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BEHAVIOR OF A COMPOSITE ENDOMYCORRHIZAL INOCULUM IN THE RHIZOSPHERE OF FIVE MYCOTROPHIC SPECIES

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Abstract

This work reports the results of the evolution of a composite inoculum containing 70 species in the rhizosphere of five mycotrophic plant species namely beans, maize, barley, wheat, and sorghum. The number of endomycorrhizal species isolated is respectively as follows: 19, 12, 11, 13, 12, one month after their inoculation. Analysis of the spores' communities of endomycorrhizal fungi found in these mycotrophic species rhizosphere showed the dominance of *Glomus intraradices*, *Gigaspora* sp1, *Glomus versiforme* and *Acaulospora* sp1. Moreover, the comparison between the AM fungi of the initial inoculum species (70 species) and those isolated revealed the appearance of 10 species and the disappearance of 51. However, 19 species are faithful and capable of maintaining a constant activity with the roots of the plants studied.

Key words : Barley, bean, behaviour, citrus, endomycorrhizal composite inoculum, maize, rhizosphere, sorghum, time, wheat.

Introduction

Citrus plants are a mycotrophic species (Wang, 2012; Wang *et al.*, 2015; Artib *et al.*, 2016). In 2012, Wang reported the presence of 12 species in the citrus rhizosphere in southern China (*Claroideoglomus claroideum*, *Claroideoglomus etunicatum*, *Glomus tenebrosum*, *Glomus tortuosum*, *Pacispora chimonobambusae*, *Funneliformis geosporum*, *Glomus* sp2, *Glomus* sp3, *Glomus aggregatum*, *Rhizophagus intraradices*, *Pacispora franciscana*, *Acaulospora scrobiculata*). In Morocco, the first studies on the diversity of arbuscular mycorrhizal fungi in the rhizosphere of several species of the genus Citrus revealed the presence of 70 species belonging to 10 genera (Artib *et al.*, 2016): *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), 8 families (*Glomaceae*, *Gigasporaceae*, et *Acaulosporaceae*, *Diversisporaceae*, *Pacisporaceae*, *Dentiscutataceae*, *Scutellosporaceae*, *Entrophosporaceae*) and 3 orders (Glomerales, Gigasporales, Diversisporales).

The soil collected from the rhizosphere of the Citrus species studied (Artib *et al.*, 2016) probably contains other species that didn't sporulate. Indeed, an inoculum multiplied on barley roots and inoculated to Citrus aurantium plants may have improved the development of root and vegetative growth of the plants (Artib *et al.*, 2017). Similarly, 23 species belonging to the genera *Glomus* (8 species), *Acauolospora* (9 species), *Scutellospora* (3 species), *Pacispora* (2 species) and *Gigaspora* (1 species) have sporulated in the rhizosphere of inoculated plants of *Citrus aurantium* L.

In order to demonstrate the presence of other endomycorrhizal species, a composite inoculum constituted of rhizospheric soil of the Citrus studied (Artib *et al.*, 2016) was multiplied using other mycotrophic plant species, cases of bean, maize, barley, wheat and sorghum. These mycotrophic species may probably favour the appearance of other species that were not encountered during the first analyses of the soil collected from the Citrus species rhizosphere.

Materials and Methods

A composite endomycorrhizal inoculum consisting of 70 different morphotypes and probably others (Artib *et al.*, 2016) was used to inoculate five species of mycotrophic plants: maize, sorghum, wheat, barley and bean. This inoculum is originating from the rhizosphere of eleven varieties of citrus rootstocks, planted in 1986, in a test plot at the National Institute of Agronomic Research in the Gharb region.

The experimental protocol was performed as a random block. Two lots of plants were made with a quantity of 38 g of seeds for each plant species.

Lot 1 : Control plants (T) transplanted into pots filled only with disinfected Mamora sand.

Lot 2 : Plants inoculated with mycorrhizae, then transplanted into plastic pots filled with a mixture of 50% of composite endomycorrhizal inoculum and 50% of sand disinfected in the oven at 250°C for 2 hours.

