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Research Article

Study of Arbuscular mycorrhizal fungi diversity in the rhizosphere of citrus grown in Morocco

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ABSTRACT

Arbuscular mycorrhizal fungi identification and evaluation of the mycorrhizal rate in roots were conducted respectively from the spores collected from soil samples and roots taken from the citrus rhizospheric soil of eleven sites in a parcel situated at El Menzah, INRA, Kénitra, Morocco.

The analyses of the results showed the presence of different structures characterizing endomycorrhizae at all sites. The mycorrhizal frequency in citrus roots varied between 73% to *Citrus aurantium* L. (Bigaradier), Troyer citrange (*C. sinensis* x *Poncirus trifoliata*) and 100% to Swingle citrumelo (*Citrus paradisi* X *Poncirus trifoliate*) varieties. The highest mycorhizal intensities were in the order of 53.2% for Troyer citrange and 43.26% for Swingle citrumelo and the lowest value was 34.56% which was recorded in the two sites of Flying dragon (*Citrus trifoliate*). Furthermore, the arbuscular contents recorded in the *Citrus aurantium L.*, Soh jhalia (*C. jambhiri*), Citrange 61-16-1 are respectively 30.89, 28.82 and 28.11%, and the lowest was observed in Flying dragon (18.51%). The vesicular content varies between 0.04% for *Citrus volcameriana* and 3.18% for Troyer citrange. The spore's density in the citrus rhizospheric soil varies between 240 for *Citrus volkameriana* and 48 spores / 100 g of soil for *Citrus aurantium* L..

The identification of isolated spores allowed to note the presence of 70 species belonging to 10 genera: *Glomus* (38 species), *Gigaspora* (3 species), *Acaulospora* (16 species), *Entrophospora* (2 species) , *Redeckera* (1 species) , *Pacispora* (2 species), *Dentiscuta* (1 species) , *Funelliformis* (1 species), *Claroideoglomus* (1 species), *Scutellospora* (5 species) and 8 families (*Glomaceae, Gigasporaceae, Acaulosporaceae, Diversisporaceae, Pacisporaceae, Dentiscutataceae, Scutellosporaceae, Gigasporaceae and Entrophosporaceae*) and 3 orders (*Glomerales, Gigasporales and Diversisporales)*. The study of the natural diversity of arbuscular mycorrhizal fungi in the citrus rhizosphere is a preliminary step for the development of an endomycorrhizal inoculum that can be used in nurseries to obtain citrus plants more robust and resistant to different pathogens and stresses after transplantation.

Keywords: Morocco, Citrus, Rhizosphere, arbuscular mycorrhizal fungi (AMF) and Diversity.

INTRODUCTION

Citrus trees, belonging to the Rutaceae family, subfamily of Aurantioideae are represented by 16 species[1,2] and originating in Southeast Asia[3,4]. The citrus industry remains among the largest agricultural speculations both in volume and value, with a world production amounting to 73 MT[5].

The citrus sector plays a big role in the Moroccan economy; it is the main source of revenue for 13,000 producers[6]. However, this sector is facing multiples biotic, abiotic, technical and commercial constraints[7]. The economic, aesthetic and biologic value of the fruits is affected by aging plantations, communicable diseases by grafting or by vectors, climatic conditions and plant health[8 - 13].

The Green Morocco Plan is a comprehensive strategy that focuses on innovative policy for upgrading competitiveness of the Moroccan citrus industry to make it more modern and better integrated into the world market, otherwise, tripling the volume of production (32 million tons / year) and doubling exports (1.3 million t / year) in 2020[6].

For this reason, the development of new vigorous plants production techniques, able to be adapted to different pedoclimatic conditions once implanted, promote healthier farming systems, reduce the use of chemical inputs and ensure the profitability of crops and the quality of the environment becomes necessary. It is for this purpose that mycorrhizal symbiosis in favor of agrimuculture was found important.

Mycorrhizal symbiosis is reflected in the formation of a mycorrhiza resulting from the intimate association of a root and a fungus (myco = fungus; rhizae = root)[14] whose major role is the collection and transportation of nutrients to the plant, especially phosphorus[15-20].

Mycorrhization is one of the biological means to improve yields of crops, inter alia citrus[16,21] by the intervention in the mineral nutrition and water acquisition[17,22-27]. Several studies have shown that mycorrhizal symbiosis also enhance the growth of young plants[28-31] and create morphological and physiological changes which help plant to tolerate environmental stresses[32].

In Morocco, research on arbuscular mycorrhizal fungi and their application in citrus cultivation are very rare. The aim of this work was to study the diversity of these fungi in the rhizosphere of eleven varieties of citrus rootstocks.

MATERIALS AND METHODS

Prospections and sampling

The collection of soil samples was performed at the rhizosphere of eleven varieties of citrus rootstocks planted in 1986 in a test plot of the National Agricultural Research Institute in the Gharb region. The samples were taken from the rhizosphere of citrus tree (5 trees per site at a rate of one kg of soil per shrub) at a depth of 0 to 20 cm and a composite sample of soil was realized per site. Very fine roots, more likely to be mycorrhizal and more easily observable under the microscope were taken together with the soil.

Mycorrhizal rates inside roots

The roots observation was prepared according to the method of Koske and Gemma[33]. They were first washed with water; the finest roots were then cut into a length of 1 cm then immersed in a solution of 10% KOH (potassium hydroxide) and placed in the water bath at 90 °C for one hour to eliminate cytoplasmic contents. At the end of this period, roots were rinsed and transferred in a solution of H_2O_2 (hydrogen peroxide) for 20 min at 90°C in the water bath until the roots became white. Roots were then rinsed, after this; they were dyed with cresyl blue[34], at 90°C for 15 min. After the final rinse, thirty pieces of dyed roots of 1 cm length were randomly selected and mounted, in groups of 10 to 15 segments, in glycerine between slide and coverslip[35]. The remaining roots were kept in glycerol acid.

The slides were examined under a microscope, each fragment being thoroughly checked over its entire length, at magnifications of x100 and x400 to observe and to note the mycorrhizal structures: arbuscules, hyphae, vesicles, external hyphae, intra and intercellular hyphae and even the endophytes structures. Vesicular and arbuscular frequencies and content of the endomycorrhizal fungi inside the roots were measured assigning a mycorrhization index ranging from 0 to 5[36].

Spores extraction

The spores were extracted by the method of wet sieving described by Gerdemann and Nicolson[37] (1963). In a beaker of 1L, 100g of each composite soil sample was submerged in 0.5 L of tap water and it was stirred with a spatula for 1 minute.

After 10 to 30 seconds of settling, the supernatant was passed through four superimposed sieves with decreasing meshes (500, 200, 80 and 50 Mm). This operation was repeated two times. The selected content by the screen 200, 80 and 50 microns was divided 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 solution of 40% sucrose in each centrifuge tube[38]. The mixture was quickly stirred and the tube was handed back into the centrifuge for 1 min at 9000 RPM. Unlike the first centrifugation process, the supernatant was poured into the sieve mesh of 50 microns; the substrate was rinsed with distilled water to remove the sucrose, and then disinfected with an antibiotic solution (streptomycin). The spores were then recovered with distilled water in an Erlenmeyer flask.

Species richness and appearance frequency

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

Statistical analysis

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

RESULTS

Characterization of mycorrhizal species isolated from citrus trees

In all study sites, citrus roots were mycorrhizal. Different structures characterizing arbuscular endomycorrhizae were observed: arbuscule, vesicles, intracellular and extracellular hyphae and endophytes (Fig. 1).

