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Diversity of arbuscular mycorrhizal fungi (AMF) associated with cotton (*Gossypium hirsutum* L.) growing in the Far-North region of Cameroon

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The present study was carried out with the aim of highlighting the diversity of arbuscular mycorrhizal fungi (AMF) associated with the rhizosphere of cotton grown in the Far-North region of Cameroon. To achieve this, composite soil and root samples were taken in six fields, chosen according to edapho-climatic conditions and types of cultural practices. After entrapping the glomales in the greenhouse, the roots of the trap plants were thinned and stained to assess colonization. The spores were isolated by wet sieving and their identification was made after analysis of their morpho-anatomical structures; then the diversity was evaluated through the calculated index. The results obtained have revealed the presence of AMF in all sites surveyed with a maximum abundance of 432 spores per 100 g of soil. Despite the low diversity of AMF that exist, four genera have been identified: *Acaulospora*, *Gigaspora*, *Glomus* and *Scutellospora*. A significant dominance of *Glomus* spp. (42%) was found. A strong correlation was found between soil physico-chemical parameters and abundance, as well as between species richness. This study confirms the presence of AMF strains in the cotton rhizosphere cultivated in this zone. The exploitation of this AMF could lead to a controlled production of local fungal inoculum, adapted to the edaphic and climatic conditions of the region, for a sustainable agriculture in Cameroon.

Key words: Cotton, rhizosphere, arbuscular mycorrhizal fungi (AMF), diversity, Far-North, Cameroun.

INTRODUCTION

Microorganisms represent the majority of living organisms in the soil compartment, thus constituting an important part of the planet's genetic diversity (Leake et al., 2004). Among these microorganisms, arbuscular mycorrhizal fungi (AMF) establish symbiosis with approximately 80% of the vascular plant species in all terrestrial biomes (Smith et al., 2010). These are considered to be the key elements in the functioning of terrestrial ecosystems,

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particularly for their ability to promote plant development in degraded environments through the establishment of a symbiotic association with their roots called mycorrhizae (Abbas, 2014). This key role attributed to them is due to the influences they may have in the biological and geochemical processes that govern ecosystems, namely the acquisition of nutrients for the plant (Smith et al., 2010), the improvement of plant growth (Megueni et al., 2011), the improvement of soil quality (Caravaca et al., 2002) and increasing plants ability to resist to soil pathogens, as well as their tolerance to biotic and abiotic stresses (Dalpé, 2005). Also mycorrhizal symbiosis increases plants tolerance to salinity, heavy metals, drought stress and low pH (Hassan et al., 2011). Despite their multiple benefits, AMF are untapped and almost ignored by Cameroonian farmers. In the Far-North region of Cameroon, cotton growing is the main cash crop for many populations and even contributes to food security (Folefack et al., 2014); but it is subject to several soil constraints (Olina et al., 2008). Indeed, this region located in a Sudano-Sahelian agro-ecological zone of Cameroon is characterized by a harsh and unstable climate; but also, by very low level of soil fertility (Tsozue et al., 2015). This constitutes the main constraint of declining agricultural production in this zone. In addition, factors that favor the decline of soil fertility in this area are, anthropogenic pressure on, cultural practices characterized by chemical fertilizer inputs below or above recommended doses and without restitution residues (Olina et al., 2008). Thus, under these conditions, the variability of the endomycorrhizal potential of soils in this region should be exploited to optimize agricultural production in general and cotton production in particular. In addition, to better control inoculation trials and make them accessible to the public, it is important to identify native AMF to estimate their competitiveness in the soil. Furthermore, with the exception of the work done by Tobolbai et al. (2018) on maize and Ngonkeu et al. (2013), the current data on the identity of glomales in this area are few. It is in this context that it has seemed interesting to evaluate the specific diversity of arbuscular mycorrhizal fungi associated with cotton (*Gossypium hirsutum* L.) grown in the Far-North region of Cameroon. Specifically, it involved (a) the identifications and quantification of different AMF species associated to cotton, (b) assessment of their distribution and diversity on cotton rhizosphere in the studied area.

MATERIALS AND METHODS

Physical description of study sites and soil sampling

Based on their edapho-climatic conditions and types of cultural practices, six sites (Dargala, Dogba, Doukoula, Laf, Tokombere and Zidim) were selected in the Far-North region Cameroon. Located in the northern cotton zone, they are characterized by a dry tropical Sudano-Sahelian climate, with an annual temperature and rainfall of 27°C and 800 mm, respectively.

