloba*, *Racocetra minuta* and *Rhizophagus fasciculatus* and those of the Mograne B site are *Acaulospora excavata*, *Acaulospora* sp7, *Entrophospora infrequens*, *Gigaspora margarita*, *Glomus aggregatum*, *Glomus* sp12.

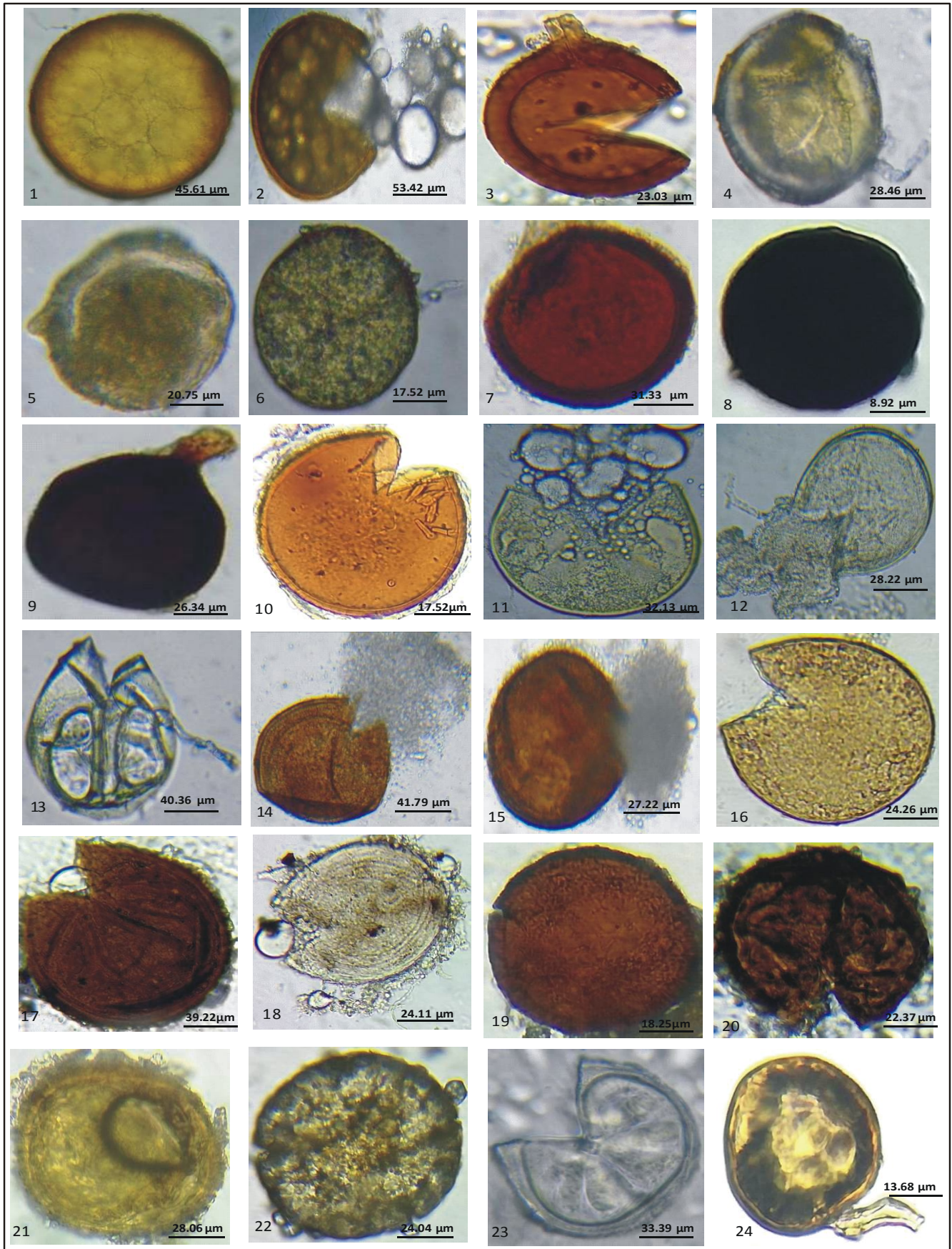


Fig. 6 Continue...