The roots of citrus rootstocks have recorded significant mycorrhizal frequency (Fig. 2), the maximum value was 100% at Swingle citrumelo and the minimum value is 73% at *Citrus aurantium* L.and Troyer citrange.

Concerning the mycorrhizal intensities which correspond to the percentage of mycorrhizal root cortex (Fig. 3), the highest values were observed in the root of Troyer citrange (53.2%) and Swingle citrumelo (43.26%) and lower in Flying dragon (34.56%) and Swingle citrumelo IF (34.30%).

Furthermore, the arbuscular contents (Fig. 4) are important in the *Citrus aurantium* L., Soh jhalia, and Citrange 61-16-1 respectively 30.89, 28.82 and 28.11%. However, those observed in Fyling dragon are in the order of 18.51%.

The vesicular contents (Fig. 5) are very low in the sites of Troyer citrange (3.18%), Shand rough lemen (*Citrus jambhiri* L.) (1.59%), and *Citrus aurantium* L. (1.24%). They are almost nil in the other sites, Swingle citrumelo IF and Citrange 61-16-1 (0.08%), and *Citrus volcameriana* and Flying dragon (0.05%), *Citrus species india* (0.04%), Swingle citrumelo (0.07 %) and Soh jhalia (0.085%).

Regarding the estimation of the spore's density in the rhizosphere of citrus growing in the studied sites (Fig.6), the average spore's number recorded, varies between 240 spores / 100g of soil at the *Citrus volcameriana* and 48 spores/100g of soil at the *Citrus aurantium* L..

Variations are important between sites of the same parcel, case of *Citrus volcameriana*, Swingle citrumelo IF, Citrange 61-16-1, *Citrus aurantium* L., respectively 240, 108, 42, 48 spores / 100g of soil.

The lower spores number varying between 58 and 67 spores / 100g of soil, was noted at the sites Troyer citrange, Citrumelo 57-98-506, Swingle citrumelo and Soh jhalia which showed very important mycorrhizal frequencies (between 86% and 100%) .

It is also important to note that mycorrhizal intensities generally vary between 53% and 34%.

2. Diversity of arbuscularmycorrhizal fungi (AM) spores

Preliminary identifications (Table 1) allowed to note that isolated spores belong to 70 species (Fig.7): *Entrophospora kentinensis; Acaulospora colossica; Acaulospora mellea; Dentiscutata biornata ; Acaulospora denticulate ; Funneliformis coronatus ; Glomus microcarpum ; Acaulospora spinosa ; Glomus fasciculatum ; Entrophospora infrequens ; Acaulospora morrowiae ; Glomus intraradices ; Gigaspora sp 1 ; Glomus versiforme ; Acaulospora rehmii ; Glomus constrictum ; Glomus aggregatum ; Glomus multicaule ; Glomus boreale ; Pacispora boliviana ; Rhizoglomus aggregatum ; Glomus claroideum ; Acaulospora gedanensis ; Glomus microaggregatum ; Acaulospora sp 1 ; Glomus radiatus ; Glomus etunicatum ; Glomus eburneum ; Glomus macrocarpum ; Rhizophagus fasiculatum ; Scutellospora fulgida ; Scutellospora nigra ; Scutellospora biornata ; Redeckera pulvinatum ; Glomus aureum ; Glomus corymbiforme ; Glomus rubiforme ; Glomus heterosporum ; Glomus mosseae ; Glomus sp 1 ; Glomus sp 2 ; Glomus sp 3 ; Glomus sp 4 ; Acaulospora sp 2 ; Glomus badium ; Glomus walkeri ; Glomus geosporum ; Acaulospora sp 4 ; Glomus sp 5 ; Acaulospora sp 3 ; Glomus clarum ; Claroideoglomus hamellosum ; Glomus monosporum ; Acaulospora scrobiculata ; Glomus ambisporum ; Acaulospora foveta ; Pacispora scintillans ; Gigaspora margarita ; Acaulospora longula ; Glomus fecundisporum ; Acaulospora delicata ; Glomus deserticola ; Acaulospora laevis ; Scutellospora armeniaca ; Glomus spinuliferum ; Glomus pansihalos ; Scutellospora castanea ; Glomus hyderabadensis ; Glomus formasum ; Gigaspora albida*.

According to the Oehl and Sieverding[39] classification, the species are divided into 10 genera (*Glomus,Gigaspora, Acaulospora, Entrophospora,Redeckera, Pacispora, Dentiscuta , Funelliformis, Claroideoglomus, Scutellospora*), 8 families (*Glomaceae, Gigasporaceae and Acaulosporaceae,Diversisporaceae, Pacisporaceae, Dentiscutataceae, Scutellosporaceae, Entrophosporaceae*) and 3 orders (*Glomerales, Gigasporales, Diversisporales*).

Glomus etunicatum, Glomus microcarpum, Acaulospora spinosa are respectively the dominant species in Swingle citrumelo IF, their appearance frequencies (Fig. 8) varied between 1.3% and 6.86%.

Glomus heterosporum and *Glomus macrocarpum* are the dominant species in *Citrus volcameriana* (Fig. 8).

Glomus etunicatum is the dominant species in Troyer citrange, while *Glomus microcarpum*, *Glomus fasciculatum*, *Glomus intraradices*, are the dominant species in Shand rough lemen (Fig. 8).

Glomus Ambisporum, *Acaulospora colossica*, *Acaulospora laevis* are the species most observed in Flying dragon, *Glomus microcarpum* and *Glomus clarum* in Swingle citrumelo IF (Fig. 8).

Table 1
Endomycorrhizal fungi isolated from the rhizosphere of citrus

Number	Name	Form	Color	Average size	Wall size	Spore surface	Hyphae length
1	*G. microcarpum*	Oval	deep yellow	53	7.2	Smooth	12
2	*S. castanea*	Globular	brown-yellow	62	6.3	Granular	-
3	*D. biornata*	Globular	Deep brown	64	3.4	Smooth	-
4	*G. versiform*	Globular	deep yellow	94.3	8.9	Smooth	-
5	*Gigaspora* sp.	Globular	light green	91	4.7	Smooth	-
6	*Rh. fasiculatum*	Globular	deep brown	93	4.2	Smooth	-
7	*G. eburneum*	Globular	light yellow	102	10.3	Smooth	-
8	*G. boreale*	Globular	light brown	64.5	11.7	Smooth	10.6
9	*E. kentinensis*	Globular	green yellow	78	4.7	Granular	-
10	*C. hamellosum*	Globular	light brown	97.4	9.6	Smooth	10
11	*P. scintillans*	Globular	deep yellow	52	3.25	Smooth	-
12	*G. intraradices*	Globular	deep brown	84	12.7	Smooth	-
13	*Glomus* sp. 4	Globular	deep yellow	74	9.6	Thorny	-
14	*Glomus* sp. 1	Globular	deep yellow	70	8.25	Thorny	-
15	*Glomus* sp. 3	Globular	yellow green	82	12.7	Thorny	-
16	*G. badium*	Globular	light brown	38	6.2	Smooth	46.8
17	*G. Claroideum*	Globular	light brown	112	10.4	Smooth	-
18	*G. aureum*	Globular	light brown	92	6.6	Smooth	18.7
19	*Glomus* sp. 4	Globular	yellow green	72	8.7	Thorny	-
20	*Glomus* sp. 2	Globular	deep yellow	80.2	5.5	Thorny	-
21	*G. fecundisporum*	Globular	light brown	92	7.9	Smooth	-
22	*G. Claroideum*	Ellipsoid	light brown	65.7	9.7	Smooth	-
23	*Gi. margarita*	Globular	brown	116	10.6	Smooth	80.5
24	*G. etunicatum*	Globular	light brown	126	11.3	Smooth	-
25	*Glomus* sp. 2	Globular	deep yellow	78	9.6	Thorny	-
26	*Glomus* sp. 4	Globular	deep yellow	76.4	8.9	Thorny	-
27	*Glomus* sp.3	Globular	yellow green	82	10.5	Thorny	-
28	*A. longula*	Oval	light brown	93	12.2	Smooth	-
29	*G. constrictum*	Globular	deep brown	65	6.9	Smooth	-
30	*G. clarum*	Oval	brown	72	3.4	Smooth	-
31	*Entrophospora* sp.	Globular	brown	79	9.25	Granular	-
32	*E. infrequens*	Globular	deep brown	65	8.9	Smooth	-
33	*G. intraradices*	Globular	green	79.7	14.3	Smooth	-
34	*A. colossica*	Globular	deep orange	76	9.5	Granular	-
35	*Rh. aggregatum*	Globular	yellow	65	7.2	Smooth	23.7