The pots are then placed in a greenhouse. After one month of cultivation (April to June), the root mycorhizal parameters of inoculated plants were evaluated using techniques adopted by Philips and Haymann (1970), Trouvelot *et al.* (1986) and Derkowska *et al.* (2008). Spore extraction from rhizospheric soil of inoculated plants was done using the wet sieving method described by Gerdemann and Nicholson (1963). Species identification was carried out on the basis of microscopic observations of morphological characters, and also with reference to the determination keys of Schenck and Perez (1990) and the INVAM website. The root mass is the agronomic parameter found in the plants after one month of cultivation.

The statistical treatment of the results obtained focused on the analysis of variance using a single classification criterion (ANOVA1).

Results

The roots of the plant species studied (maize, sorghum, wheat, barley, and beans) were found to be mycorrhizal. Indeed, different structures characteristics of arbuscular endomycorrhizae have been observed: arbuscles, vesicles, extracellular hyphae and endophytes (fig. 1).

The roots of the plants studied recorded significant mycorrhizal frequencies (fig. 2), 96.6% was noted in the roots of sorghum, wheat, barley and 93.3% in those of maize and beans.

The highest values of mycorrhizal intensities which correspond to the percentage of the root cortex mycorrhized (fig. 2) were noted in the roots of barley (62.5%) and sorghum (59.03%) and the lowest values in those of wheat (50.2%) and beans (56.3%).

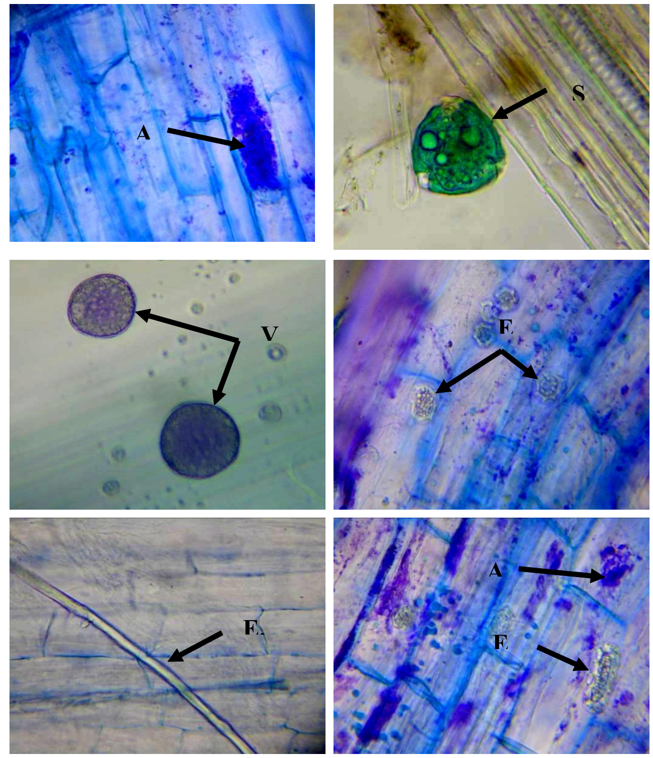

Fig. 1 : Mycorhizal roots of maize, sorghum, wheat, barley and beans with arbuscles (A); extra hyphae (EH), spores (S); vesicles (V) and endophytes (E) (G ×400).

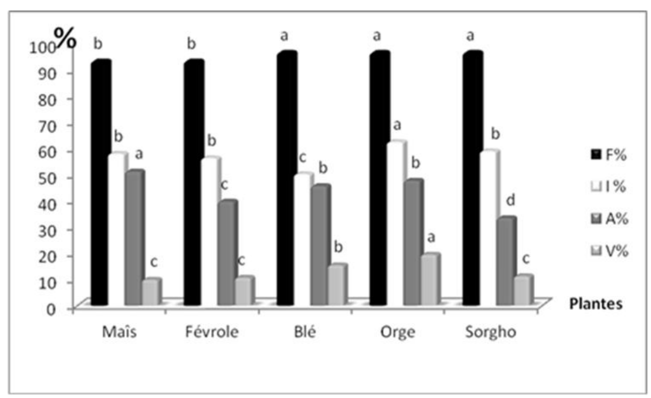

Fig. 2 : Evaluation of the mycorrhizal rate of the five inoculated plant species. Mycorrhizal frequency (F), mycorrhizal intensity (I), arbuscular content (A) and vesicular content (V).