The samples were taken between November 2017 and January 2018, a transition period between the rainy and dry seasons. The choice of sampling plots (Table 1) mainly concerned the type of crop (monoculture), the cropping system (non-use of chemicals input), the appearance of the plot (plot slightly degraded by erosion and not on a slope), the history of these over the last two years and the separation with neighboring fields (preferably nearest the organic production lands or enough distance end from conventional lands). The samples were taken according to the zigzag method described by Barker (1985). At each selected site, 5 to 10 kg of soil was collected at 10-20 cm depth, near the root rhizosphere of cotton, at different points of the site. Then, the soil samples were sieved (sieve 2 mm mesh size) in order to remove the large size particles and homogenize to obtain representative 10 kg of composite soil sample. These samples were packaged in plastic bags and stored at room temperature until use.

Determination of the physicochemical characteristics of soils

Physical and chemical properties were analyzed in the Faculty of Agronomy and Agricultural Science of the University of Dschang (Cameroon). Soil sample was air-dried and ground to pass through a 2-mm sieve. Soil pH was determined according to Pansu and Gautheyrou (2003). Organic carbon was assessed after the method by Walkley and Black (1934) and soil nitrogen (N) by the Kjeldahl method (MacDonald, 1977). Soil cationic exchange capacity (CEC) and total phosphorus (P) were determined using the method by Duchaufour (1977) and Pansu and Gautheyrou (2003) respectively. Soil available phosphorus was determined after Olsen (1952) and soil textural classes using French standards of soil classification (ISO, 1991). The exchangeable bases (Ca^{2+}, Mg^{2+}, K^{+}) were extracted using the ammonium acetate (NH4OAC, pH: 7) and determined by flame atomic absorption spectrophotometry.

Trapping of endomycorrhizal spores and assessment of root mycorrhization

The greenhouse trapping method of Morton (1992) was chosen using soils sampled as an inoculum to allow the eventual hatching of all spores and their development. A local variety (CMS variety) of corn (*Zea mays* L.) was used as a trap plant. Plastic pots of 10 L volume containing a mixture (v / v) of soil used as inoculum and sand previously sterilized with a dry heat at 120°C temperature for 5 h in an oven were used as a culture substrate. After eight weeks of greenhouse cultivation, the aerial plant of maize plants was dissociated and removed, underground part. Mycorhization criteria were evaluated, for each sample, on 30 fine root fragments stained according to the method (Philips and Hayman, 1970) and mounted in glycerol on three slides each. Under optical microscope, these root fragments were carefully examined along their entire length to record the mycorrhizal structures. The parameters noted were frequency (F), root mycorrhizal intensity (M) and arbuscular contents (Trouvelot et al., 1986).

Extraction and enumeration of spores

Spores were extracted according to the wet sieving method described by Gerdemann and Nicholson (1963). A 100 g sample of dry soil was suspended in 1 L of tap water. The suspension obtained was transferred to a series of five sieves with decreasing mesh size (1, 250, 100, 63 and 50 µm). The suspension of spores in the sieves was centrifuged for 3 min at 4000 rpm and at a temperature of 4°C with sucrose (60%). Then the suspensions were

Table 1. Geographical coordinates of the different study sites.

Sites	Latitude (°)	Longitude (°)	Altitude (m)
Dargala	10°31'57.65''N	14°36'15.84''E	351
Dogba	10°46'18.68 N	14°18'27.90''E	402
Doukoula	10°11'49.17''N	14°97'46.79''E	357
Laf	10°25'47.53''N	14°21'70.11''E	480
Tokombéré	10°86'16.17''N	14°14'63.94''E	360
Zidim	10°29'18.72''N	13°58'40.90''E	539

placed in a Petri dish with grid bottom for observation and counting. This observation was made with the EUROMAX edublue brand binocular loupe, while separating the various morphotypes with a micropipette. Spores were considered viable if they had a clear content under an optical microscope, with an intact wall. They were classified according to their morphological characteristics (size, color, shape, consistency, wall structure and attachment of the hyphal suspensor), then quantified. The spores were mounted on glass slide in PVLG with Melzer and the identification of species were made using the identification keys of Schenk and Perez (1990); as well as the INVAM (International Culture Collection of Arbuscular and Vesicular Arbuscular Mycorrhizal Fungi), http://invam.caf.wvu.edu/fungi/taxonomy/species/id.htm.species were ranked according to Redecker et al. (2013).