Table 1(cont.)
Endomycorrhizal fungi isolated from the rhizosphere of citrus

Number	Name	Form	Color	Average size	Wall size	Spore surface	Hyphae length
36	*A. colossica*	Globular	deep orange	69.8	5.4	Smooth	-
37	*A. laevis*	Globular	brown	81	12.5	Smooth	-
38	*Acaulospora.* sp 2	Oval	orange	85	14.7	Smooth	-
39	*Acaulospora.* sp 3	Globular	yellow	92	6.5	Smooth	-
40	*G. spinuliferum*	Globular	deep yellow	66	8.25	Smooth	-
41	*G. eburneum*	Globular	light yellow	76	7.9	Smooth	-
42	*G. spinuliferum*	Globular	orange	79	10.2	Smooth	-
43	*A. rehmii*	Globular	orange	101	9.7	Smooth	-
44	*G. aggregatum*	Globular	orange	98.8	11	Smooth	-
45	*A. gedanensis*	Globular	yellow	92	10.5	Smooth	-
46	*Rh. aggregatum*	Globular	light yellow	66	12	Smooth	-
47	*G. intraradices*	Globular	deep yellow	47.6	13.25	Smooth	37
48	*G. microcarpum*	Ellipsoid	orange	106	5.4	Smooth	-
49	*G. etunicatum*	Oval	orange	98	10.3	Smooth	-
50	*A. morrowiae*	Globular	deep yellow	102	17.5	Smooth	-
51	*A. foveta*	Globular	orange	97	30.6	Granular	-
52	*Scutellospora.* sp	Oval	yellow	93	10.7	Smooth	-
53	*A. delicata*	Globular	brown	53.25	20.7	Smooth	-
54	*G. boreale*	Globular	black	92.6	2.5	Smooth	40
55	*G. radiatus*	Globular	brown	99	8.4	Smooth	-
56	*Acaulospora.* sp 1	Globular	yellow	90.2	8.3	Smooth	-
57	*G. deserticola*	Globular	orange	60	6.7	Smooth	-
58	*Acaulospor* sp 1	Globular	brown	74.4	2.3	granular	-
59	*G. multicaule*	Globular	deep brown	72	8.5	Smooth	-
60	*G. intraradices*	Globular	light yellow	80.8	10.3	Smooth	-
61	*G.fecundisporum*	Globular	light yellow	54	6.2	Smooth	-
62	*G. fasciculatum*	Globular	orange	56.6	3.2	Smooth	-
63	*G. mossea*	Globular	orange	60.4	7.6	Smooth	-
64	*G. ambisporum*	Globular	yellow	52	3.7	Smooth	82,4
65	*Glomus.* sp 5	Oval	yellow	102	8.5	Smooth	54,3
66	*G. heterosporum*	Globular	deep brown	123	10.6	Smooth	20.5
67	*G. heterosporum*	Globular	deep brown	130	12	Smooth	-
68	*G. fecundisporum*	Globular	yellow	98	8.4	Smooth	-
69	*G. hyderabadensis*	Globular	light brown	94	10.5	Smooth	-
70	*G. formasum*	Globular	light brown	103	9.4	Smooth	-
71	*Acaulospora.* sp4	Oval	yellow	95	10.2	Smooth	103
72	*Acaulospora.* sp5	Globular	yellow	98.5	7.6	Smooth	40.5

Table 1 (cont.)
Endomycorrhizal fungi isolated from the rhizosphere of citrus

Number	Name	Form	Color	Average size	Wall size	Spore surface	Hyphae length
73	*G. corymbiform*	Globular	orange	102	9	Smooth	20.7
74	*G. rubiform*	Globular	brown	92.6	10.7	Smooth	19.8
75	*G.mossea*	Globular	orange	96.7	9.8	Smooth	-
76	*G. multicaule*	Globular	brown	54	6.7	Smooth	30.9
77	*G. macrocarpum*	Globular	orange	65	5	Smooth	-
78	*G. intraradices*	Globular	deep yellow	53.9	7.8	Smooth	79.7
79	*G. aggregatum*	Globular	deep brown	59	6.7	Smooth	-
80	*Acaulospora.* sp4	Globular	yellow	63.8	6.7	Smooth	40.4
81	*Acaulospora.* sp4	Globular	yellow	105	9.3	Smooth	-
82	*A. foveata*	Globular	orange	116	9.7	Smooth	-
83	*G. walkeri*	Globular	orange	96.7	6.9	Smooth	102.4

Note : *A – Acaulospora ; E – Entrophospora ; Gi – Gigaspora ; G – Glomus ; P - Pacispora ; S – Scutellospora ; R- Redeckera ; D- Dentiscuta ; F- Funelliformis ; C- Claroideoglomus*

