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Diversity of Arbuscular Mycorrhizal Fungi Associated with Maize and Peanut Crop in Northern Côte d'Ivoire

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Abstract

Arbuscular Mycorrhizal Fungi (AMF) are known to be more efficient and effective in helping the growth of plants. Understanding the diversity and community structure of AMF is important for optimizing their potential role in the functioning of terrestrial ecosystems. However, AMF diversity is less explored in tropical areas especially in northern CI, where agriculture is often encountered low yields. In this regard, exploring of AMF in these soils was conducted to look at the population of AMF indigenous. Rhizospheric and non-rhizospheric soils were collected from peanut and maize fields in different localities of the Korhogo area in northern Côte d'Ivoire. The density and Morphological diversity of AMF spores associated with these crops were determined in these soils. Then the effect of corn and peanut crops on the morphological diversity of AMF spores was also evaluated. Results showed that maize has significantly improved spore density of soil more than a peanut. The morphological identification of AMF spores associated with peanut and maize made it possible to list eleven species divided into five genera and three spore families including *Gigasporaceae* (36,36 %), *Acaulosporaceae* (18,18 %) and *Glomeraceae* (45,46 %). A better distribution of these different morphotypes has been observed in the rhizospheric soils of both crops, with a pronounced effect observed in the maize crop.

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Introduction

As for developing countries, the economy of Côte d'Ivoire is based primarily on agriculture. Maize and peanut are two food crops that are sources of income for the local population in the country. Maize (*Zea mays* L.) is a cereal, belonging to the grass family (Kellogg, 2001). It is the third most cultivated cereal in the world after rice (*Oryza sativa* L.) and wheat (*Triticum aestivum* L.) and cultivated for its carbohydrate-rich seeds. Peanut (*Arachis hypogaea* L.) is a legume cultivated for its high protein and oil content. Its fatty and amino acid composition and its taste and flavor are important features attributed to it (Asibuo *et al.*, 2008). It is considered to be one of the most important oilseed crops worldwide. This plant is also cultivated in rotation to improve the nitrogen content of the soil and thus contributes to its fertility. They are both used in both human and animal feed. The main growing area of these crops is Korhogo in the northern country. However, This area of CI is characterized by low soil productivity and low crop yields. Besides environmental concerns caused by the expensive chemical fertilizers (Dobermann and Cassman, 2004) support researchers for new sustainable strategies to promote soil fertility and improve crop production. In this context, exploitation of soil microbial communities such as arbuscular mycorrhizal fungi (AMF), to improve food quantity and quality (Barea, 2015), has been considered as safe, inexpensive and environmentally friendly. AMF establishes a mutual relationship with plant roots which benefit from water and nutrients that fungus collects in the soil; in turn, it feeds on carbon allocated by the plant (Parniske, 2008).

They contribute to the mineral nutrition of plants even during drought and other environmental stress (Martínez-García, 2010). Several studies have demonstrated the stimulatory effect of these symbionts on growth parameters in grasses and legumes (*Smith et al.*, 1998) and in the decomposition of organic matters. Positive effects of AMF and phosphorus application were observed on the growth and phosphatase activities of peanuts (Doley and Jite, 2012). The literature has shown an abundance and morphological diversity of AMFs in different agroecological zones. In CI, investigations on the diversity of AMF are focused on forest areas and very few studies have been carried out in savannah areas, particularly in the north of the country. So far, no studies have been carried out on the diversity of AMF spores associated with maize and peanut in Côte d'Ivoire, more accurately in the Korhogo area. The reserch aims to study the abundance and diversity of arbuscular mycorrhizal fungi associated with the crop of maize and peanut in the Korhogo area. We are also studying the effect of these crops on the morphological diversity of these spores.

Material and methods

Soil sampling

Soils were collected in October 2016, in peanut and maize monoculture fields in Takali (9°25 N; 5°35 W) located in Korhogo in northern Côte d'Ivoire. For each crop, a composite sample of rhizospheric soil under maize or peanut was collected by removing five plants and shaking the soil surrounding the roots. Composite soil used as a control was also collected in bulk without any vegetation. These soils were used to study the density and diversity of AMF beneath maize and peanut. Physical and chemical parameters of this composite soil were determined at the Centre de Recherche en Océanographie (CRO) of Abidjan, Côte d'Ivoire. The pH (water) was measured in the supernatant of a soil / distilled water mixture in a ratio of 1:2.5. Organic and mineral matter content was determined according to Moreno *et al.* (2001). Contents of total nitrogen (N) and phosphorus (P) were quantified according to Bremner (1960) and Sherrell and Saunders (1966), respectively by atomic absorption spectrometer after digestion with concentrated sulfuric acid. Potassium (K) was analyzed employing argon plasma ionization source mass spectrometer (ICP-MS) according to Rao and Talluri (2007) method.