The following parameter were evaluated

The density of spores, corresponding to the number of spores per 100 g of dry soil; the species richness (S) represents the total number of species present in a site; the Shannon index, which expresses diversity by taking into account the number of species and the abundance of individuals within each of these species:

$$H' = - [(ni / N) \log2 (ni / N)];$$

With: ni = number of individuals of a given species (i) in a site; N = total number of individuals of all species of the site; Log2 = logarithm based on 2.

Piélou's equitability index (E), which is the regularity of the distribution of species:

$$EQ = H' / Log2 S;$$

With: S = Shannon diversity index, S = total number of species. Simpson's diversity index (1-D), it measures the probability that two randomly selected individuals belong to the same species:

$$1-D = Ì \{[ni (ni-1)] / [N (N-1)]$$

With: n-i = number of individuals in species i; N = total number of individuals.

Statistical analyzes

The values of the various parameters evaluated were calculated as an average of three repetitions using the EXCEL 2010 spreadsheet and the graphs were also made using the same spreadsheet. All data were processed by one-way analysis of variance using the Statgraphics plus version 5.0 software and the main component analysis was performed by the xl stat pro software. For multiple comparisons, the averages of the different variables were compared by the Duncan test ($p < 0.05$).

RESULTS AND DISCUSSION

Physicochemical characteristics of cotton rhizosphere soil

The distribution of mineral fractions varied among different sites (Table 2); these soils are of lumpy nature, characterized by sandy-loamy structures with a large predominance of sand compared to clay and silt. Furthermore, in the Doukoula soil, clay is predominant (56%), unlike Dargala where silt is the most dominant (47%). The pH of soil samples from the different sites was essentially acid with average values between 5 and 6. Contents of mineral elements vary from one soil to another but remain, therefore quite weak. The Dogba site is moderately rich in assimilable phosphorus (71 ppm), unlike the other soil poor in this element and whose content was less than 45 ppm. The total nitrogen (0.12 to 0.48%) and organic matter (4.81 to 1.15) levels were significant in the sites surveyed, but the Dogba site remained poor in organic matter (1.15%). Concerning the exchangeable bases (Ca, Mg and K), data indicate a great variability of their contents in different soils.

Mycorrhization of trap plants

Microscopic examination of maize roots (*Zea mays* L.) was used as a trap plant, after eight weeks of greenhouse culture and no trace of mycorrhizal fungi was observed in the control pots. In addition, all the other root samples were densely colonized by fungi and the cytological organization of these mycorrhizae was essentially arbuscular. The average mycorrhizal frequency has always been very high, ranging from 90 to 100% depending on the sites surveyed; but no significant difference was revealed among them (Table 3). Despite some differences reported for mycorrhization intensity values, particularly for Laf (34.60 ± 4.08%), Doukoula (32.6 ± 8.07%), and Dargala (31.66 ± 5.6%), this difference also remained insignificant at the 5% threshold according to ANOVA. Similarly, for different arbuscular contents, the site factor showed no significant effect on the presence of arbuscular structures in the roots observed.

Table 2. Physical and chemical characteristics of soils collected from study sites.

Sites	pH-H_2O	pH-KCl	Sa (%)	Si (%)	C (%)	OM (%)	N (%)	Ca (ppm)	Mg ppm)	K (ppm)	P (ppm)
Dargala	6.6	5.8	44	47	12	2.81	0.4	135	167	32	45
Dogba	6.4	5.77	57	15	26	1.15	0.32	520	110	60	71
Doukoula	6.8	5.12	27	23	56	3.34	0.25	750	216	65	41
Laf	6.01	5.66	18	55	29	3.05	0.42	598	115	96	27
Tokombéré	6.5	5.24	57	37	10	2.98	0.48	101	95	27	15
Zidim	5.98	4.95	73	16	14	4.81	0.12	150	60	45	43

C: Clay; Si: Silt; OM: Organic matter; Sa : Sand; N: Total N; P: Phosphorus.

Table 3. Quantification of various root colonization parameters of greenhouse maize plants.