Fig. 6 Continue...

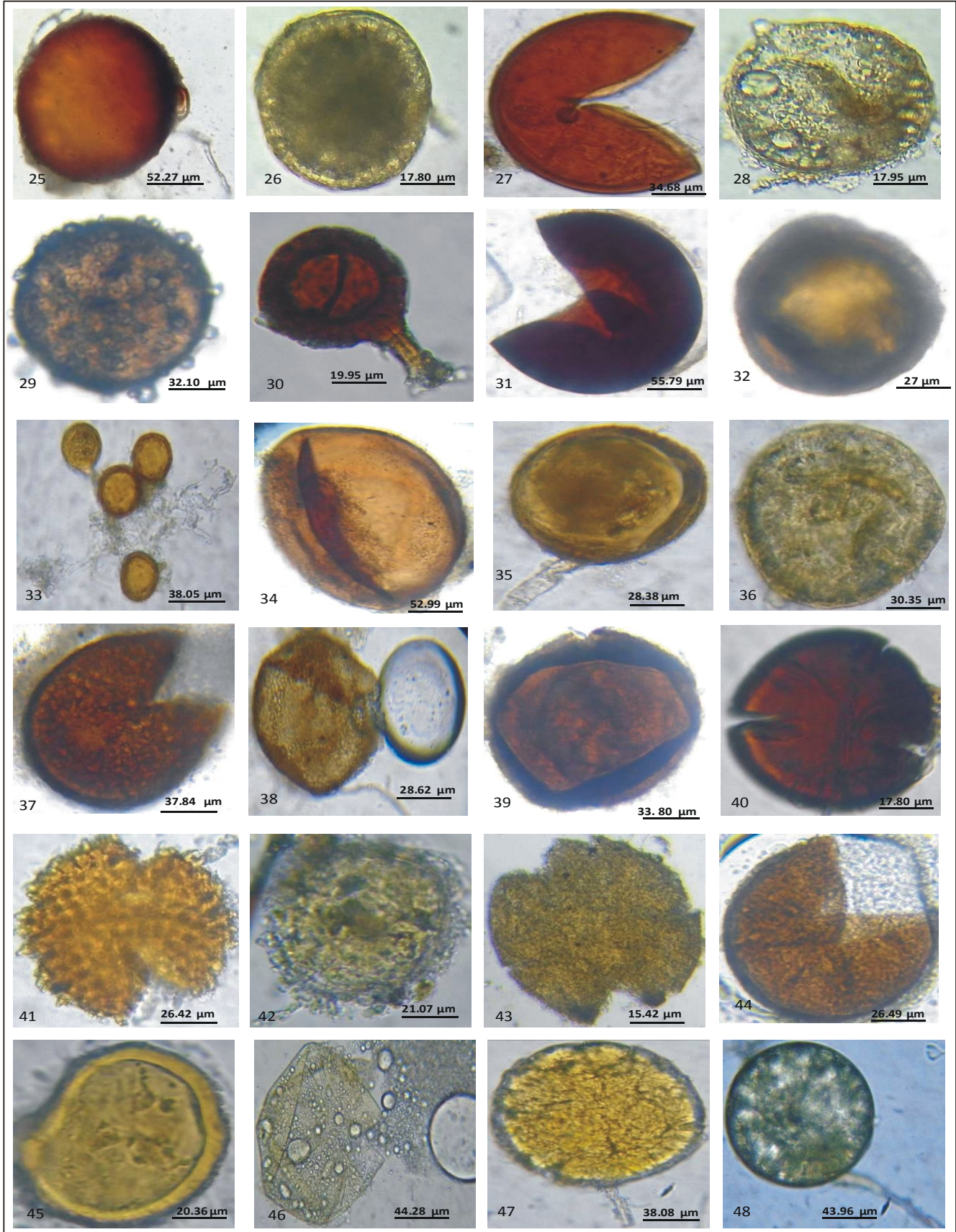


Fig. 6 Continue...

