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Research Article

DIVERSITY OF ARBUSCULAR MYCORRHIZAL FUNGI IN THE STRAWBERRY RHIZOSPHERE IN MOROCCO

Bahouq Madiha and Douira Allal*

Laboratoire de Botanique, Biotechnologie et Protection des Plantes, Faculté des Sciences, Université Ibn Tofail, Kénitra Maroc (Morocco)

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ABSTRACT

Our study on arbusular mycorrhizal fungi of the strawberry plant was performed in various parcels (G, M and H) in the perimeter of Loukkos. The considered parameters were mycorrhizal colonization of strawberry roots, spore's density and specific richness. In all the roots samples, the presence of various characteristic structures of endomycorrhizal arbuscular fungi was revealed. The intensity of mycorhization varied between 71 and 12 % in the three parcels (G, M and H). Spore's density was respectively 52, 46 and 44 spores by 100 g of ground in parcels G, M and H. Four genera were identified: *Glomus, Acaulospora, Gigaspora and Scutellospora.* Genera *Glomus* spores were the most observed. The parcel with a strong mycorrhization shows a good vigor and a good phytosanitary state of plants.

INTRODUCTION

In Morocco, the strawberry plant is an important culture with high added value (Anonymous, 2015). It is cultivated in two perimeters: Loukkos (70%) and Gharb (30%). A part of 70 % of production is intended for the export (Anonymous, 2014). Strawberry culture needs important inputs of fertilizers, fungicides, insecticides, fumigating, qualified labor and plants. Strawberry is one of the rosaceae which requires cold for development, suitable for the Mediterranean, subtropical and tropical climate (Anonymous, 2015).

Strawberry culture is subjected to a wide range of diseases and enemies (El Kaissoumi *et al.,* 2016). The most dreaded are: the mildew, the powdery mildew, the decay intoxicates, acaroids, nematodes (Mouden *et al.,* 2013). By its nature, the ground accommodates a multitude of microorganisms acting according to various modes of action especially interaction, parasitism, antagonism and symbiosis. Mycorrhization was described as the most interesting symbiosis between plant and telluric microorganisms (Bever, 2002). This mode of interaction has an important role in the functioning and performance of their host plants (Hartnett *et al.,* 1993). Additional positive effects of mycorrhizal fungi on the host plants functioning were reported; including increased disease resistance, improved water relations, and acquisition of other soil nutrients (Augé *et al.,* 2015 ; Pozo and Azcón-Aguilar, 2007).

Also in 2000, Miller observed that roots covered by mycorrhizae have a better development with a high number of fine side roots (Miller *et al.,* 2000). Mycorrhizal colonization intensifies the capacity of the roots absorption of water and mineral elements from the soil (Anonymous, Daniel Tessier and Christian Mougin, 2011). The mycelium grows inside roots cells; thereby forming arbuscular structures which facilitates the assimilation of water and nutrient elements by plants and makes the development of agents more difficult (Derkowska *et al.,* 2008).

The role of the AMF was described as a fundamental connection between the plant and the soil (Al areqi *et al.,* 2013; Miller *et al.,* 1994; O' Neil *et al.,* 1991).

The diversity of arbuscular mycorrhizal fungi (AMF) is a major factor contributing to the maintenance of plant biodiversity, and ecosystem functioning (Van der heijden *et al*., 1998).

The endomycorrhizal fungi in the rhizosphere of the strawberry plant have never been studied in Morocco. The present study aims to determine the diversity of these fungi on the strawberry roots in the perimeter of Loukkos / Morocco.

Corresponding author:* **Douira Allal
Laboratoire de Botanique, Biotechnologie et Protection des Plantes, Faculté des Sciences, Université Ibn Tofail, Kénitra Maroc (Morocco)

MATERIALS AND METHODS

Soil and roots samples

The soil samples were taken from the rhizosphere of the strawberry plant in three land plots at the level of various sites in the perimeter of Loukkos. In every site, five kilograms (kg) of the soil were taken from five locations chosen in a random way. All samples were taken from the area around the roots. A composite sample was prepared from five samples collected at the level of every site.