Density of AMF spores

AMF spores density was determined in rhizospheric and non-rhizospheric soils of maize and peanut, as

well as in non-crop soils. AMF spores were extracted by the wet sieving method described by Gerdemann and Nicolson (1963) on soil samples previously dried and sieved using a 2 mm diameter sieve. 50 g of soil were diluted into 1 L of tap water in a beaker, after 1 min of decantation, the supernatant was passed through a series of superimposed sieves of decreasing mesh. The operation was repeated three times to collect the maximum number of spores. After collecting the contents of each sieve in a test tube, a density gradient was created by successively adding to the bottom of each tube, 3 mL of 20% and 60% sucrose. The tubes were then centrifuged at 3000 rpm for 3 min at 4 ° C. Then the supernatant from each tube was overturned into the 50 µm mesh sieve and then rinsed thoroughly to remove the sucrose.

The spores were collected by size, in a tube, then observed and counted by morphotype with a binocular magnifying glass using a squared Petri dish and an electric counter. Three replicates were performed for each soil sample and the average was taken into account. Density was determined as the average number of spores for each sample per 50 g of soil.

Morphological diversity of AMF

Trapping of AMF in the greenhouse: AMF spores present in study soils are often under stressful conditions due to soil sampling techniques, transport and storage. To have spores with an intact wall and of good quality, they must be put in favorable conditions, that is to say, multiply them with their host plant. In general, to carry out this study, maize is used as the mycotrophic plant (which allows the multiplication of AMF spores). However, in our study, we also used peanuts to trap these. The corn and peanut seeds were previously disinfected respectively in a bleach solution for 3 min and a 70% calcium hypochlorite solution for 8 min and rinsed 6 times with sterile water. They were sown into 1000 mL crop pots, containing sterile desalinated sand and approximately 200 g of study soil as inoculum. The pots were watered daily and after three months of the crop the plants were harvested and the growing medium was used for spore extraction.

Morphological identification of AMF spores: After harvest, the spores were extracted by the wet sieve method described by Gerdemann and Nicolson (1963). The spores were processed manually according to morphological characteristics such as color, shape, size and the presence of sporogenous cells. The batches of morphologically identical spores obtained, after trapping, were observed with a binocular magnifying glass. Samples were mounted between the slide and coverslip in PVLG (Koske and Tessier, 1983) to obtain a permanent mounting or PVLG supplemented with Melzer v / v reagent (Morton, 1988) which reacts to contact with spore tissue, by coloring some of them from pink to purplish-pink depending to their chemical composition.

The spores observed were described and compared to the specimens from the INVAM collection to identify them. All of the data obtained were compared with the original description of the reference crop database published on the website http://invam.caf.wvu.edu. According to Bâ *et al.* (1996), characteristic structures such as the color, the shape, the presence of sporogenic cell, the suspensory bulb, make it possible to identify the genera of spores. It is therefore impossible to identify spores based on morphological criteria when their characteristic structures are absent. The characteristic criteria of the families and genera described on the site http://fungi.invam.wvu.edu, allowed us to identify the genera and families to which these spores belong.

Results

Morphological diversity of arbuscular mycorrhizal fungi associated to maize and peanut crop

The different morphotypes of AMF spores observed, after trapping and morphological identification, are comparable in the three localities. A total of eleven (11) morphotypes belonging to five (5) genera were identified thanks to their characteristic structures depending on the shape, size and color, in each of the three localities. *Glomus* is the genus the most

represented and the most diversified with five (5) morphotypes encountered, i.e. an abundance rate of 45.46%, followed by the genera *Gigaspora* and *Scutellospora* each representing two (2) morphotypes, i.e. a rate of 18, 18% for each of them. Finally, the least represented genera are *Acaulospora* and *Entrophospora*, each represented by one (1) morphotype, ie a rate of 9.09% for each genus. These five (5) genera belong to three large families which are the family of *Glomeraceae* represented by 5 morphotypes (45.46%); the family of *Gigasporaceae* with 4 morphotypes (36.37%); and that of *Acaulosporaceae* containing 2 morphotypes, or 18.18% of the morphotypes encountered (Fig.1).