Fig. 6 Continue...

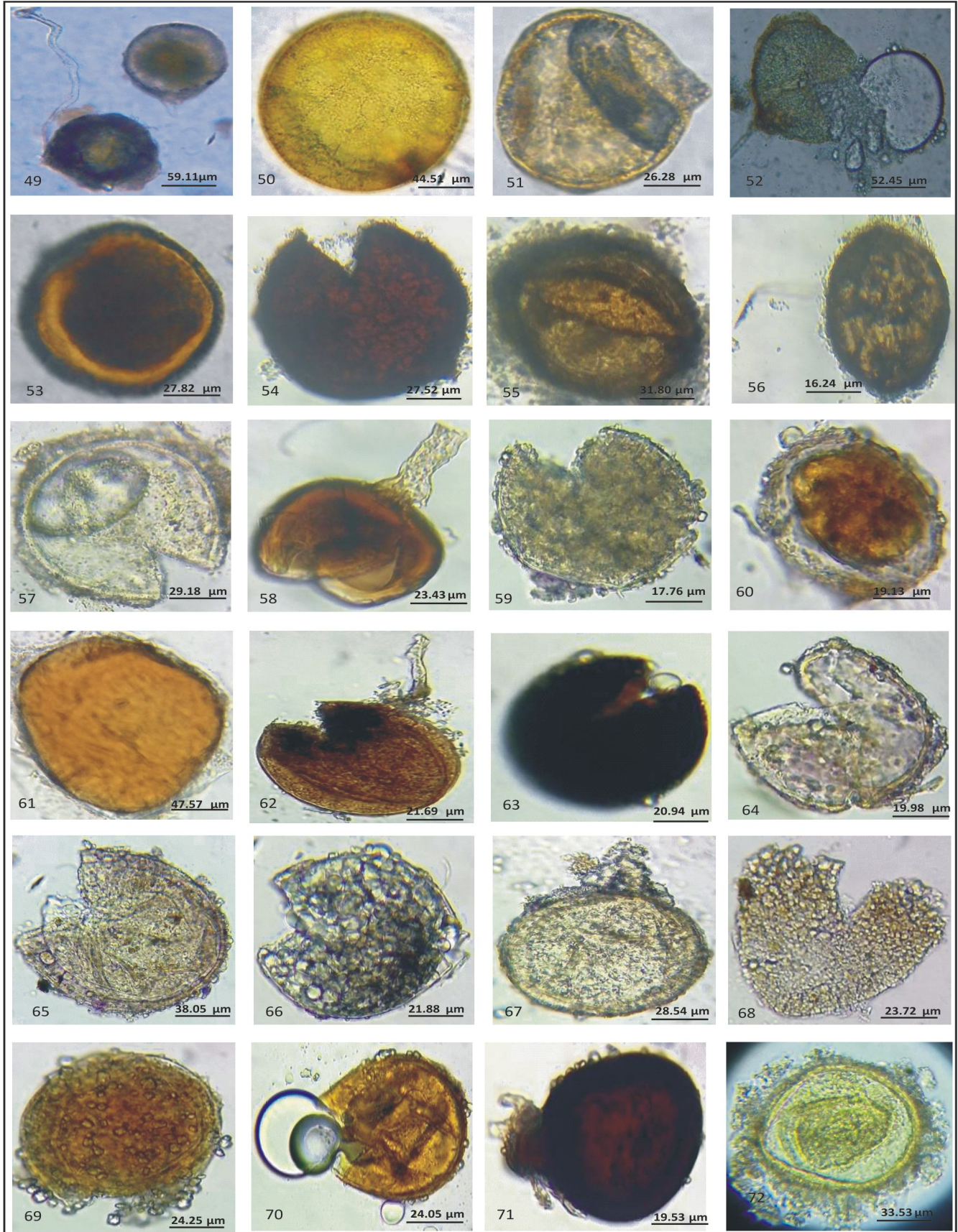


Fig. 6 Continue...