Determination of the soil physico-chemical characteristics

The main physico-chemical characteristics of thesoil (pH, electric conductivity (EC), limestone, organic matter (O.M.), organic carbon (O.C.), nitric nitrogen (Nit.N.), nitrogen ammoniacal (Amo. N.), mineral nitrogen (Min.N.), phosphor (P) and potassium (K) were determined by the conventional analysis made in the "Office Regional de Mise en Valeur Agricole du Gharb (ORMVAG)" laboratory.

Roots coloring

Roots were removed from the soils particles by an abounding rinsing in the tap water in a sieve. Then, only the finest small roots were selected.

According to Philips and Hayman (1970) technique, roots were cut in fragments of 1 to 2 cm, and put in flasks of 10 % potassium hydroxide solution (KOH). These flasks were then placed in the water bath for 15 min at 90°C. Roots fragments were then cleared by adding some drops of H_2O_2 to the KOH solution. After 15 min, fragments were rinsed with the distilled water then colored witb Cresyl's bluesolution (0.05 %).

Evaluation of the mycorrhization rate

The evaluation of the mycorrhization parameters was realized by the observation of thirty fragments with 1 cm root's length, chosen randomly to quantify mycorrhizal structures inside of them (Trouvelot *et al.,* on 1986). These fragments were observed in groups of 10-15 in a drop of glycerin water between slide and coverslip (Kormanik and Mc Graw, 1982). Every fragment was carefully verified at a magnification ×100 and ×400 in all its length.

Mycorrhization frequency, mycorrhization intensity, and the arbuscular and vesiclar contents of the arbuscular mycorrhizal fungi inside the root bark were measured by attributing an index of mycorrhization going from 0 to 5 (Derkowska *et al.,* 2008): 0: Absence; 1: tracks; 2: less than 10 %; 3: from 11 to 50 %; 4: from 51 to 90 %; 5: more than 91 %

Mycorrhization Frequency (F %)

F % = 100 × (N0 - n0)/N
Where, N: number of observed fragments and n0: number of fragments non mycorrhized.

Mycorrhization Intensity (M %)

M % = (95 n5 + 70 n4 + 30 n3 + 5 n2 + n1)/N
Where, n = number of fragments affected of the indication 0, 1, 2, 3, 4 or 5

Arbuscular Content (A %)

In % = (100 mA3 + 50 mA2 + 10 mA1) / 100
Where mA3, mA2, mA1 are the % affected respectively notes A3, A2, A1,
With, mA3 = (95 n5 A3 + 70 n4 A3 + 30 n3 A3 + 5 n2 A3 + n1 A3) / N. Also, for A1, A2,

In this formula, n5A3 represents the noted number of fragments 5 with A3; n4A3 the noted number of fragments 4 with A3;
A0: no arbuscules; A1: few arbuscules 10 %; A2: moderately abundant arbuscules 50 %; A3: abundant arbuscules: 100 %.

Vesicles Content (V %)

V % = (100 mV3 + 50 mV2 + 10 mV1) / 100
Where mV3, mV2, mV1 are the % affected respectively notes V3, V2, V1,
With, mV3 = (95 n5 V3 + 70 n4 V3 + 30 n3 V3 + 5 n2 V3 + n1 V3) / N. Also, for V1, V2,
In this formula, n5V3 represents the noted number of fragments 5 with V3; n4V3 the noted number of fragments 4 with V3;..
V0: no vesicles; V1: few vesicles 10 %; V2: moderately abundant vesicles 50 %; V3: very abundant vesicles: 100 %.

Spores Extraction

Gerdemann and Nicholson (1963) method's was adopted to extract spores from the soil around the roots. In a 1 liter (L) beaker, 100 grams (g) of each soil was 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 a sieve of 315 microns mesh size. The same soil sample was again submerged, stirred, and the wet sieving was repeated 3 times. Deposition in the used sieve contained the maximum of spores; it was recovered with 6 mL of distilled water and transferred to centrifuge tubes. After 5 minutes of the first centrifugation at 2000 revolution per minute (RPM), debris and the supernatant were discarded and the pellet was suspended in a 4 mL solution of sucrose 50%.