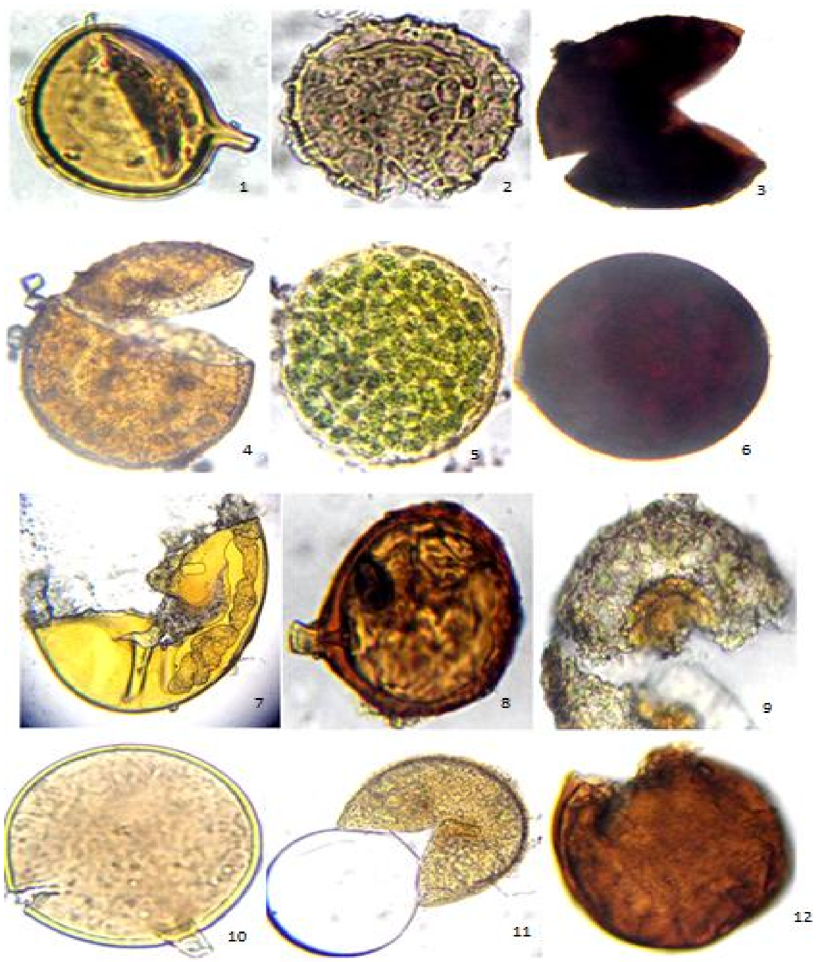

Fig. 1(1-12)
Endomycorrhizal fungi isolated from the rhizosphere of citrus

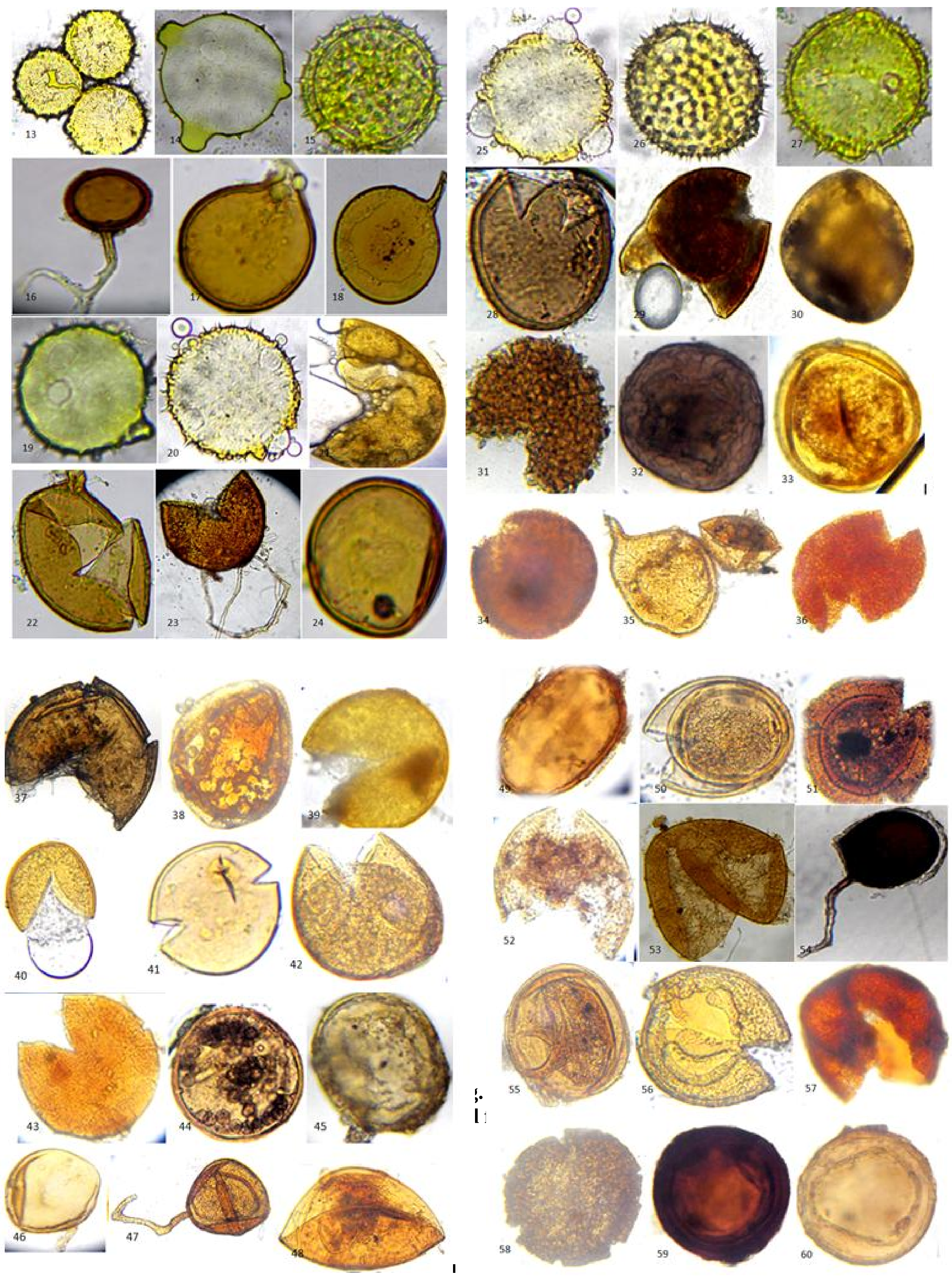

Fig. 1(13-60)
Endomycorrhizal fungi isolated from the rhizosphere of citrus

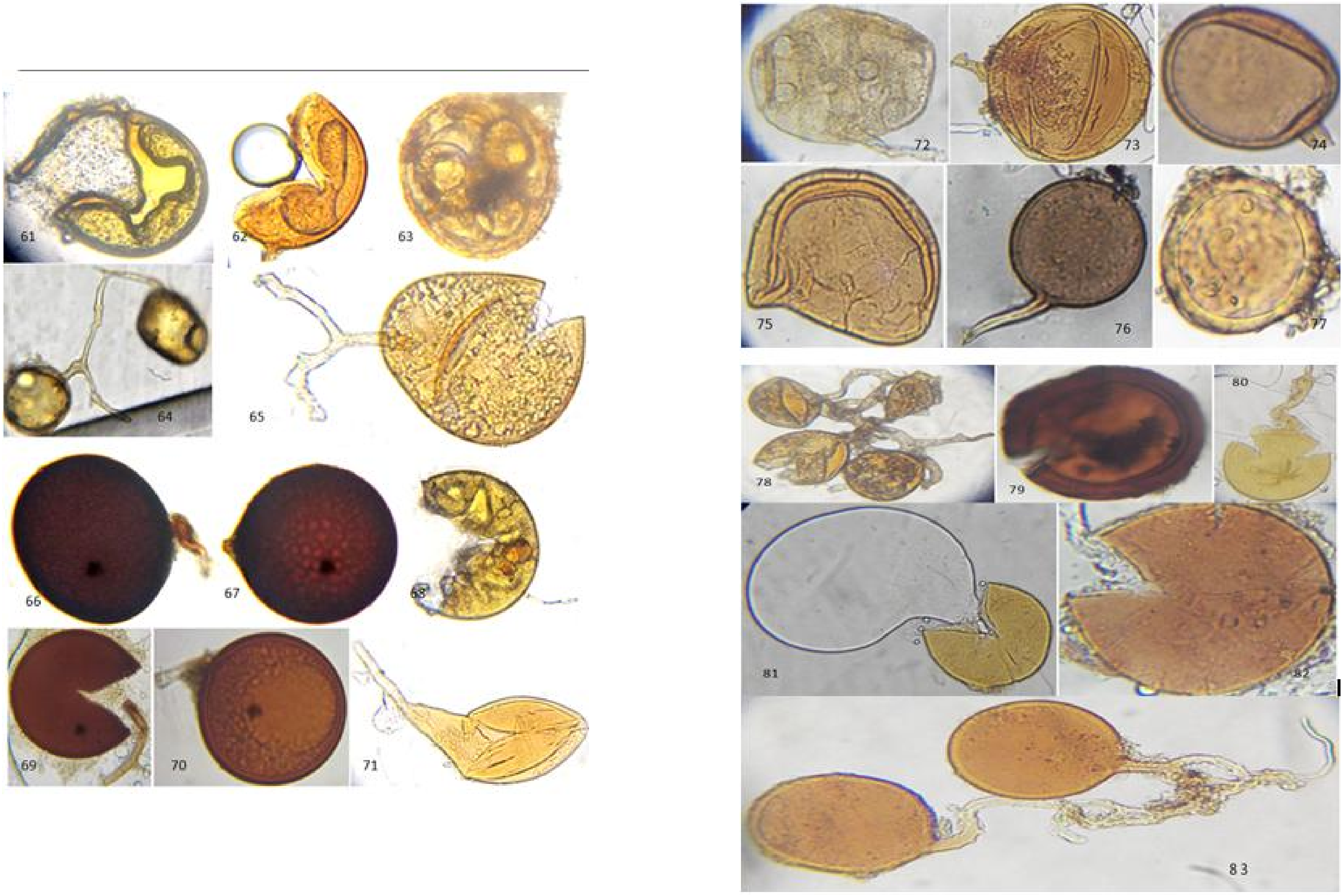

Fig. 1(61-83)
Endomycorrhizal fungi isolated from the rhizosphere of citrus

In citrange 61-16-1, species that seem to dominate are *Glomus eburneum, glomus versiforme* and *glomus versiforme*. *Glomus Claroideum* and *Glomus aureum* dominated at *Citrus species india*. At Swingle citrumelo, *Glomus multicaule* and *Glomus mosseae* are the most encountered species (Fig. 8).
Glomus multicaule, *Glomus etunicatum* were reported as dominant species in Soh jhalia, and *Glomus intraradices* dominated in the *Citrus aurantium* L. (Fig. 8).
Species richness (Fig. 9) also varies depending onthe sites. 22 species in the rhizosphere of Swingle citrumelo IF, 15, 14, 13 and 2 species respectively in those of *Citrus volcameriana* and Citrange 61-16-*1*, Troyer citrange, Shand rough lemen and Flying dragon, Swingle citrumelo and *Citrus aurantium* L..

DISCUSSION