The same species, genera and families of spores (Table 1) were encountered in the three localities with a predominance of species of the family *Glomeraceae*.

Table 1. Different morphotypes of AMF spores encountered.

Families	Genera	Morphotypes encountered
Gigasporaceae	*Gigaspora*	*Gigaspora sp1*
		Gigaspora sp2
	Scutellospora	*Scutellospora Sp1*
		Scutellospora Sp2
Glomeraceae	*Glomus*	*Glomus sp1*
		Glomus sp2
		Glomus sp3
		Glomus sp4
		Glomus sp5
Acaulosporaceae	*Acaulospora*	*Acaulospora sp*
	Entrophospora	*Entrophospora sp*

Family of Gigasporaceae

In this family, four (4) morphotypes were found. These are two morphotypes of the genus *Gigaspora* and two other morphotypes of the genus *Scutellospora*. This family is characterized by large spores with diameters greater than 200 µm, with the presence of sporogenous cells. The genus *Scutellospora* can reach diameters of more than 500 µm and can be seen with the naked eye. Fig. 2 below shows the photographs of the different morphotypes observed in this family.

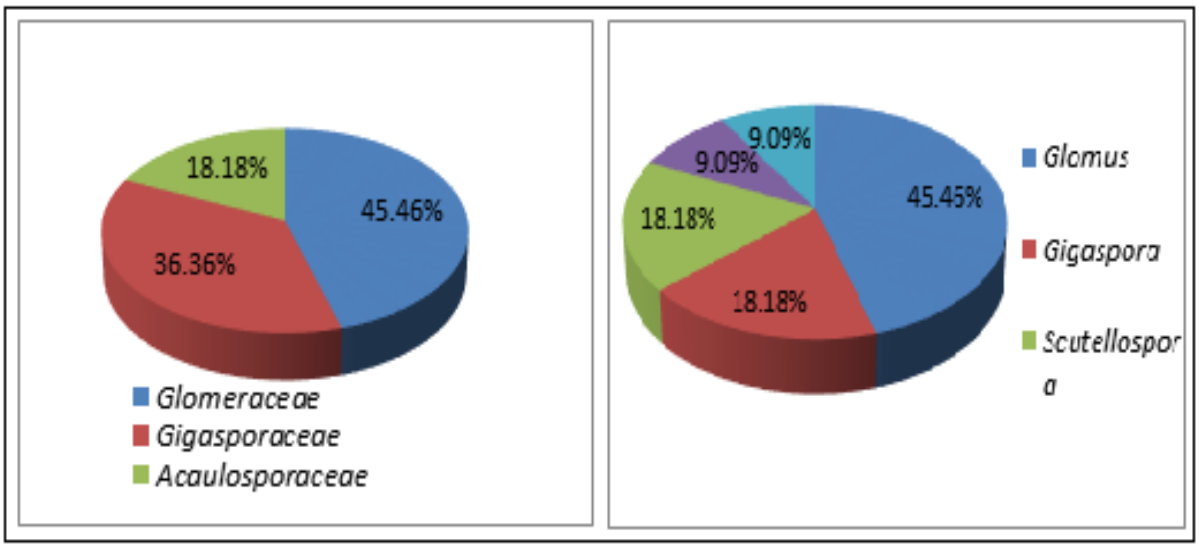

Fig. 1. Distribution of different morphotypes of spores according to families and genera

Family of Glomeraceae

Five (5) morphotypes (Table), all belonging to the genus *Glomus*, were found. This family is distinguished from others by small spores (whose diameters are less than 200 µm) of brown color, with very fine suspensory hyphae. Fig. 3 below shows the photographs of the different morphotypes of the genus *Glomus* observed belonging to this family.