After agitation, a second centrifugation was performed for 1 minute at 2000 RPM and a 3th one was realized for 1 minute at 3000 RPM. Spores existing in the supernatant were passed through the sieve and the pellet was discarded. Spores in the sieve were rinsed with distilled water to remove the sucrose, and then disinfected with a solution of streptomycin. Spores were then recovered with 5 milliliter (mL) of distilled water in an Erlenmeyer flask. Finally, endomycorrhizal spores were quantified to estimate their number in 100 g of soil.

A preliminary identification of spores' genera and species was based on morphological characters proposed by Berch (1986), Schenk and Smith (1982), Schenck and Perez (1987), Morton and Benny (1990), Walker (1992), Dalpé (1995), Mukerji (1996), and available information in various databases (Anonymous, 2011).

Specific richness and occurrence frequency

Species richness was defined as the total number of the observed species per site collection.

Occurrence frequency of species corresponded to the percentage of sites where each species is detected.

Statistical analysis

Statistical study was based on the analysis of variance in single criteria (ANOVA1).

RESULTS

Physico-chemical Properties of the soil

The soil physico-chemical characteristics collected from the plant rhizosphere of the three parcels are presented in table 1. pH values vary between 7.79 (site M) and 7.98 (site H); carbon rates are around 0.55; the total nitrogen rate fluctuates between 145.56 ppm (M) and 212.20 parts per million (ppm) (H).

Table 1 Physico-chemical characteristics of soil

Site	pH	CE mmhos/cm	Limestone %	O.M %	C.O %	nitrogen ppm			P ppm	K ppm
						Amo.	Nit.	Min.		
G	7.96	0.15	0.12	0.95	0.55	24.48	133.92	158.40	31	176
M	7.79	0.11	0.00	0.91	0.53	19.08	126.48	145.56	21	205
H	7.98	0.09	0.00	0.96	0.56	21.24	190.96	212.20	25	88

Contents in organic matter do not exceed 0.96 %; phosphorus content varies between 31 ppm (G) and 21 ppm (M). Contents in comparable potassium also oscillate between 88 ppm (H) and 205 ppm (M). Limestone is present in the site G (0.12%).

Mycorrhizal characterization of the strawberry plant

In all studied sites, the roots of the strawberry plant were all mycorrhized. Various characteristic structures of arbuscular mycorrhizal fungi (AMF) were observed: arbuscules, hyphae intra and extracellular (Fig. 1), vesicles (Fig. 2) and spores (Fig. 8).

Mycorhization frequency varies from 12 % (site G) to 71 % (site H) (Fig. 3). The highest values of mycorrhization intensity were observed in the site H (46 %) and the lowest level was in the site G (5 %) (Fig.4). Arbuscular content was very low in site G (7 %) and higher in site H 56 % (Fig. 5). Vesicles content reached 5 % in the site G (Fig. 6). The mean of AMF spores density was respectively 52 in soil (H) and 44 spores in soil (M) (Fig. 7).

Spores diversity

Preliminary and temporary identifications allowed noting that isolated spores belong to 3 families (Glomaceae, Gigasporaceae, and Acaulosporaceae), distributed to 4 genera (*Glomus, Gigaspora, Acaulospora, Scutellospora)* and to 6 species of Glomales: *G. intraradices, G. mossaeae, Glomus* sp., *Acaulospora colossica, Gigaspora margarita and Scutellospora scutata* (Fig. 8 and 9).

Specific richness varied according to parcels. There were 6 species at the site G and 5 at each of the two other sites (Fig. 10). *Glomus intraradices* and *Glomus mossaeae* were the most dominant species; their occurrence frequency reached respectively 37 and 27 % (Fig. 11).