The surveys carried out in the rhizosphere of eleven citrus rootstocks varieties planted in 1986 in a test plot of the National Agricultural Research Institute of the Gharb region, have shown that the roots of all these varieties are bearers of endomycorrhizal structures: vesicles, arbuscules, internal and external hyphae and endomycorrhizae. The presence of these structures characterizing endomycorrhizae classifies citrus fruits as a mycotrophic species[40].
The root mycorrhizal frequencies are very high in Citrumelo Swingle (100%); Citrumelo57-98-2 (93%); Citrange 61-16-1 and Soh jhalia (83%); *Citrus aurantium* L. and Troyer citrange (73%). However, the rhizosphere of these varieties was poor in spores (spores number ranges from 48 to 58 spores per 100 g of soil). However, it seems that there is a correspondence between the mycorrhizal frequency (ranging from 96% to 76%) and the spore's number observed in the rhizosphere of Swingle citrumelo IF; *Citrus volcameriana*; *Citrus species india*; Flying dragon, respectively 108; 240; 98; 85 spores per 100 g of soil.

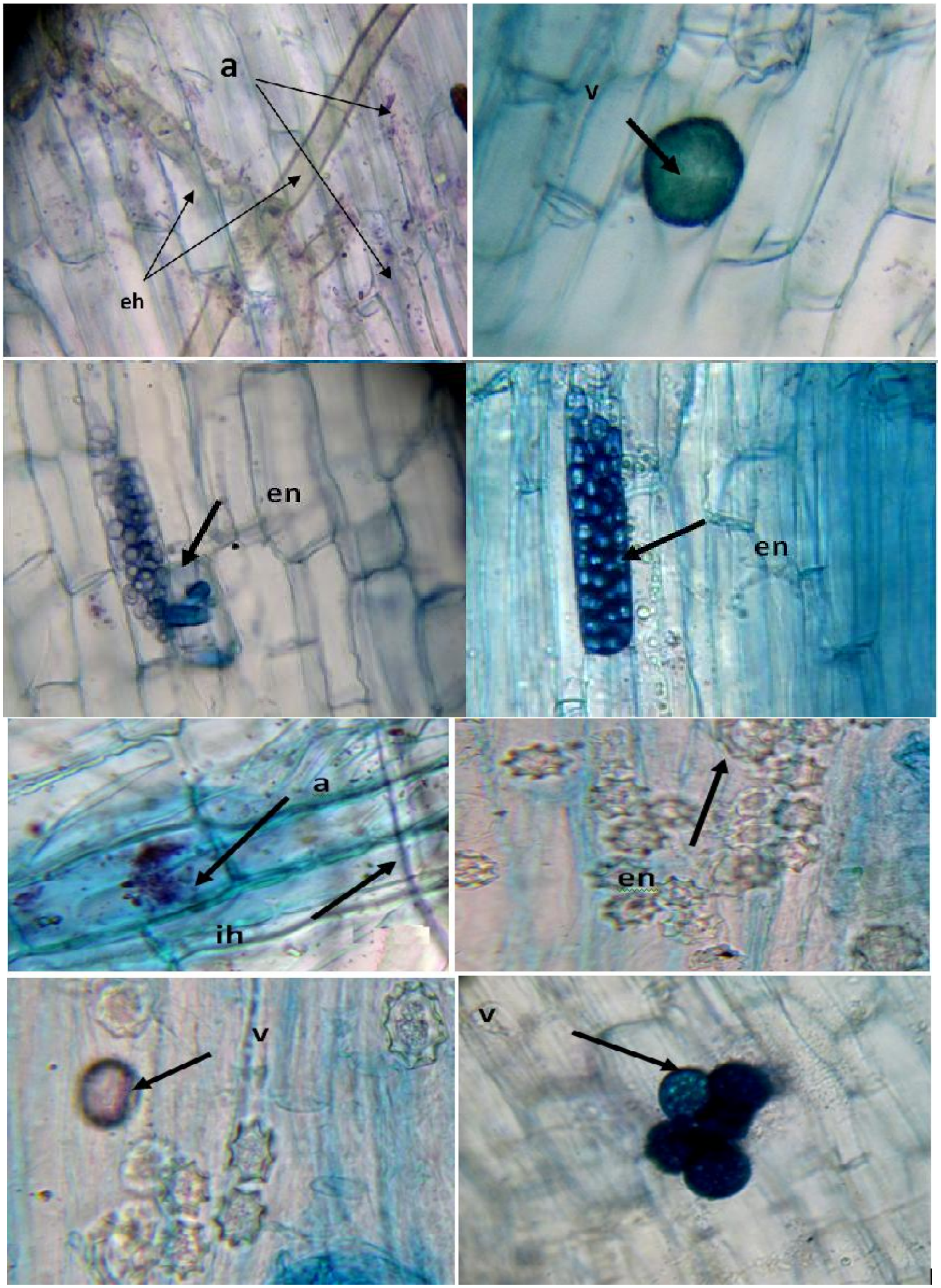

Fig. 2
Citrus roots presenting structures of arbuscular mycorrhizae: arbuscles (a); hyphae extra (eh) and intra-radicular (ih), spores (s); vesicles (v) and endophytic (en). (G. × 400).