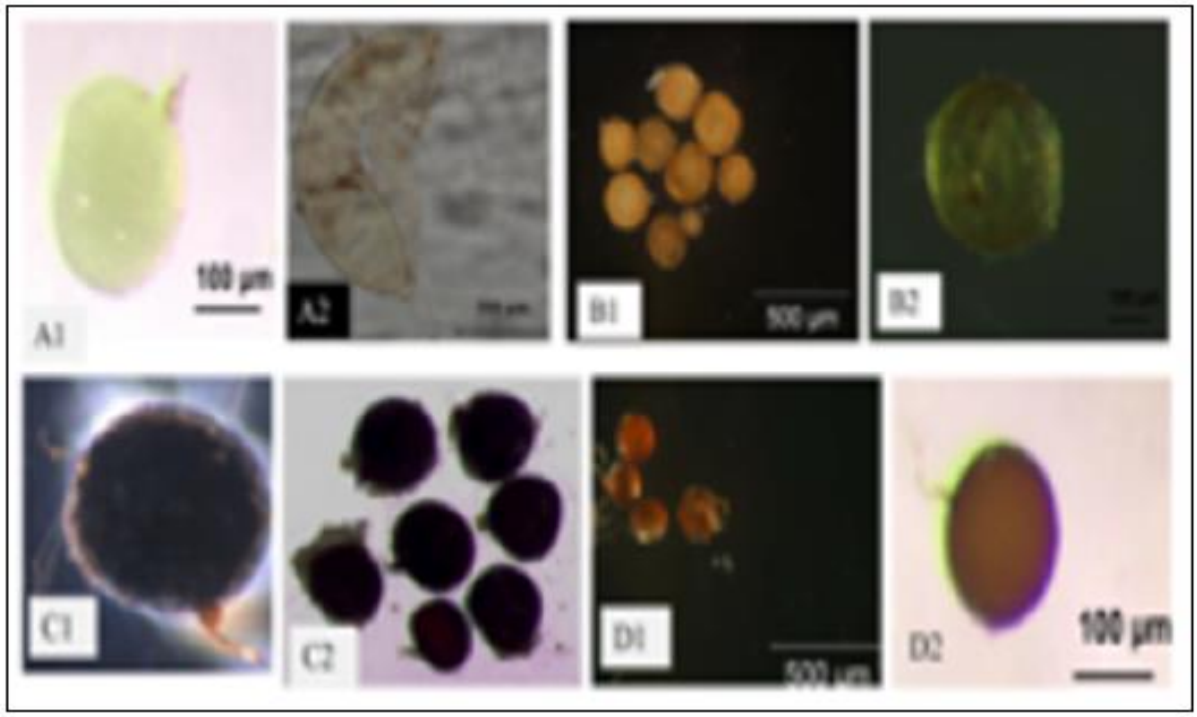

Fig. 2. Spores of *Gigasporaceae* family. A1 : *Gigaspora sp1* (white-yellow) ; A2 : *Gigaspora sp1* crushed in PVLG ; B1 : *Gigaspora sp2* ; B2 : *Gigaspora sp2* (golden yellow) ; C1 et C2 : *Scutellospora sp1* (black, presence of suspensory bulb) ; *Scutellospora sp2* (brown, suspensory hypha).

Family of Acaulosporaceae

This family is characterized by small spores, whose diameters vary between 30 and 80 µm. They are white or yellow. Two morphotypes *Acaulospora sp* and *Entrophospora sp* have been identified in this family. Fig. 4 shows the photographs of the different morphotypes observed in this family.

Effect of maize and peanut crops on the morphological diversity of AMF spores

The spores were counted according to their morphotype (size, color, shape) in non-crop soils (NCS) and rhizospheric soils of maize (SM) and peanut (SP) for each locality. The results showed that in each locality, the density of the least represented morphotypes, in non-crop soils, increases under the influence of the two crops (Fig. 5). This effect is more pronounced with the maize crop. In fact, in locality 1, the density of spores of the genus *Glomus* (the most abundant), increased from 51% (NCS) to 42% (SP) under the influence of peanut and from 51% to 31% (SM) under the influence of maize. The same trend was observed in the other two localities. It decreases from 66% (NCS) to 34% (SP) and from 66% (NCS) to 27% (SM) respectively under the peanut and maize crop in locality 2; as well as from 54% (NCS) to 47% (SP) and from 54% (NCS) to 50% (SM) in locality 3. We also notice that in locality 2, the morphological diversity of AMF spores is higher under crops (peanut and maize) than in the non-crop soil compared to the other two localities. However, some morphotypes such as *Scutellospora sp1* almost non-existent in the non-crop soils of the three localities increased from 1 to 2% and from 1 to 4% respectively in the rhizospheric soils of peanuts and maize in locality 1. In locality 2, this morphotype increased from 1 to 5% and from 1 to 6% respectively in the rhizospheric soils of peanuts and maize. However, in non-crop soils,