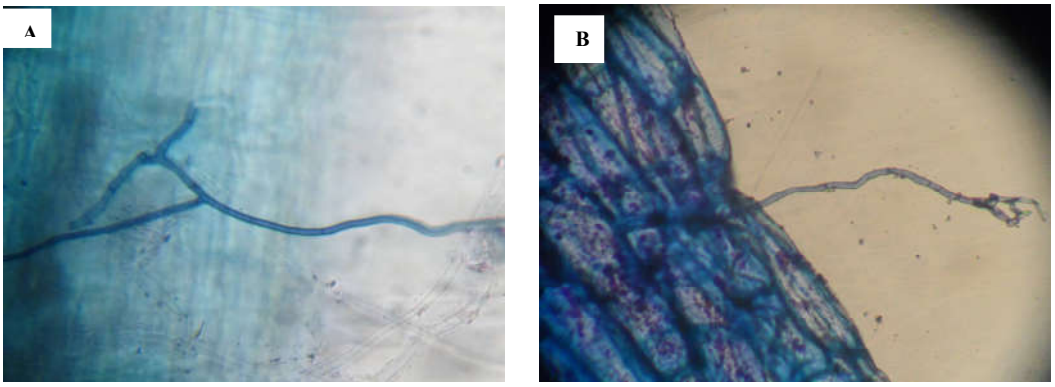

Fig 1 Roots of mycorrhizal strawberry roots presenting various structures of the arbuscules (A): hyphae extra and intra-roots (B) (G. ×400).

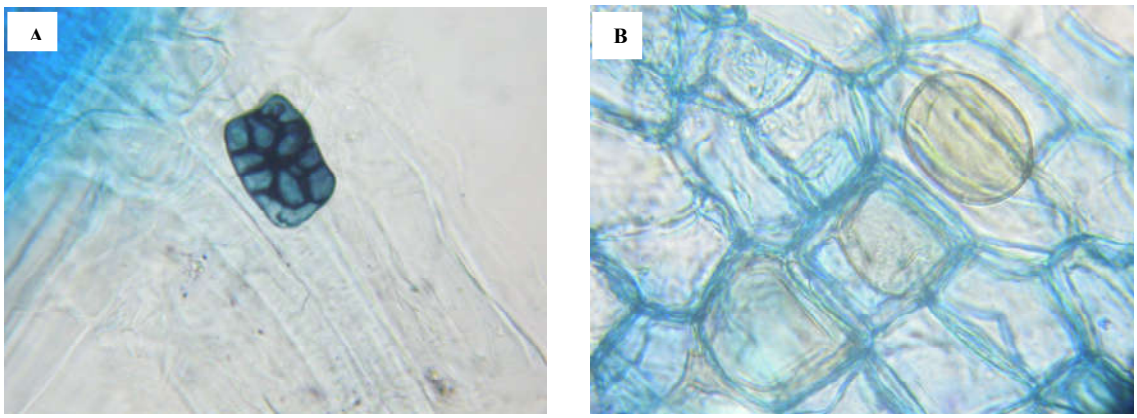

Fig. 2 Round vesicles are formed between roots and spores cortex cells of AMF (A); rectangular vesicles are formed between roots cortex cells (B) (G. ×400).

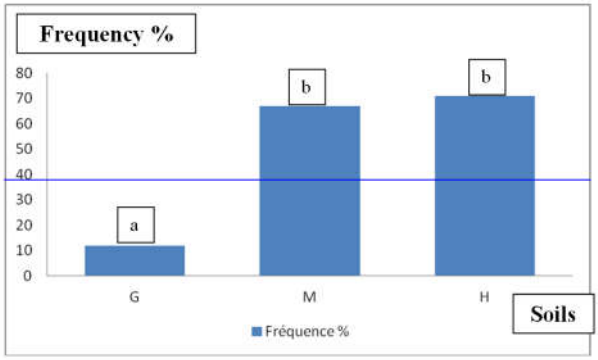

Fig. 3 Mycorrhization Frequency of the mycorrhizal species in the study sites.

Two results with the same letter do not differ significantly at the threshold of 5%.

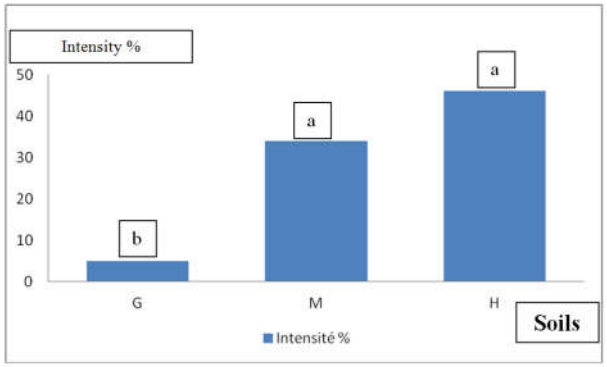

Fig 4 Mycorrhization Intensity of mycorrhizal species in the study; sites.