Sites	Frequency (%)	Intensity (%)	Arbucular content (%)
Dargala	90 ± 10.00	31.66 ± 5.60	18.69 ± 3.15
Dogba	100 ± 0.00	26.96 ± 4.64	18.42 ± 3.87
Doukoula	100 ± 0.00	32.6 ± 8.07	21.76 ± 8.7
Laf	93.33 ± 5.77	34.26 ± 3.13	16.42 ± 5.3
Tokombéré	96.66 ± 5.77	26.3 ± 4.9	12.87 ± 5.19
Zidim	93.33 ± 5.77	28.07 ± 1.95	20.6 ± 4.33
Moyenne	95.55 ± 4.03	29.97 ± 3.29	18.13 ± 3.17
P-Value	0.271 ^{Ns}	0.649 ^{Ns}	0.440 ^{Ns}

Ns: not significant at the 5% threshold.

Table 4. Composition of arbuscular mycorrhizal fungi according to the taxonomic classification of Redecker et al. (2013).

Family	Genrera	Species	Occurence (%)
Glomaceae	*Glomus*	*Glomus aggregatum* Schenck and Smith emend. Koske *Glomus hoi* Berch and Trappe *Glomus manihotis* Howeler, Sieverd and Schenck *Glomus* sp.	42
Gigasporaceae	*Scutellospora*	*Scutellospora cerradensis* Spain and Miranda *Scutellospora gregaria* Schenck and Nicolson emend. Walker and Sanders *Scutellospora nigra* Redhead, Walker and Sander	26
	Gigaspora	*Gigaspora margarita* Gerd and Trappe	2
Acaulosporaceae	*Acaulospora*	*Acaulospora* sp.1 *Acaulospora* sp.2	19
Unknow	Myco Brun-orange		11

Composition of the AMF of the prospected soils

On the basis of the morphological features and on some identification keys of reference works, 10 AMF have been described and one morphotype has not been identified (Myc Brown-orange). According to the taxonomic classification of Redecker et al. (2013), the most isolated spore species belong to Glomaceae family (4 species), Gigasporaceae (4 species) and Acaulosporaceae (2 species) (Table 4). On the four isolated genera (Acaulospora, Gigaspora, Glomus and Scutellospora), only seven species were identified with specific taxa and the identification of the other four was restricted to the genus.

The *Glomus* genus has been represented by four species (Figure 2), such as *Glomus aggregatum*