The arbuscular contents (fig. 2) are important in maize, barley, wheat; respectively 51.26, 47.77 and 45.71%, and those observed in beans and sorghum are in the order of 39.79% and 33.5%. Vesicular contents are very low in maize (9.97%), beans (10.72%), and moderately high in barley (19.55%) and wheat (15.47%).

Root mycorrhization was accompanied by spore development in the rhizosphere of inoculated plants. The

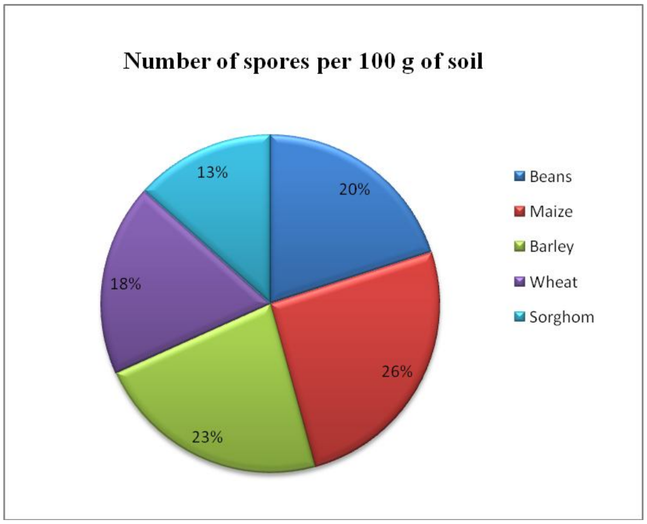

Fig. 3 : Number of spores per 100 g of soil isolated from the rhizosphere of mycotrophic plants studied after one month of cultivation.

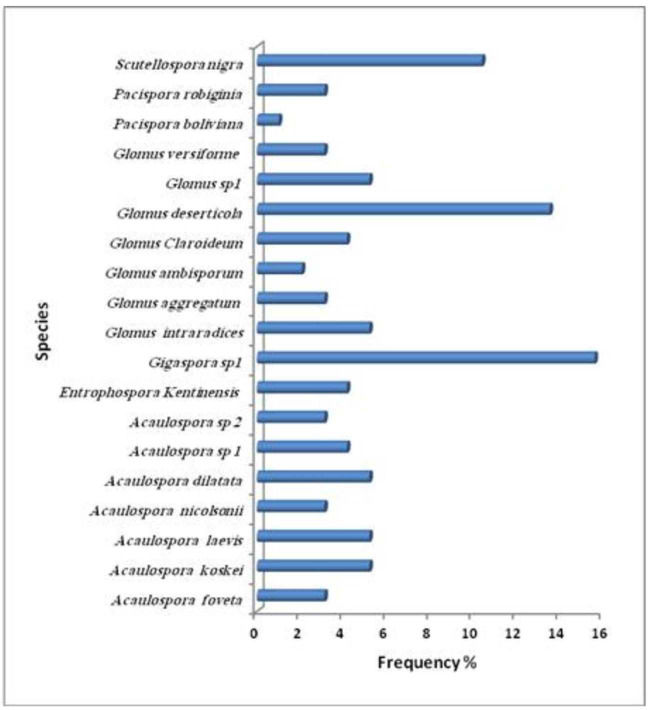

Fig. 4 : The appearance frequency of endomycorrhizal species isolated from the rhizosphere of Bean plants after 1 month of cultivation with AM fungi.

density of the endomycorrhizal fungi spores in the rhizosphere of inoculated plants depend on the plant species studied (fig. 3).

After 1 month of cultivation, Microscopic observation revealed the presence of 19 species in the rhizosphere of the bean, with a sporal density of 96 spores/100g of soil. *Gigaspora* sp1, *Glomus deserticola* and *Scutellospora nigra* are the most dominant species (fig. 4).

The average number of spores found in the rhizosphere of maize plants was 123 spores/100g of soil (fig. 5). This number was represented by 12 species of endomycorrhizal fungi, the most dominant were *Acaulospora* sp 1, *Glomus boreale* and *Scutellospora fulgida*. The number of spores recorded in the rhizosphere of barley plants was 108 spores/100g of soil, represented by 11 species and *Glomus macrocarpum* was the dominant (fig. 6).