Two results with the same letter do not differ significantly at the threshold of 5%.

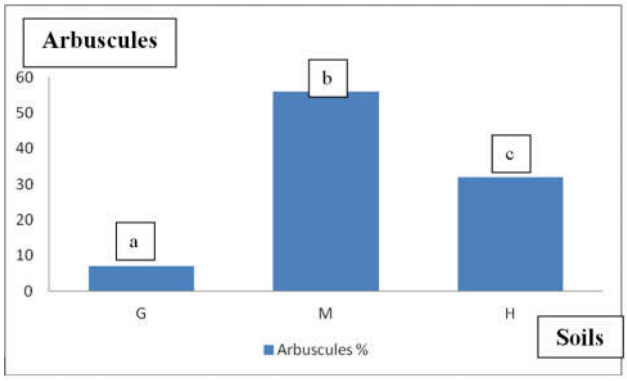

Fig 5 Arbuscular content of mycorrhizal species in the study sites.

Two results with the same letter do not differ significantly at the threshold of 5%.

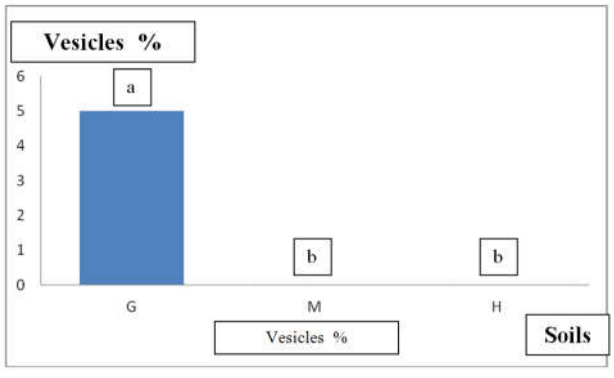

Fig 6 Vesicular content of the mycorrhizal species in the study sites.

Two results with the same letter do not differ significantly at the threshold of 5%.

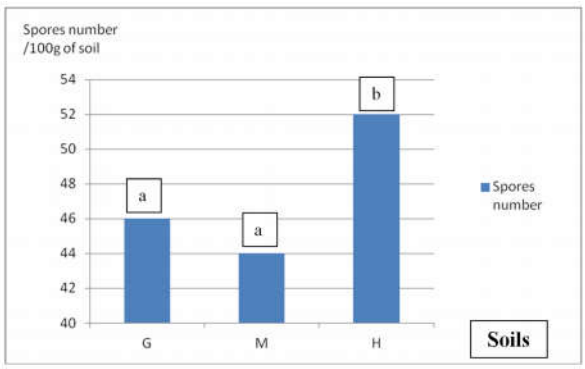

Fig 7 Spores Density in the study sites

Two results with the same letter do not differ significantly at the threshold of 5%.

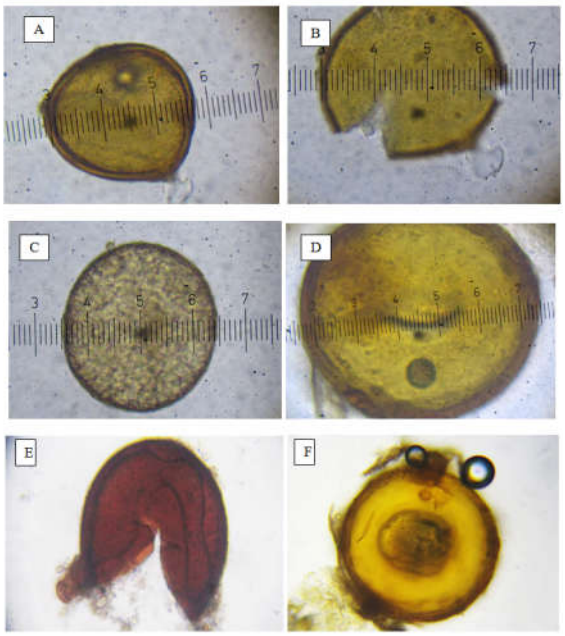

Fig 8 Species of the Glomus genera isolated from the plant rhizosphere: spore of *Glomus intraradices* (A); *Glomus mosseae* (B); *Glomus* sp. (C); *Gigaspora margarita* (D) *Acaulospora colossica* (E), *Scutellospora scutata* (F) (G. ×400).