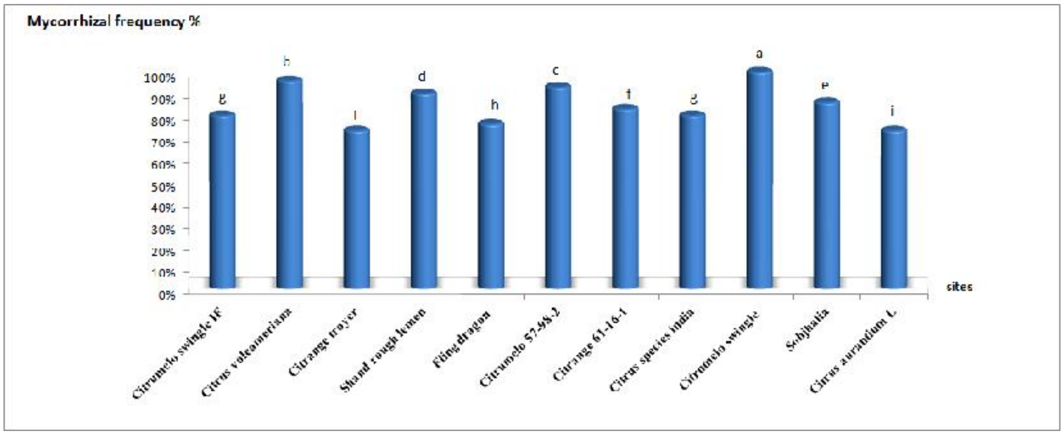

Fig. 3
Mycorrhizal frequency of citrus roots in the study sites

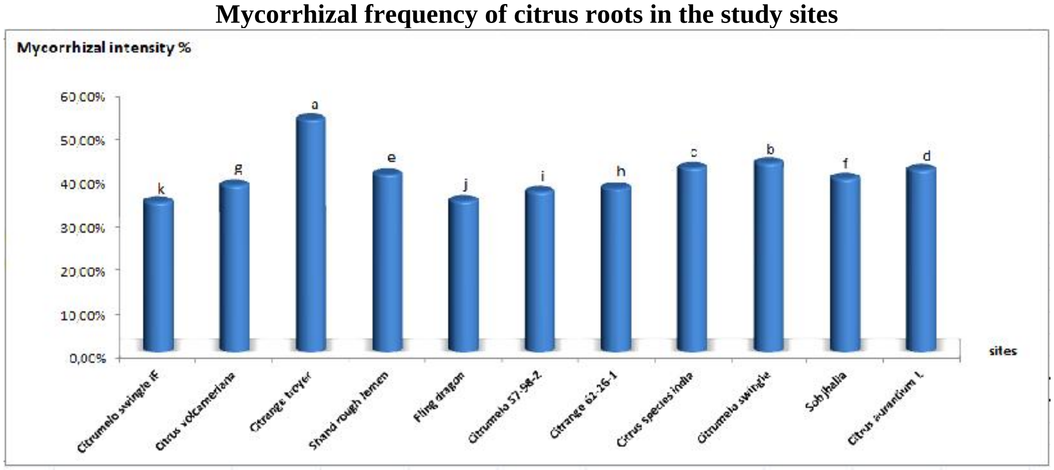

Fig. 4
Mycorrhizal Intensity of citrus roots in the study sites

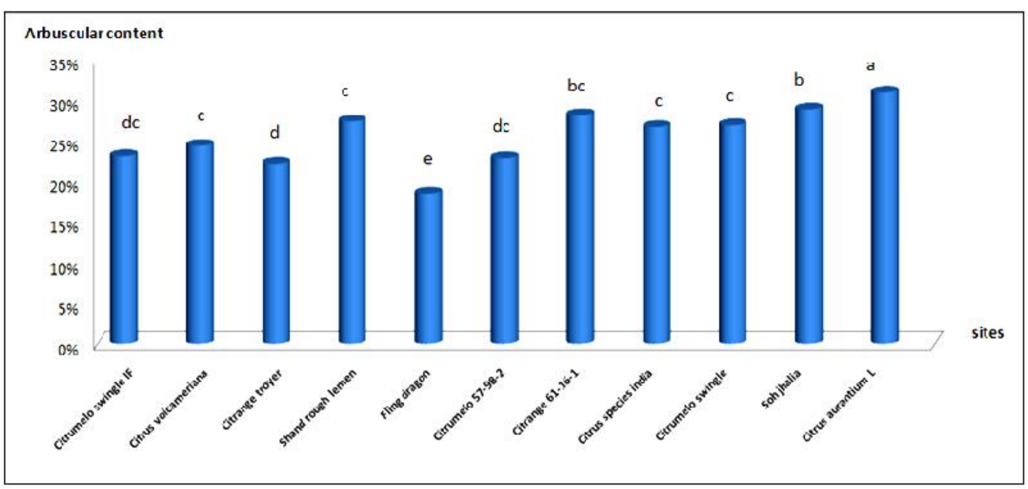

Fig. 5
Arbuscular content of citrus roots in the study sites

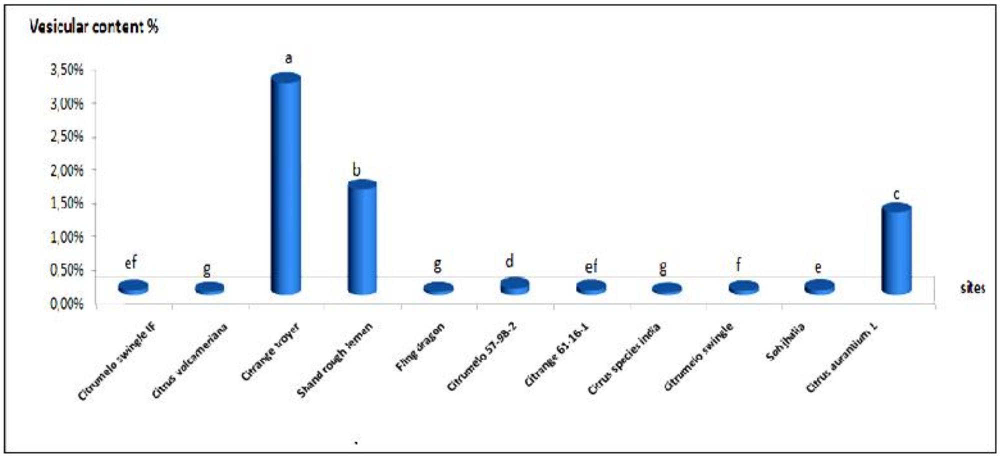

Fig. 6
vesicular content of citrus roots in the study sites

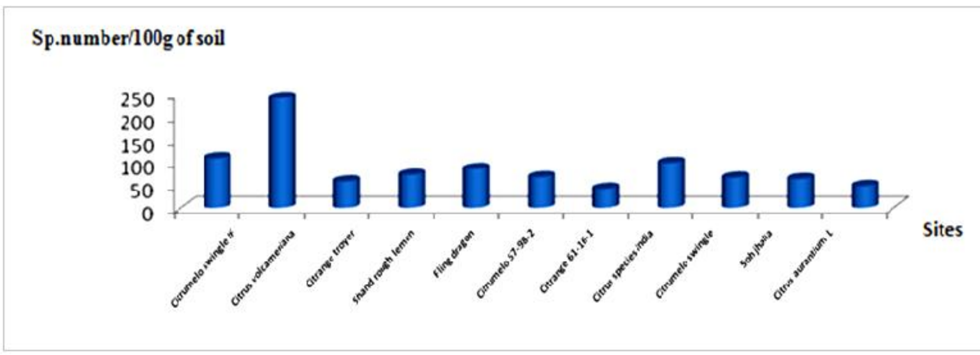

Fig. 7
Density of AM fungi spores in the citrus tree rhizosphere in the study sites

In all instances, according to Diagne and Ingleby[41]; Walker and Mize[38]; Mukerji and Kapoor[42], it is risky to approach the infectious activity of CMA of a floor given to the spores number present in this soil. Sporulation may depend on the species of CMA, edaphic characteristics of the soil and climate conditions. According to Jasper *et al*.[43], the weak relationship between the endomycorrhizal formation and the spore's quantity they have isolated is due to the fact that some propagules would be dormant.