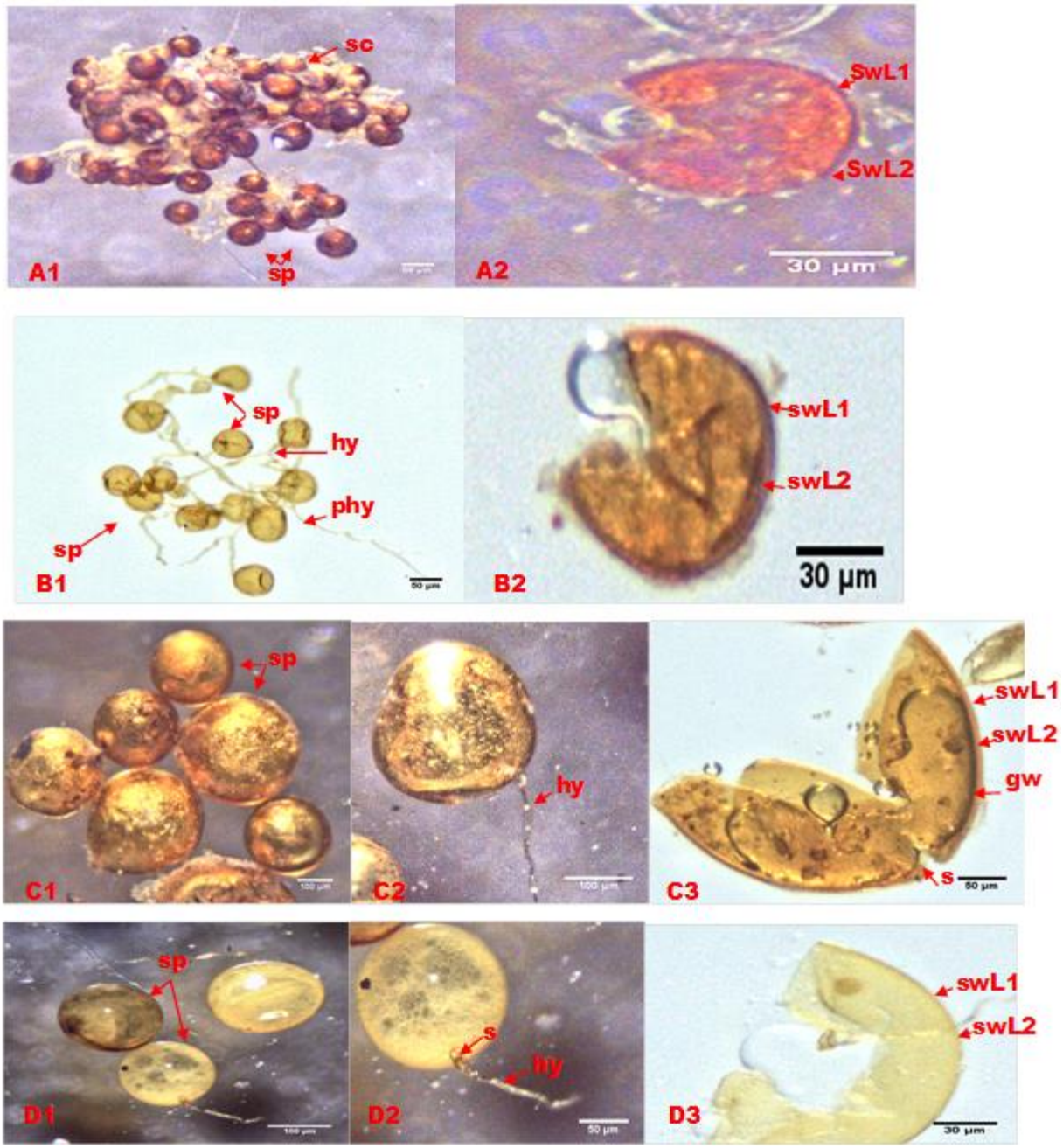

Figure 1. Morphological and anatomical characteristics of *Glomus* genus with; A: *Glomus aggregatum* B : *Glomus hoi*; C: *Glomus manihotis* ; D: *Glomus* sp.: A1 B1: sporocarp; C1 and D1: isolate spores C2, D2: hyphal structure on single spore ; A2, B2, C3, D3: wall structure of spore in PVLG with Melzer ; (hy) subtending hypha; (phy) central hyphal plexus; (sc) sporocarp; (s) septum; (sp) whole spore ; (sw) spore wall ; (swL1) outer layer of spore wall; (swL2) inner layer of spore wall.

[Schenck & Smith emend. Koske (Figure 2A), *G. hoi* [Berch & Trap (Figure 2B)], *G. manihotis* [Howeler, Sieverd & Schenck (Figure 2C) and *Glomus* sp. (Figure 1D). This were the most abundant in the region with nearly 42% of isolated spores (Table 4). These fungi consist of aggregates of several compact spores (Figure 1A1) or not, called sporocarp. Otherwise some species like *G. hoi* were characterized by a free sporocarp with constituted spores connected each to other by a more or less extensive hyphal plexus (Figure 2B1). Of a shiny and more or less rough appearance, *G. Manihotis* spores on the other hand, are rather solitary in the soil and can