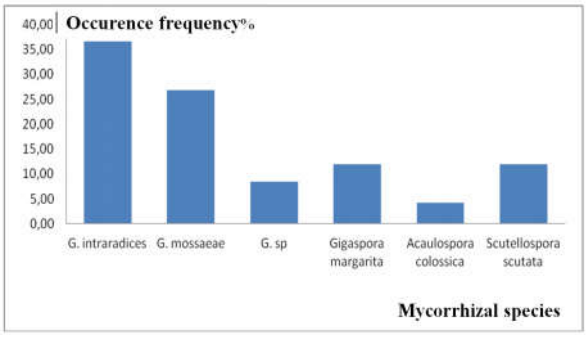

Fig 9 Occurrence Frequency of the mycorrhizal species in the all study sites.

DISCUSSION AND CONCLUSION

The results showed that parcel M and H were more colonized by mycorrhizae than parcel G. The highest arbuscular content was registred in parcel M. Vesiclar content was raised in parcel G compared to the other parcels. Mycorrhization frequency and intensity were more important in parcel M. Spores number registered in parcel H exceeded those observed in the two other soils.

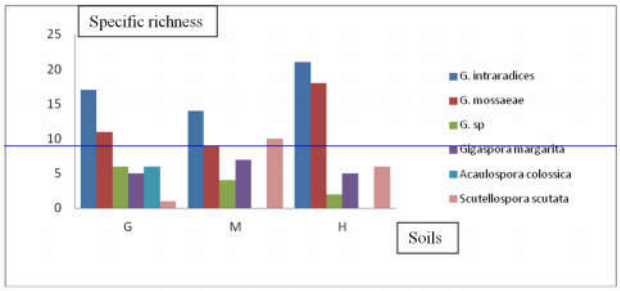

Fig 10 Specific richness in all study sites

The variability of mycorrhization parameters between the three soils could be explained by the difference of the physico-chemical properties and the mode of culture adopted in every soil (Boullard 1990).

Soils M and H present a lower salinity as well as content raised in nitrogen compared to the soil G. These two soils showed important mycorrhization colonization.

Studies indicate that the AMF could have a role in the phosphor assimilation by the plant (Boullard, 1990).

Recent results have shown that Phosphorus (P) , in agreement with previous data on the same AM system (Breuillin *et al.,* 2010 ; Nouri *et al.,* 2014), and nitrate can potentially exert negative regulation on AMF, while sulfate, Mg^{2+}, Ca^{2+}, and Fe^{3+} have no effect.

These results are the same as what Harley and Smith (1983) have found, Vivekanandan and Fixen (1991) stipulating that mycorrhization frequency is raised in soils with a low rate of total phosphorus.

According to Mousain *et al.* (1979), the concomitant increase of the contents in phosphorus and in nitrogen observed in the air systems of mycorrhized pines, shows that these took bigger quantities of these elements than non mycorrhized ones. The stimulation of mycorrhized plants growth is often associated, indeed, with a beneficial effect of AMF symbioses on the nutritional phosphate by plants-hosts (Hatch, 1937; Mousain, 1989; Bolan, 1991).

Our results converged to other authors' findings which demonstrated that mycorrhizal frequency in the roots of trees growing in an organic orchard was higher than in the roots of trees grown by the conventional method (Forge *et al.,* 2001).

The total number of the observed spores was more important in soil H compared to the two others. This can be due to the cumulative effect of the low level of phosphorus (P). Various studies indicated the negative effect of soil nutrient on the presence and the development of the AMF (Smith *et al.,* 1997). The soils H present a high level of mycorrhization colonization, which can explain the increased number of observed spores.

Besides, the infectious potential of a soil with AMF depends not only on the number of the present spores in this soil but also on their quality and their capacity of adaptation and infectivity (Gianinazzi-Pearson *et al*., 1988).