AMF spore morphotypes of the genus *Glomus* were more higher compared to morphotypes of other genera. In fact, in locality 1, the genus *Glomus* representing 59% while the morphotypes of the genera *Gigaspora, Scutellopora, Acaulospora* and *Entrophospora* only represent respectively 11, 13, 11 and 6% of the morphotypes encountered. The same observation is found in the two other localities, where the morphotypes of the genus *Glomus* represent more than half (72% in locality 2 and 62% in locality 3 in non-crop soil) of the morphotypes encountered. Under maize and peanut crops, the trend is the same except that the morphotypes of the genus *Glomus*, admittedly the most numerous, but only represented less than half of the morphotypes encountered. Indeed in locality 2, the genus *Glomus* contains 44% of the morphotypes while the genera *Gigaspora, Scutellopsora, Acaulospora* and *Entrophospora* only represent respectively 8, 17, 18 and 13% in the rhizospheric soils of peanuts. The same observations were found in the other two localities and under the two crops.

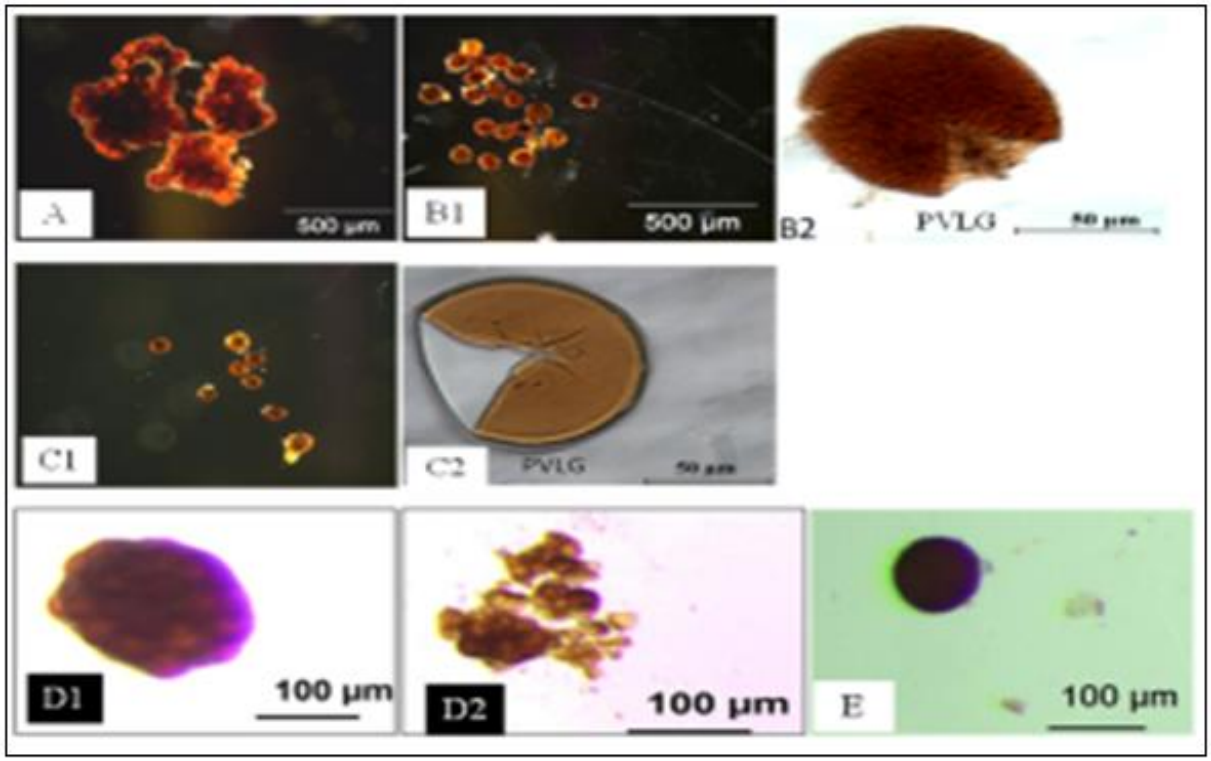

Fig. 1. *Glomeraceae* family spores. A : *Glomus sp1* (dissociable cluster) ; B1 : *Glomus sp2* (isolated spores) ; B2 : *Glomus sp2* (in PVLG, rough appearance) ; C1 : *Glomus sp3* (isolated spores) ; C2 : *Glomus Sp3* (in PVLG, smooth appearance) ; D1 : *Glomus sp4* (inseparable cluster) ; D2 *Glomus sp4* (crushed inseparable cluster) ; E : *Glomus sp5* (blackish appearance).