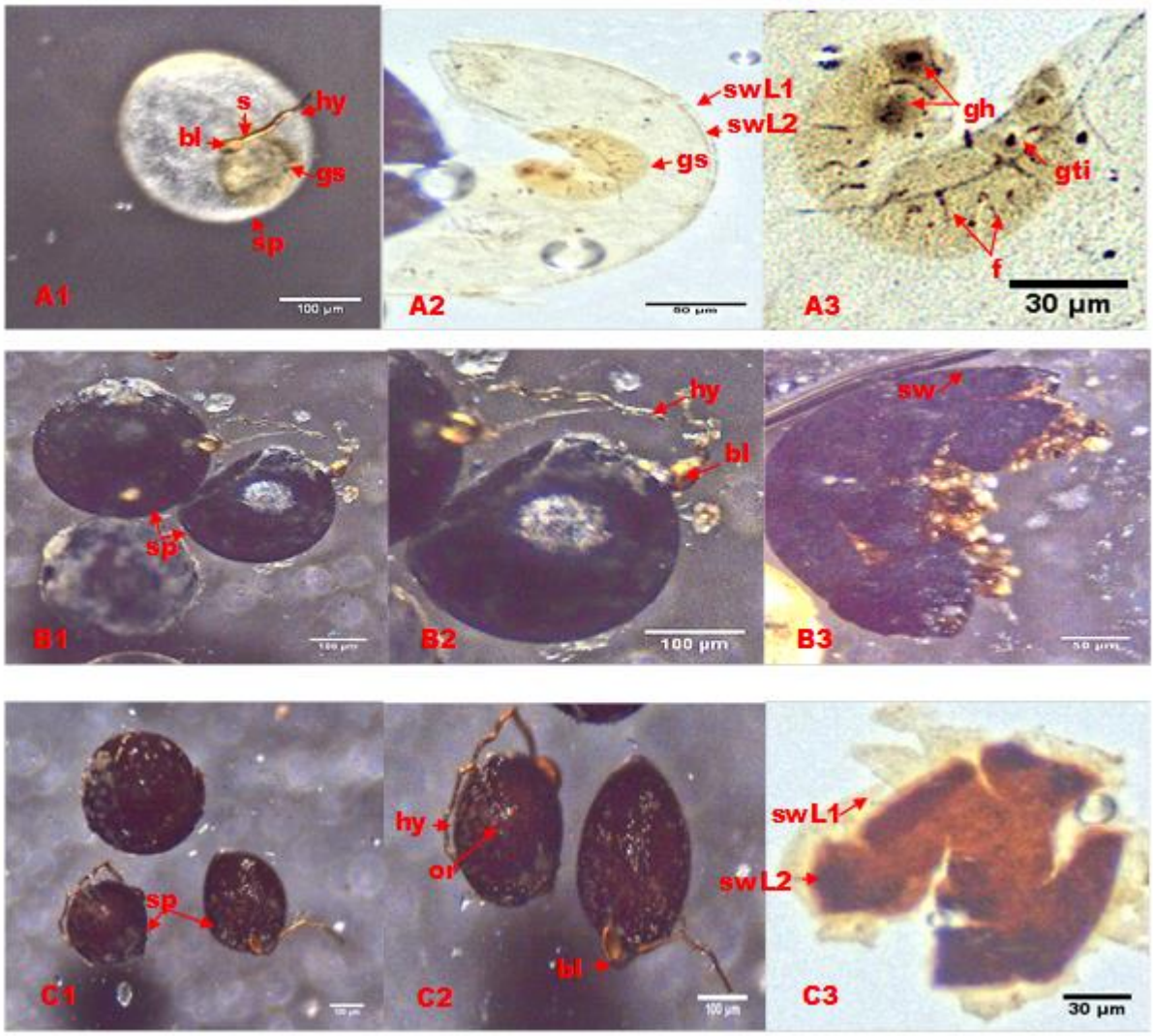

Figure 2. Morphological and anatomical characteristics of *Scutellospora* genus with; **A:** *Scutellospora cerradensis*; **B:** *Scutellospora gregaria*; **C:** *Scutellospora nigra* : **B1, C1:** isolate spores; **A1, B2, C2:** hyphal strurture; **A3:** germination shield; **B2, C3:** wall structure of spore in PVLG with Melzer. (bl) bulbous suspensor; (f) folds; (gh) germ hole; (gs) germination shield; (gti) germ tube initiation; (hy) subtending hypha; (or)ornament; (s) septum; (sp) whole spore ; (swL1) outer layer of spore wall; (swL2) inner layer of spore wall.

reach up to 250 µm in diameter (Figure 2-C1), with narrow and long hyphae (Figure 2C2). Unlike the latter, *Glomus* sp has a thin, cylindrical suspensory hypha, but it slightly thickens at the point of contact with the spore (Figure 2D2).

The *Scutellospora* genus has been represented by three species such as *Scutellospora cerradensis* [Spain and Miranda (Figure 3A)], *Scutellospora gregaria* [Schenck & Nicolson emend. Walker & Sanders (Figure 3B)] and *Scutellospora nigra* [Redhead emend. Walker & Sander (Figure 3C)]. Representing 26% of isolated spores (Table 4), these species are characterized by large spores with diameters ranged from 250 to 400 µm and by bulbous hyphae (Fig 3B2 and C2). Moreover, the main criterion of distinction of the different species is essentially based on the presence of germination shield (Figure 3A3) and whose structure is specific to each taxon.