Fig. 6 Continue...

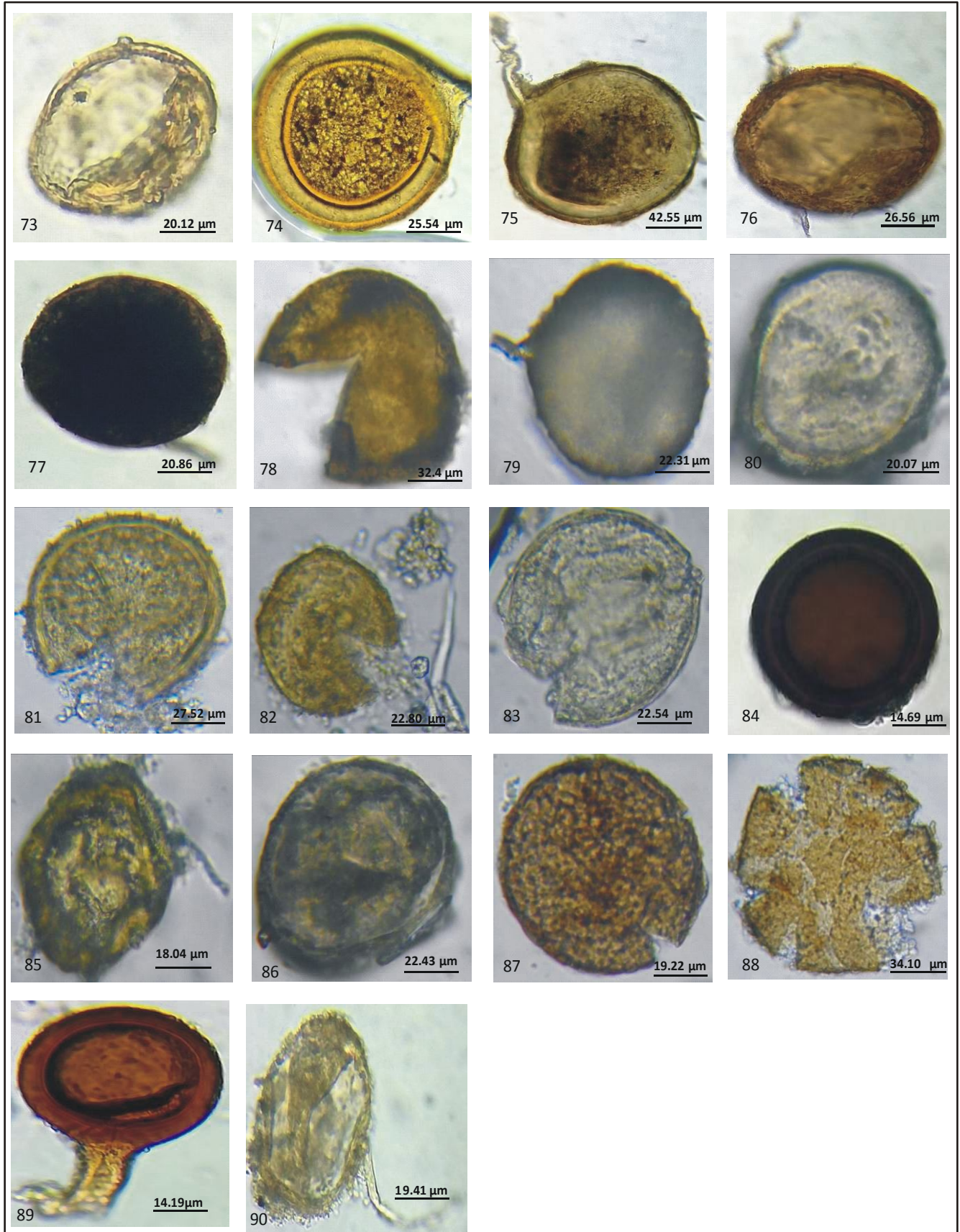


Fig. 6: Fungal species isolated from the barley rhizosphere.