Spore densities observed in the rhizosphere of citrus varieties rootstock studied (42 to 240 spores per 100 g of soil are medium compared to the bibliographic data reported by some authors. Thus, Weissenhorn[44] reported 150-200 spores per 100 g of dry soil collected from polluted agricultural soil by atmospheric deposition. Sieverding[45] 120 spores per 100 g of soil under a manioc monoculture, 132 in a rotation culture and 360 in a savane. In the rhizosphere of the argan tree in Morocco 188 spores / 100 g soil (Toufalazte), and 28 and 44 spores / 100g of soil (Tiznit and Ait Melloul)[46] and *Acacia albida* in Senegal (775-1240 spores per 100 g of soil) [47]. Bouamri[48] noted densities that vary between 295 and 1900 spores per 100 g of soil in the rhizosphere of palm Tafilalt. Sghir[49] reported for that species in the regions of Draa Tafilat, a number of spores ranging from 80 to 132 spores per 100 g. Gould[50] reported a number of spores ranging from 4-1576 spores per 100 g of soil in careers soils restored at different times after revegetation. Zuberer and Mott[51] ravealed spores densities reaching 9050 to 11470 spores per 100 g of soil in the mulberry tree soil. El Asri[52] observed 84-160 spores per 100g of soil and Talbi [53] noted 92 spores/100g of soil in the Carob tree rhizosphere, Populus alba in northwest of Morocco (78 spores/100g of soil)[54]. However, at the *Casuarina* sp. rhizosphere, showed a very low number of spores was recorded from 2 to 22 spores per 100 g of soil[55]and in the rhizosphere of *E. maritimum* the spore's number varied between 10 to 20 spores / 100 g of soil [56].

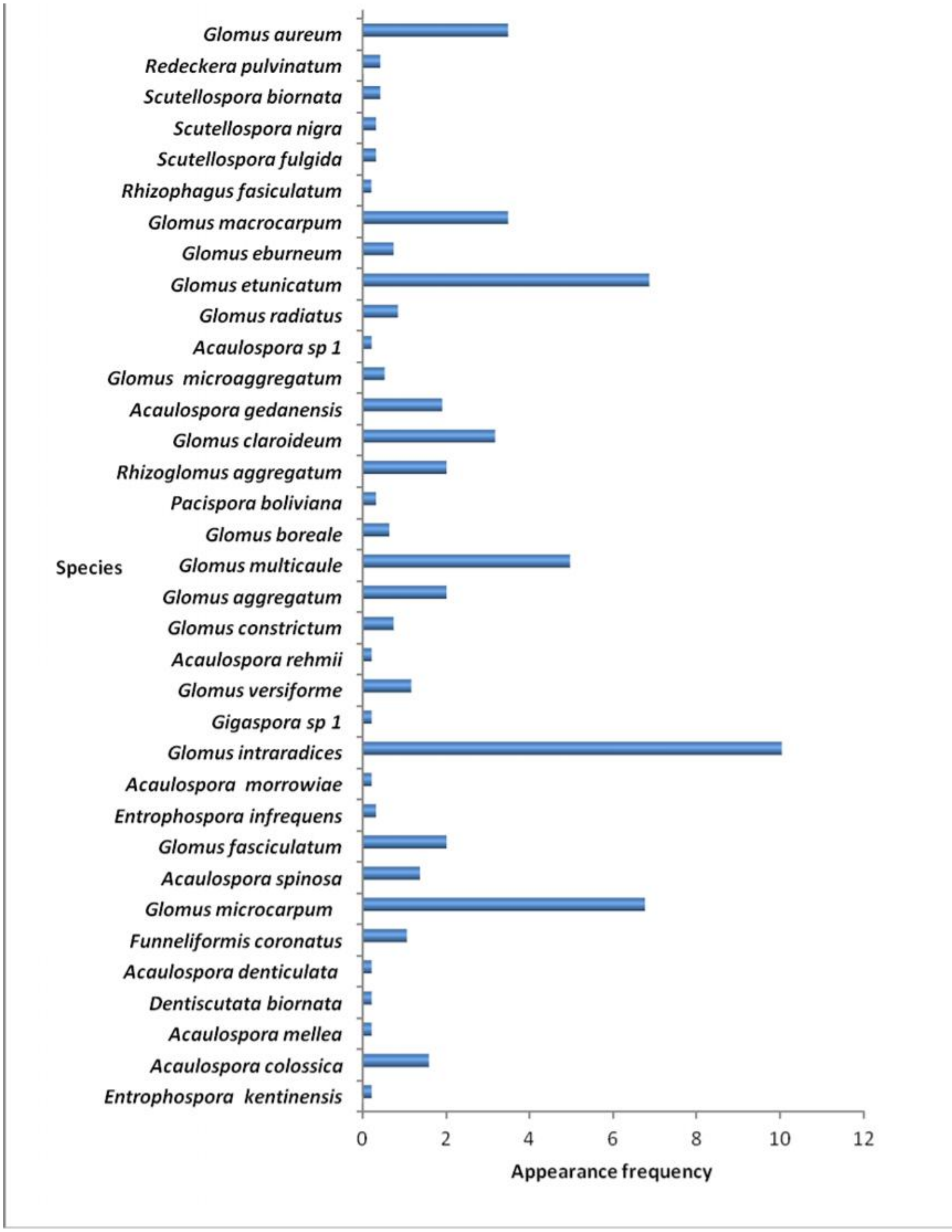

Fig.9
Appearance frequency of mycorrhizal species at each study sites.