Discussion

The first descriptions of AMF were based on the morphological characters of the spores (color, shape, size, ornateness) which led to the classification of AMF into six genera according to Morton and Benny (1990). Thus, most of the information on AMF diversity came from temperate areas. Exploration attempts in the tropical areas have started more recently. In this present study, the morphological identification made it possible to distinguish eleven (11) morphotypes of AMF spores distributed in five (5) genera and three (3) large families of spores, the most represented of which is the *Glomeraceae* family with five (5) morphotypes of *Glomus*, i.e. an abundance rate by 45.44%. The genera *Gigaspora* and *Scutellospora* each presented a total of 2 morphotypes or abundance rates of 18.18% for each genus. The genera *Entrophospora* and *Acaulospora* are each represented by only 1 morphotype, corresponding to an abundance rate of 9.1% for each

genus. Indeed, several studies on the density and diversity of spores have shown that the spores of the genus *Glomus* are the most abundant in several natural ecosystems. This predominance of species of the genus *Glomus* is due to a better adaptation of this genus to environmental stresses (Lenoir *et al.*, 2016) and to their ability to mend their mycelial filament during work carried out on soils (Blaszkowski *et al.*, 2002). The distribution and abundance of morphotypes of the genus *Glomus* found in this study are comparable to those observed in several eco-geographic areas including Algeria (Driai, 2016), Burkina Faso (Bâ *et al.*, 1996), Morocco (Bouamri *et al.*, 2006) and Senegal (Ndoye *et al.*, 2012).

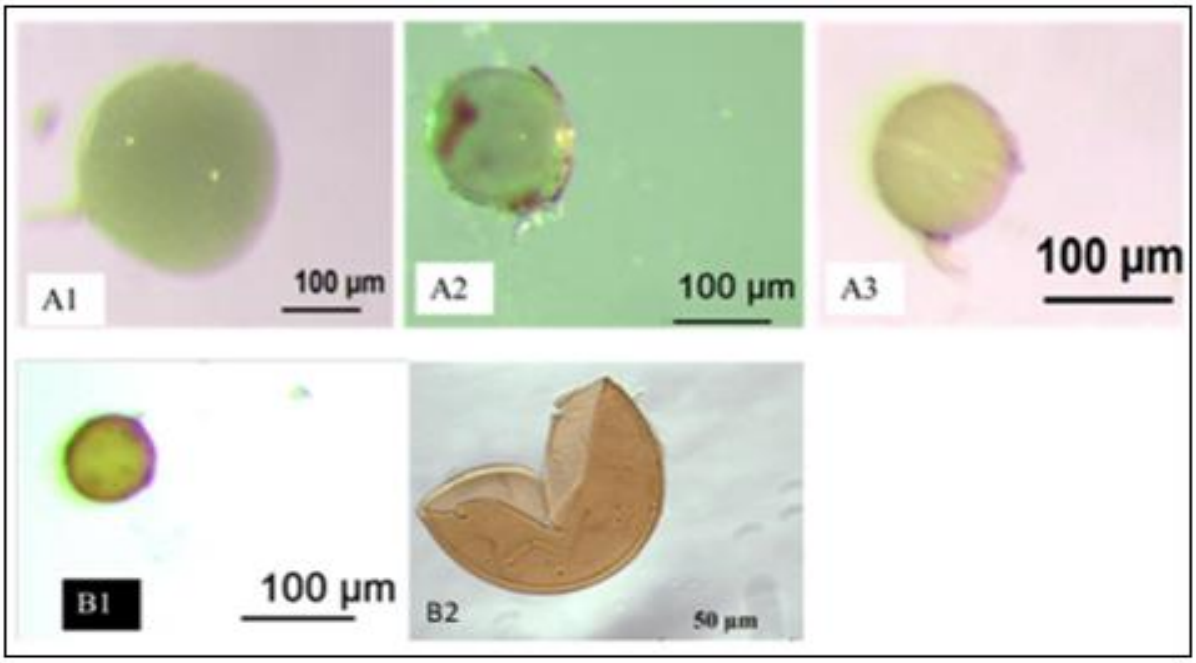

Fig. 4. Spores of *Acaulosporaceae* family. A1, A2 et A3 : *Entrophospora sp* (white) ; B1 : *Acaulospora sp* (yellow) ; B2 : *Acaulospora sp* (in PVLG).