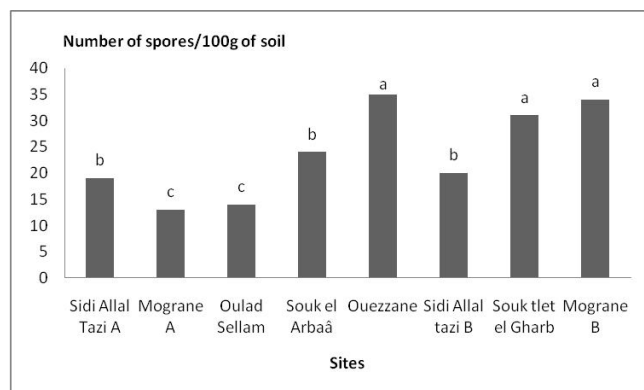


Fig. 6: Density of spores (number of spores / 100g of soil) of AM fungi in the rhizosphere of barley roots in the studied sites.

Discussion

Soils are generally complex and specific environments. The loss of one or more of their properties degrades their ability to produce biomass. Environmental factors such as physicochemical characteristics, pH and soil moisture influence microbial populations (Anderson and Domsch, 1993; Stotzky, 1997).

The used parameters to estimate the degree of colonization of barley roots by endomycorrhizal fungi vary from one site to another. Thus, the values of the frequency of mycorrhization (F) and those of the intensity of root colonization (M), the highest can reach 100% respectively (Sidi Allal Tazi B, Souk Tlet el Gharb and Mograne B sites) and 17.26% (Mograne B site). The lowest values of the frequency and intensity of mycorrhization were observed at the roots of barley plants from the Sidi Allal Tazi A sites and at Souk el Arbaâ. Furthermore, the contents of arbuscules and vesicles are very variable, the highest were recorded in the roots of barley plants from the site of Mograne B. Jerbi *et al.*, (2018) studied the variation of the natural biomass of arbuscular mycorrhizal fungi in the rhizosphere of the main Poaceae in northern Tunisia. These authors noted that the abundance of arbuscular mycorrhization is a function of the physicochemical characteristics of the soil (soil texture, pH, electrical conductivity, total and active lime content, organic matter content) and the host plant. They also reported that the most colonized species by AMFs is barley: 93.5% of the plants sampled and common wheat is the least mycorrhizal among the studied species.

Analysis of the spore communities of endomycorrhizal fungi found in the rhizosphere of barley plants shows a variation in spore density from one site to another, the highest average being around 35 spores / 100 g at the Ouazzane site. This observed variation can be attributed to the spore formation process and the degradation of their germination (Smith, 1980), the sampling season

(Gemma *et al.*, 1999), the soil and climatic variations (Koske, 1987; Johnson *et al.*, 1991) and soil microorganisms (Dalpé, 1989).

The typical morpho diversity of MACs depends on the quality of the soil and the ability of the spores to adapt. Their symbiotic activity is variable depending on the functional specificity of each soil strain which can be specialized for a given function or be generalist (Ngonkeu *et al.*, 2013).

The counting of mycorrhizal fungi spores has shown a predominance of the *Glomus* genus. This dominance has also been found in Nigeria (Redhead, 1977), in Burkina Faso (Ba *et al.*, 1996), in Senegal (Diop *et al.*, 1994), in the soil of certain forests in Benin (Houngnandan *et al.*, 2009), in the soil of certain orchards in Quebec (Dalpé *et al.*, 1986), in Malaysia, in the rhizosphere of *Octomelus sumatrana* and *Anthocephalis chinensis* (An Na Yan *et al.*, 2011).