Differences in specific diversity and spores number can be explained by the characteristics of every soil as well as the used cultivation methods. This result was also reported and verified by Santos (2011), and the high diversity of AMF strawberry colonization was also noted by Gavériaux (2012).

Fluctuation in the number of observed spores can be also attributed to the formation process, the seeding, the degradation (Chauhan *et al.,* 2010), the date of the sampling (Jamil *et al.,* 2003), climate change, ground, and existing microorganisms (Miller *et al.,* 1994). Hatimi and Tahrouch (2007) demonstrated that the mycorrhization depends on the evolution of the soil nutrients and showed that the production of spores is important during the blooming of plants and decreases at the end of season when plants are in terminal phase and physiologically change of roots.

Furthermore, sporulation may depend on the AMF species, edaphic characteristics of the soil, and climate conditions. According to Jasper *et al.* (1991), the weak relationship between the endomycorrhizal formation and the quantity of the isolated spore's is due to the fact that some propagules would be dormant.

According to Le Tacon (1978), AMF fungi were not specific and there was no blank ground where there was no endophytes, but the AMF differ in their infective powers and numbers that appeared more or less important depending on the plant host and the competition between different endomycorrhizal species.

Our results showed that the *Glomus* genus was dominant. This report was brought back at the level of other studies (Charest *et al.,* 1993; Porras Soriano *et al.,* 2002). That was demonstrated by other studies in Latin America (Cruz, 1989; Lopes *et al.,* 1983), southwest of Ethiopia (Jefwa *et al.,* 2009; Muleta *et al.,* 2008), the rain forest of Xishuangbanna, China (Zhao, 2001), dry lands and semi dry of the North of Jordan (Jamil *et al.,* 2003) and the majority of the coffee tree fields in Yemen (Areqi *et al.,* 2013).

Authors have associated the high incidence Glomus spores genus with their capacity to produce an important number of spores in a reduced time compared to other genera (*Gigaspora, Scutellospora*) (Dell' Amico *et al.,* 2002; Jefwa *et al.,* 2009**)**. In other studies, it was found that the species of the Glomus genus are good colonists of many plants including strawberry (Lidia Sas Paszt *et al.,* 2011; Gavériaux, 2012). Our results showed that the isolated spores belong to 4 genera (*Glomus, Gigaspora, Acaulospora, Scutellospora*) distributed to 6 species of Glomales: *G. intraradices, G. mossaeae, Glomus* sp., *Acaulospora colossica, Gigaspora margarita* and *Scutellospora scutata.*

In Poland, Lisek *et al*. (2012) noticed the presence of the *Glomus* genus, Scutellospora and Acaulospora on strawberry roots. In the selected soil of strawberry fields of Southern Quebec, Muamba Funakoshi Didier *et al*., (2003) detects *Glomus mosseae* and *G. macrocarpum* at two of the three sampling sites. *G. constrictum* and *Sclerocystis rubiformis* were detected initially in the soil of strawberry fields, but could not be recovered throughout successive bait cultures.

The variation of the observed species was associated with the degree of colonization, the presence or the absence of the AMF in the same soil, and the environmental factors (Alexander *et al.,* 1989).

In fact, agricultural practices affect mycorrhizal colonization and various symbioses generally. Negative effect of some of these practices was demonstrated; especially fumigation technique (Buttery *et al*., 1988), pesticides, and fertilizers application (Titus *et al.,* 2000; Wilson and Williams, 2008).

Our study demonstrated the large mycorrhizal colonization of all roots samples which was affected by the soil characteristics. Spores diversity was noted by the dominance of *Glomus* genus. This study of the natural diversity of arbuscular mycorrhizal fungi in the strawberry rhizosphere in Morocco is a starting point to develop inoculants suitable to get strawberry plants that may be more robust and resistant against pathogens and stress after transplanting. The knowledge about the functional diversity of Arbuscular Mycorrhizal Fungi (AMF) is an important ecological issue that deserves greater research efforts especially when trying to use them in horticulture. Strawberry diversity of AMF is unexplored in Morocco, and this study has contributed to the available knowledge.

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