The dominance of spores from the *Glomeraceae* family has been reported in other studies carried out on different habitats such as geothermal sites (Appoloni *et al.*, 2008), tropical forests (Wubet *et al.*, 2004), and soils (Daniell *et al.*, 2001) but also in soils contaminated by trace metal elements (Schneider *et al.*, 2013). Typically, a high diversity of AMF spores is found in meadows and pastures, while intensive cropping areas often contain fewer species (Oehl *et al.*, 2011).

This can also be attributed to the sporogenous characters of these species. Indeed, it has been shown that AMF of the genus *Glomus* produces more spores than those of *Scutellospora* and *Gigaspora* in the same environment (Bever *et al.*, 1996). Besides, frequent tillage would affect the species of *Gigaspora, Scutellospora* and *Racocetra* more because they have difficulty connecting their broken mycelial filaments, compared to species of the genus *Glomus* (de la Providencia *et al.*, 2005).

However, this morphological diversity of different AMF spores varies under the influence of crops. Indeed in locality 2, the *Glomus* morphotypes representing 72% of the spores of the soil outside cover, represented only 44 and 41% respectively in the rhizospheric soils of peanuts and those of maize. The same trends were observed in the other two localities. These observations would be due to the presence of plant species, which, thanks to their root exudates such as the released flavonoids, stimulate the development and multiplication of dormant spores. These spores only multiply in the presence of a host plant and are more diversified under the maize rhizosphere.

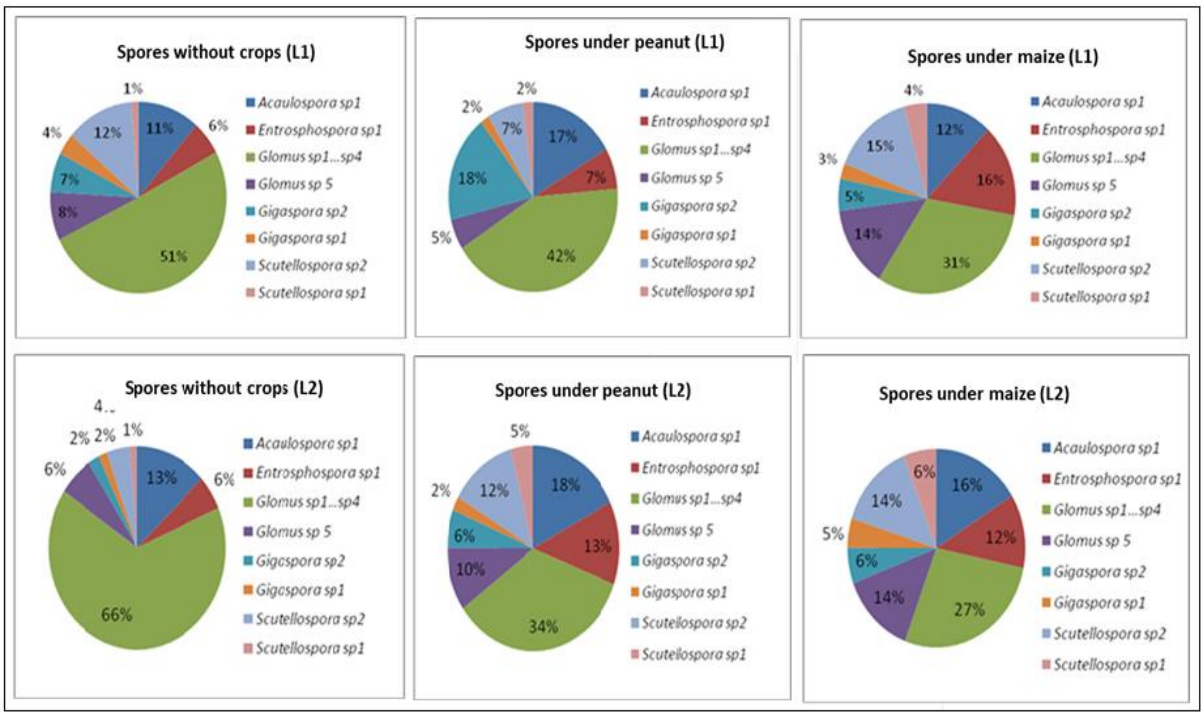

Fig. 5. effect of maize (*Zea mays*) and peanut (*Arachis hypogaea*) crops) on the diversity and distribution of different morphotypes of AMF spores. L1 : locality 1 of Fodontchon ; L2 : locality 2 of Takali.