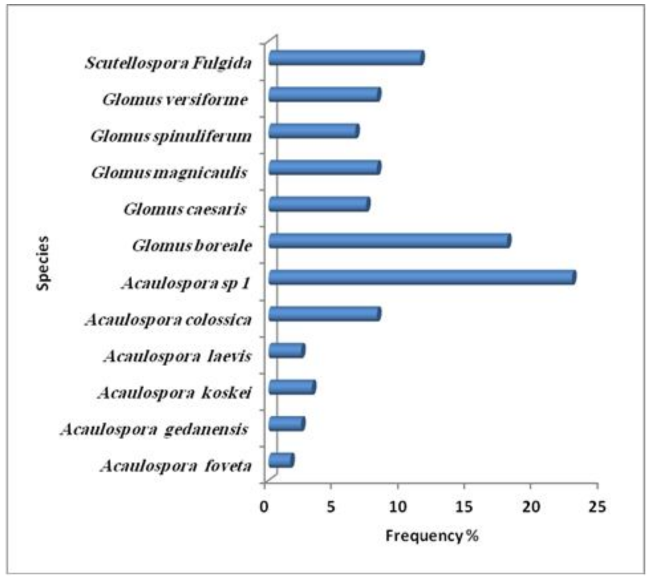

Fig. 5 : The appearance frequency of endomycorrhizal species isolated from the rhizosphere of maize plants after 1 month of cultivation with AM fungi.

Moreover, in the rhizosphere of wheat plants, the number of spores reached 88 spores/100g of soil, represented by 13 endomycorrhizal species; *Gigaspora* sp1 and *Glomus intraradices* were the most dominant (fig. 7). The number of spores in the rhizosphere of sorghum plants was 64 spores/100g soil, represented by 12 species; *Glomus intraradices*, *Acaulospora foveta* were the dominant species (fig. 8).

A comparison of different species of the original inoculum AM fungi (70 species) with those isolated from the rhizosphere of the different mycotrophic species studied revealed the appearance of 10 other species (*Acaulospora koskei*, *Acaulospora nicolsonii*, *Acaulospora dilatata*, *Acaulospora* sp 1, *Acaulospora* sp 2, *Entrophospora* sp 1, *Glomus caesaris*, *Glomus magnicaulis*, *Glomus* sp1, *Pacispora robiginia*) and non-sporulation of 51 species.

In addition 19 species (*Acaulospora foveta*, *Acaulospora gedanensis*, *Acaulospora laevis*, *Acaulospora colossica*, *Entrophospora Kentinensis*, *Gigaspora* sp1, *Glomus intraradices*, *Glomus aggregatum*, *Glomus ambisporum*, *Glomus boreale, Glomus Claroideum, Glomus deserticola, Glomus*

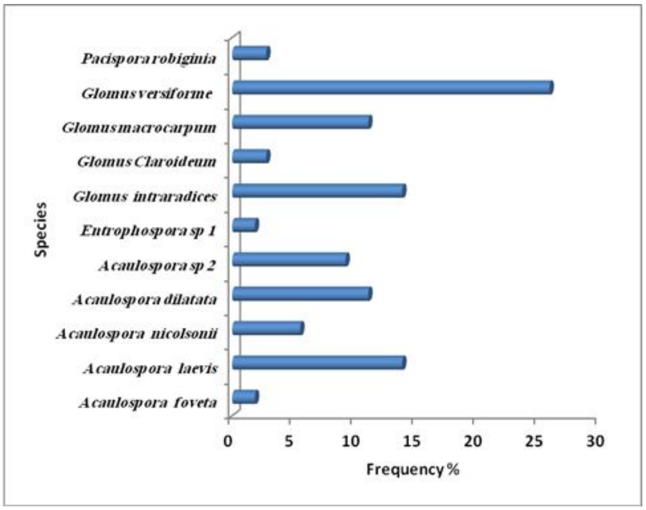

Fig. 6 : The appearance frequency of endomycorrhizal species isolated from the rhizosphere of barley plants after 1 month of cultivation with AM fungi.