The genera *Acaulospora*, *Gigaspora* and *Glomus* have already been observed in the Sudanian zone of Burkina Faso under the plantations of *Acacia halosericea* and *A. mangion* (Ba *et al.*, 1996), in the Moroccan coastal dunes of Souss Massa (Hatim and Tahrouch, 2007), in soils under argan trees (Sellal *et al.*, 2017) and in the rhizosphere of *Casuarina* sp. in Morocco (Tellal *et al.*, 2008). Several AMF species including, *Glomus mosseae*, *G. hoï*, *G. versiformae*, *G. diaphanum*, *G. geosporum*, *G. cladonius*, *G. clarum*, *Ascaulosporum* spp., *Archacospora* spp., *Paraglomus* spp. and others are often reported in association with rice roots resulting in an increase in the root surface for better acquisition of nutrients (Zhang *et al.*, 2005; Gao *et al.*, 2006; Raimam *et al.*, 2007; Rajeshkannan *et al.*, 2009; Fernández *et al.*, 2011).

The work of Cimen *et al.*, (2010) found that applying AMFs to lettuce plants in the greenhouse increased leaf yields by more than 200% compared to uninoculated plants. On clover plants a 65% increase in biomass was observed (Ben khaled *et al.*, 2003) whereas in watermelon cultivation, Hamza, (2014) reported an increase of 29% and 31% in three months, respectively, in the weight of aerial and root dry matter of plants inoculated with AMF compared to plants not inoculated. In their work in Niger, Laminou *et al.*, (2009) have shown that inoculating AMFs (especially *G. intraradices*) with plants allows them to increase their total biomass yield. The mycorrhizal symbiosis provides the plant mainly with phosphorus (Ezawa *et al.*, 2005) which is a nutrient little available to plants due to its reduced mobility in the soil and its partial solubility but also promotes access to Nitrogen complex forms (Govindarajulu *et al.*, 2005; Cruz *et al.*, 2007).

Inoculation of plants with *Glomus mosseae* allows better growth of clover plants than inoculation with other mycorrhizal fungi. Thus mycorrhization by this fungal isolate has resulted in a marked improvement in the number of leaves formed, the increase in leaf area and the height of the plant, in the face of a water deficit. Likewise, dry matter productions are improved compared to control plants. Similar responses have been reported for onion and lettuce inoculated with *Glomus fasciculatum* (Hirrel *et al.*, 1980; Ruiz-Lozano *et al.*, 1995; Tobar *et al.*, 1994) and corn inoculated with *Glomus mosseae* (Kothari *et al.*, 1990).

According to Brundrett, (1991), rich soils in colloids are favorable for the formation of mycorrhizae. Soils rich in organic matter can stimulate the functioning of arbuscular mycorrhizal fungi (Ryan *et al.*, 1994).

The soils of the aforementioned sites of the barley rhizosphere include a mycorrhizal biodiversity translated by the different types of spores dominated by the genus *Glomus* and *Acaulospora*.

The endomycorrhizal species found in the rhizosphere of barley plants can be exploited to promote the growth and protection of cereals against fungal diseases. Indeed, according to Wehner *et al.*, (2010), plant protection is ensured by mycorrhizal colonization which results from a combination of stimulation of plant growth by better nutrition and compensation by symbiosis for damage caused by phytopathogenic agents.

Among the most feared fungal diseases of cereals, root rot induced by a variable fungal complex consisting of the species of the *Fusarium* genus, *Bipolaris sorokiniana* and *Curvularia spicifera* (Qostal *et al.*, 2019b and Qostal *et al.* 2019a). The potential of arbuscular mycorrhizal fungi (MACs) as biocontrol agents has been described in the case of various root infections (Fiorilli *et al.*, 2011; Castellanos-Morales *et al.*, 2012). In this sense, an endomycorrhizal inoculum has shown great efficacy against *Fusarium solani*, the pathogenic agent responsible for root rot in chickpea plants (El Hazzat *et al.*, 2019). Mycorrhization of chickpea plants reduced infection of chickpea plants by *F. solani* and increased activity in the soil of the plant rhizosphere, sporulation of endomycorrhizal fungi was noted.

To achieve this objective, it is necessary to select certain endomycorrhizal species having both a high infectious power and a good adaptation to the different climatic and edaphic conditions of cereal cultivation.

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