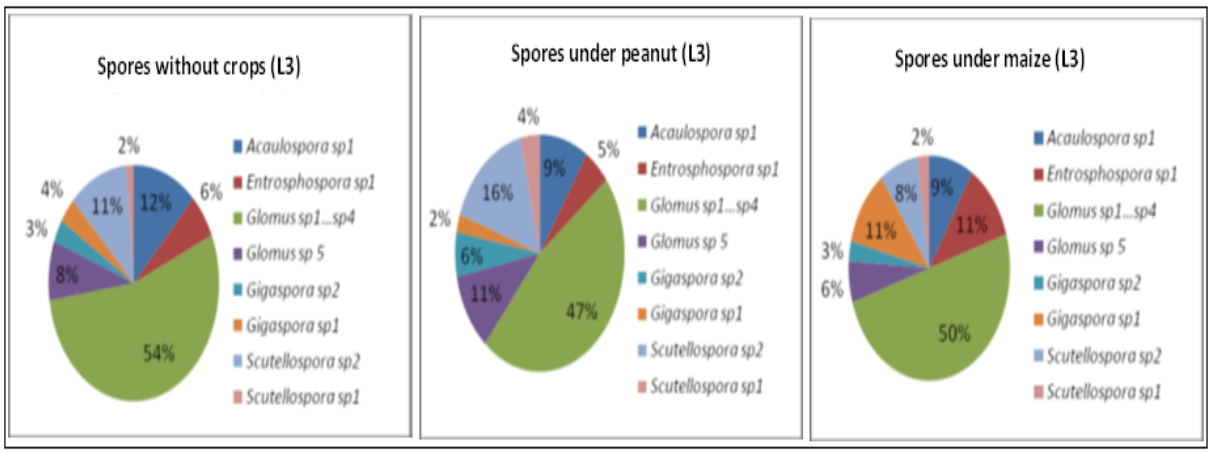

Fig. 5 (Suite) Effect of maize (*Zea mays*) and peanut (*Arachis hypogaea*) crops on the diversity and distribution of differents morphotypes of AMF spores. L3: locality 3 of Blawaha.

The spore species collected from the different localities of our study area are morphologically comparable to those described in the literature. However, they show some variations in the diameter compared to the original description (Schenck and Pérez, 1987). These morphometric variations of the spores observed in the natural state could be explained by the differences in the physic and chemical characteristics of the different ecosystems hosting the spores. Indeed, relationships have been established between the nature of fungal communities and the type of plant population (Jeffries and Barea, 2001). The morphological diversity of spore species observed in this study is comparable to that obtained in Kenya by Jefwa *et al.* (2012) and in Senegal by Ndoye *et al.* (2012) who recorded, in their investigation on the impact of cultivation practices on spores, a total of 12 and 11 species respectively. However, it remains lower than the data obtained in Côte d'Ivoire in the areas of Dabakala (northern CI) by Nandjui *et al.* (2013) and Abengourou (Eastern CI) by Voko *et al.* (2013) where they recorded the same

number of 30 species associated respectively with yam (*Dioscorea sp*) and cassava (*Manihot esculenta*). The distribution of the different species of AMF spores is comparable in the three localities and follows the same trends under the 2 different crops. This could be explained by the similar physicochemical characteristics observed in the three localities of the study area. However, in locality 3 of Blawaha, the distribution of the genus *Glomus* under maize and peanut crops is almost similar to that of the soil without cover. This finding would be due to the environmental stress conditions observed in this locality. Indeed the physic and chemical characteristics of this soil show a higher Na rate than the two other localities. Thus Oehl *et al.* (2017) have shown that soil factors can play a determining role in the diversity of AMF spores. This locality is located north of the study area, presents more pronounced aridity conditions, and suffers from lower rainfall than the other two.

Conclusion

The morphological identification of AMF spores associated with peanut and maize allowed the identification of 11 morphotypes, 5 genera (*Gigaspora, Scutellospora, Glomus, Acaulospora* and *Entrophospora*) and 3 large families of spores including *Gigasporaceae* (36,36 %), *Acaulosporaceae* (18,18 %) and *Glomeraceae* (45,46 %).

The presence of crops influenced positively the morphological diversity and distribution of the different morphotypes which are better represented under maize and peanut crops than in soils without cover. This effect is more pronounced with the maize crop. Under crops influence, the least morphotypes represented in soils outside crops, are more represented in the rhizospheric soils of the two crops. These results suggest the importance of crops in the diversification and representativeness of the different AMF morphotypes present in the soil.

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