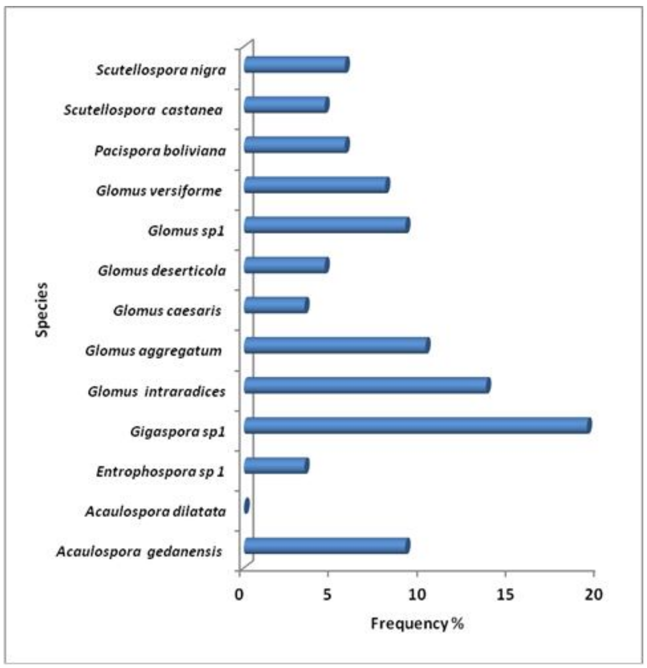

Fig. 7 : The appearance frequency of endomycorrhizal species isolated from the rhizosphere of wheat plants after 1 month of cultivation with AM fungi.

macrocarpum, Glomus spinuliferum, Glomus versiforme, Pacispora boliviana, Scutellospora castanea, Scutellospora fulgida, Scutellospora nigra) have sporulated in the roots of the plants of the studied species (fig. 9). Similarly, isolated species are represented by 43.33% of *Glomus*, 30% of *Acaulospora*, 10% of *Scutellospora*, 6.66% *Pacispora* and *Entrophospora*, and also *Gigaspora* 3.33% (fig. 10).

The roots mycorrhization of the plants species studied and the sporulation of the endomycorrhizal fungi in the rhizosphere of these plants roots were accompanied

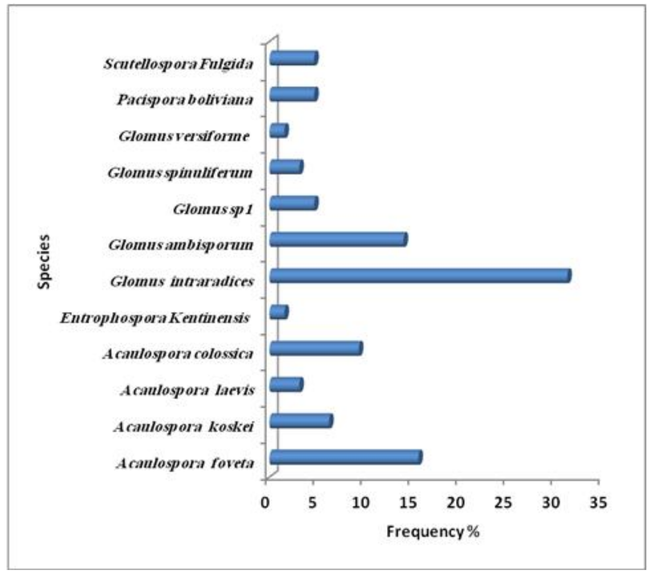

Fig. 8 : The appearance frequency of endomycorrhizal species isolated from the rhizosphere of sorghum plants after 1 month of cultivation with AM fungi.

Table 1 : Root mass of plant species studied inoculated with a composite endomycorrhizal inoculum after one month of cultivation.

Mycotrophic species	Root mass (g)		Yield (%)
	Inoculated plants	Control plants	
Beans	29.3g	18 g	62.7%
Maize	40 g	21g	90.4%
Barley	33.2 g	17 g	89.4%
Wheat	31g	16,6 g	86.7%
Sorghum	30 g	14,8g	102.7%

by a growth of the root mass of all the inoculated plants (fig. 9). The most apparent root mass is that of the inoculated maize plants (40 g against 21g in control plants with a gain of more than 90.4%) and the lowest was noted in bean plants (29.3 g compared with 18 g in control plants with a gain of 62.7%) (table 1).