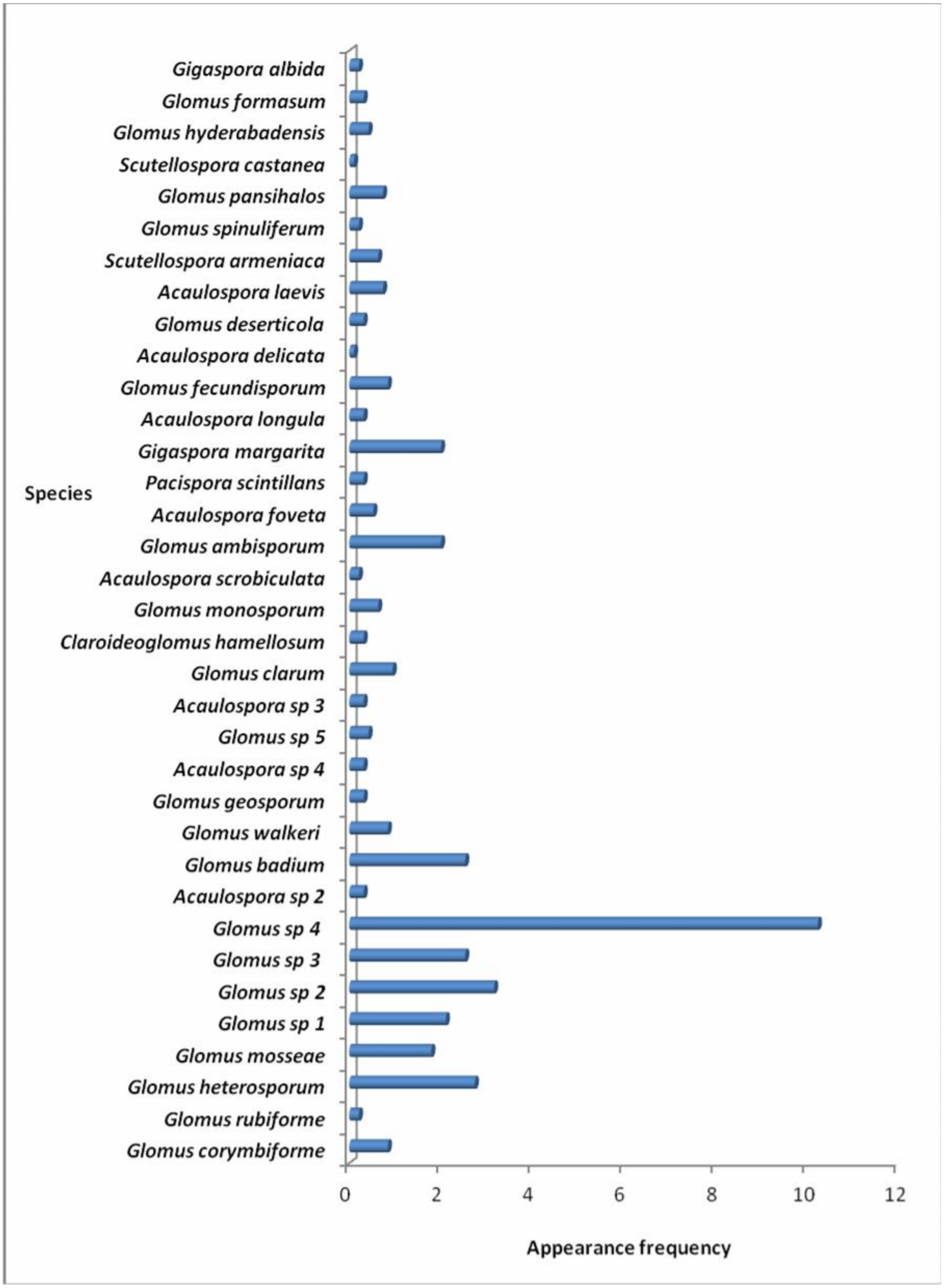

Fig.9 (cont.)
Appearance frequency of mycorrhizal species at each study sites.

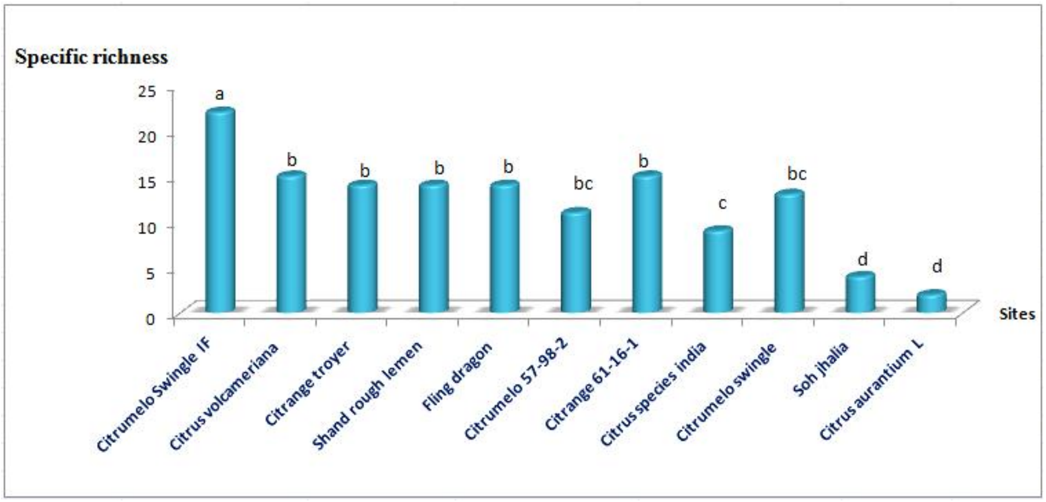

Fig. 10
Specific richness of mycorrhizal species at each site.

The differences recorded may be due to the physico-chemical and microbiological properties of soils[57, 58, 59], the micro-climatic fluctuations[60, 61], plant cover[62] and the sampling season[33, 48]. Enumeration of the spores of mycorrhizal fungi showed a predominance of the genus *Glomus*. This dominance was also found in Morocco at the rhizosphere of Argania spinosa (L.) [47] Populus alba [54] , carob tree [53,52] , the olive tree [63] oleaster[64], Date palm[,65], in Nigeria[66], Burkina Faso[67], Senegal[47], in the soil of some forests of Benin[59] in some orchards in Quebec[61], and Malaysia, in the rhizosphere of *Octomelus sumatrana* and *Anthocephalis chinensis*[68].

In this study, 70 species of (*Glomerales*, *Gigasporales*, *Diversisporales*) were detected in the rhizosphere of citrus, indicating very important species richness. Wang[40] reported only 12 species in the rhizosphere of citrus in southern China. At the other plant species rhizosphere the number never exceeded 30 species. Chliyeh [69] isolated twenty six endomycorrhizal species from the rhizosphere of two olive varieties (Haouzia and Dahbia) ; Kachkouch[59] isolated 6 species and 5 genera in the rhizosphere of the olive tree. Talbi [53]found 21 species in the rhizosphere of Carob. Abbas[70] reported the presence of 6 species of arbuscular mycorrhizal fungi (AMF) in seven Moroccan Tetraclinaies. Telllal[55] noted 10 species in the rhizosphere of *Casuarina glauca* and *C. cunninghamiana* developing in 15 sites and two nurseries in Morocco. Bouamri[48] found 15 species in the rhizosphere of Tafilalt palm after two successive rounds of trapping by sorghum and maize. In central Europe, Oehl[71] identified 12 species in the rhizosphere of the vine. *Acaulospora*, *Gigaspora* and *Glomus* genera are observed in the Sudanese area of Burkina Faso under the *Acacia halosericea* and A. Mangion plantations[66], in the Moroccan coastal dunes of Souss Massa[72], in the rhizosphere of *Casuarina* sp. in Morocco[55] and in argan trees soils [71]. Species *Claroideoglomus claroideum, Claroideoglomus etunicatum, Glomus tenebrosum, Glomus tortuosum, Pacispora chimonobambusae, Funneliformis geosporum, Glomus* sp2, *Glomus* sp3, *Glomus aggregatum, Rhizophagus intraradices Pacispora franciscana, Acaulospora scrobiculata*, have been reported at the rhizosphere of citrus in southern China[40]. Generally, nurseries produce plants with a root system which is not vigorous and slightly structured and cannot, therefore, withstand water stress they encounter after transplantation[24,71.] In this sense, Akpynar and Ortab[73] and Fikri[74] noted that *Glomus clarum* develop rapidly at the rhizosphere of different citrus rootstocks and stimulates the growth of inoculated plants. Syvertsen and Graham[75] and Farih[76] reported that plants inoculated with AMF have several advantages and it is therefore necessary to develop an inoculum production system. Indeed, its implementation appears interesting for Nurseries. In the USA, inoculation of Citrus grown in nurseries with AMF has become a common practice[77]. To achieve this objective, it is necessary to select certain species or a complex of native fungi, composed of several species, exhibiting high infectivity and good adaptation to different climatic and soil.

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