These results shows that the sporulation of the species of the composite endomycorrhizal inoculum is dependent on the mycotrophic plant tested (maize, sorghum, wheat, barley, and beans). Some species develop a symbiotic association with the roots of the inoculated species and sporulate in their rhizospheres four weeks after inoculation. This association is beneficial and allows the development of a root mass in the inoculated plants.

Discussion and Conclusion

Arbuscular mycorrhizae (AM) are important symbioses for most plant species, especially in difficult conditions where phosphorus is considered as a limiting

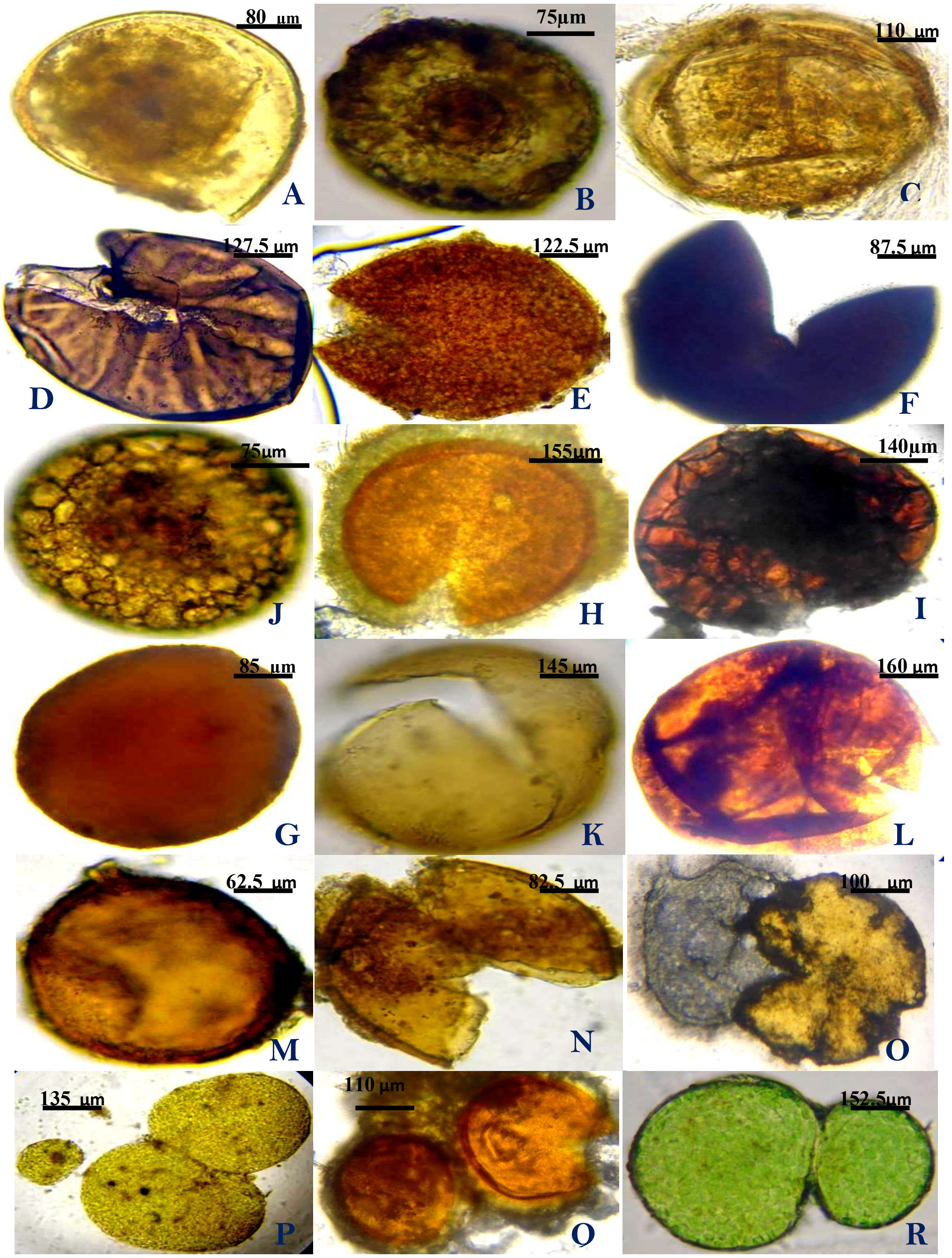

Fig. 9 : Some species of endomycorrhizal fungi isolated from the rhizosphere of maize, sorghum, wheat, barley and beans. (A) *Scutellospora fulgida*, (B) *Entrophospora kentinensis*, (C) *Glomus intraradices*, (D) *Scutellospora castanea*, (E) *Glomus versiforme*, (F) *Scutellospora nigra,* (J) *Entrophospora* sp 1, (H) *Acaulospora foveta,* (I) *Acaulospora* sp 1, (G) *Glomus deserticola* , (K) *Acaulospora gedanensis*, (I) *Glomus deserticola*, (L) *Glomus magnicaulis,* (M) *Glomus boreale*, (N) *Glomus macrocarpum*, (O) *Glomus ambisporum*, (P) *Gigaspora* sp1, (Q) *Glomus caesaris* , (R) *Giga spora* sp1.

factor. These symbiotic associations improve plant growth and development; also increase plant resistance to biotic and abiotic stresses (Peter *et al*., 2003).

The data on the root's fresh weight showed a beneficial effect after inoculation with endomycorrhizal fungi; this confirms the results reported in other plant species, Citrus (Artib *et al*., 2017), Carob tree (Talbi *et al*., 2016), *Lycium europaeum* (Touati *et al*., 2015), date palm (Sghir *et al*., 2014), tomato and olive tree (Chliyeh *et al*., 2014).

The analysis of the communities of endomycorrhizal fungi spores found in the rhizosphere of the five mycotrophic plant species studied showed the dominance of 6 species (*Giga spora* sp1, *Acaulospora* sp 1,

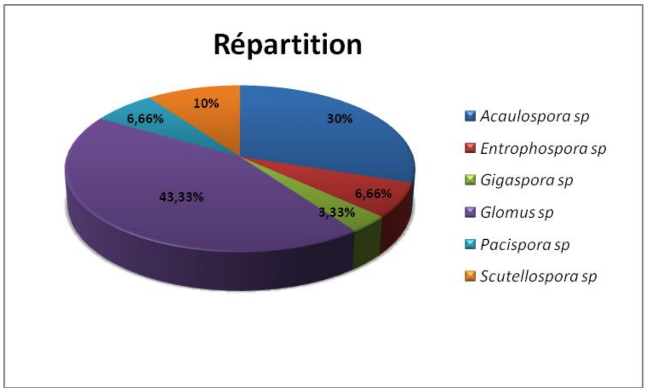

Fig. 10 : Distribution of isolated genera in the rhizosphere of mycotrophic plants.

Glomus boreale, *Glomus macrocarpum*, *Glomus intraradices*, *Acaulospora foveta*). These species belong to 3 genera (*Gigaspora*, *Glomus*, *Acaulospora*); the most dominant is the genus Glomus. This genus is also most common in the mobile dunes of Mehdia (Morocco) in the *Eryngium maritimum* rhizosphere (Hibilik et al. 2016), in the Sudanian zone of Burkina Faso under the plantations of *Acacia halosericea* and *A. mangion* (Ba *et al*., 1996), also in the rhizosphere of *Casuarina* sp. in Morocco (Tellal *et al.*, 2008), the Moroccan coastal dunes of Souss Massa (Hatim and Tahrouch, 2007) and in argan soils (Sellal *et al*., 2016).

A comparison between the AM fungi species of the initial composite inoculum (70 identified species and probably others with no sporulation) and those isolated from the rhizosphere of the mycotrophic plants inoculated by the initial inoculum revealed the presence of 29 species in the rhizosphere of these species, with the presence of 10 other species that sporulated for the first time (*Acaulospora koskei*, *Acaulospora nicolsonii*, *Acaulospora dilatata, Acaulospora* sp 1*, Acaulospora* sp 2*, Entrophospora* sp 1*, Glomus caesaris*, *Glomus magnicaulis, Glomus* sp1, *Pacispora robiginia*) and 19 species, among the 70 of the initial inoculum, have also multiplied.

It seems that some endomycorrhizal species develop and sporulate better in the rhizosphere of such or certain mycotrophic species case of *Acaulospora* sp1, *Glomus boreale* and *Scutellospora fulgida* which dominate in the rhizosphere of maize plants and *Glomus macrocarpum* in the rhizosphere of barley plants. In the rhizosphere of wheat plants, the species *Gigaspora* sp1 and *Glomus intraradices* are the dominant, while in the sorghum plants, the dominant species are *Glomus intraradices* and *Acaulospora foveta*. However, in the rhizosphere of bean plants, the species *Gigaspora* sp1, *Glomus deserticola* and *Scutellospora nigra* sporulate the most. Two species of endomycorrhizal fungi, *Glomus aggregatum* and *Glomus intraradices*, found in the rhizosphere of Citrus species (Wang, 2012; Artib *et al*., 2016) were also found in the rhizosphere of mycotrophic plants studied. *Glomus intraradices* is among the dominant species in the rhizosphere of sorghum plants, the same result has been reported by Talbi *et al*. (2016). According to Saif (1981, 1983), the highest colonization of sorghum roots by endomycorrhizal fungi was noted for oxygen levels of 16 and 21%.

Saif (1981, 1983) and Talbi *et al*. (2016) recorded the presence of *Acaulospora laevis* and *Glomus intraradices* in the rhizosphere of sorghum; this is in agreement. Furthermore, Carrenho *et al*. (2002) reported the presence of *Glomus clarum* and *Glomus claroideum* in this host plant. Saif (1986) and Howeler *et al*. (1987) noted that legumes tend to favour sporulation of AM fungi more than grasses. Thus, Howeler *et al*. (1987) noted that leguminous plants can produce more AM spores than grasses. Simpson and Daft reported the contrary, the sporulation of *Glomus clarum* in the rhizosphere of sorghum and millet is higher than in groundnuts and chickpeas. Indeed, Redmond et al. noted that some flavonoids are produced exclusively by compound legumes, which have been often linked to the attraction of the germ tubes of *Glomus margarita* and *Glomus* sp at the root level. These compounds can promote root colonization by AM fungi, influencing the germination of spores and the growth of germ tubes.

Glomus aggregatum; *Glomus ambisporum*; *Glomus boreale*; *Glomus deserticola*; *Glomus macrocarpum*; *Glomus spinuliferum*; *Pacispora boliviana*; *Scutellospora castanea*; *Acaulospora foveta*; *Acaulospora laevis*; *Acaulospora colossica*; *Acaulospora gedanensis* are persistent species reported in the primary inoculum (Citrus rhizospheric soil), after the inoculation of *Citrus aurantium* plants and that of the mycotropic plants studied.

Arbuscular mycorrhizal symbiosis does not show clear host specificity (Selosse *et al*., 2006); some species of AM fungi have a wide range of host plants, while others have been found only in the rhizosphere of a single host plant, such as Acaulospora splendida found in the rhizosphere of Quercus costaricensis (Sieverding, 1991). Newman *et al*. (1994) showed that the same AMF is capable of affecting simultaneously two adjacent plants of different species which can be linked with a common underground mycelial network.

The distribution and dominance of different species observed in all the different rhizospheres of the five plants studied showed that *Glomus versiforme* is the common specie in all these plants with variable abundance, while the other species are specific. Mycorrhizal status has been linked to plant distribution and productivity (Van der Heijden *et al.*, 2008; Hempel *et al*., 2013), also the AMF may be influenced at a small scale by the local plant community and the adjacent plant of the focal species (Hausman *et al*., 2009).

Temperature influences spore germination, AMF development, and root colonization (Maia *et al*., 2010; Wu and Zou, 2010; Barrett *et al*., 2011). Tommerup (1983) considered 20°C as an ideal temperature for the germination of *Acaulospora laevis* spores and between 15 and 25°C for hyphae growth. Siqueira and Hubbell (1985) observed that the optimal germination temperature for *Glomus versiforme* spores is 18-25°C and that temperatures above 35°C and below 15°C are inhibitive.

Conclusion

It is widely recognized that endomycorrhizae plays an important role in the uptake of nutrients and minerals by plants. According to this study, it appears that the use of different mycotrophic plant species influences the multiplication of endomycorrhizal fungi. Indeed, after the multiplication of mycorrhizae and the inoculation by a composite inoculum containing 70 species, it turned out that some species appear over time, others disappear and other species have remained faithful and capable of maintaining a constant activity with the roots of the plants studied. Therefore, it seems that over time each mycotrophic species promotes the multiplication and dominance of one or more species of endomycorrhizal fungi.

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