Gigaspora margarita (Figure 3D) was the only species of the genus *Gigaspora*, isolated from the prospected soils. This is characterized by the presence of a bulbous suspensory hypha whose point of attachment to the spore opening out from inside the spore wall (Figure 3A2). The inner wall, also called the germinal wall (Figure 3A2) is thinner, smooth, flexible and less thick compared to the previous one; it surrounds a spore content rich in hyaline lipid elements more or less dense and rather concentrated after bursting of the spore.

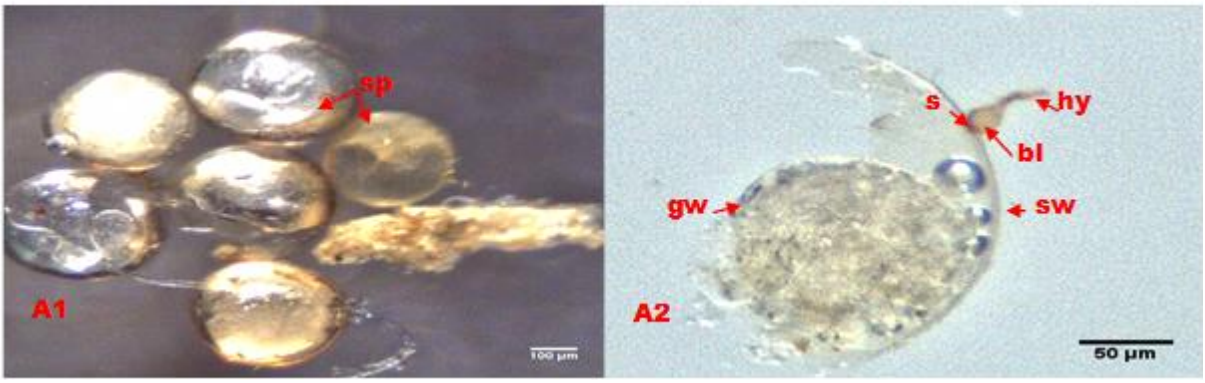

Figure 3. *Gigaspora margarita*: **A1:** isolate spores; **A2:** spore structure in PVLG with Melzer. (bl) bulbous suspensor; (gw) germination wall; (hy) subtending hypha; (s) septum; (sp) whole spore; (sw) spore wall.

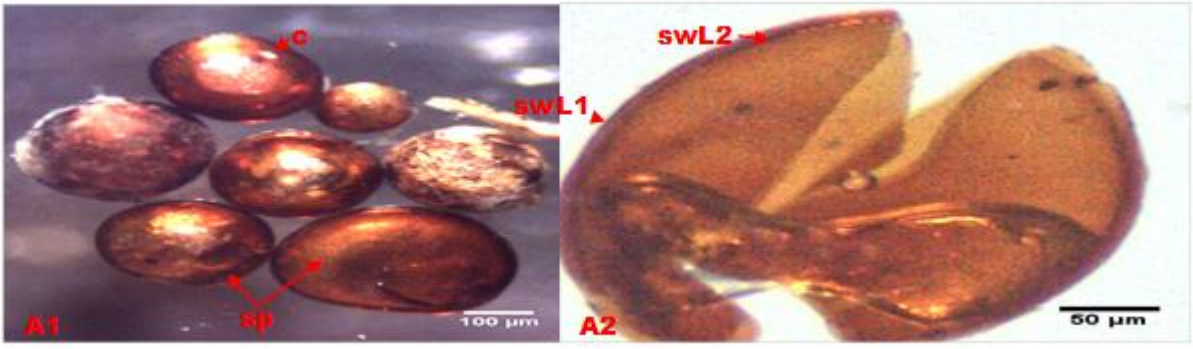

Figure 4. Morphological and anatomical characteristics of "Myco Brun-orange"spores, with: **A1:** isolated spores; **A2:** wall structure of spore in PVLG with Melzer ; (sp) whole spore ; (swL1) outer layer of spore wall; (swL2) inner layer of spore wall.

The *Acaulospora* genus constitutes 19% of isolated spores (Figure 5) and is represented by only two species whose identification is limited to the genus (*Acaulospora* sp.1 (Figure 5A) and *Acaulospora* sp.2 (Figure 5B)). Their spores appear sessile and the presence of a sporiferous saccule is one of their main characteristics (Figure 4A and B), as well as one or more germinal walls distinct from the spore walls were generally pore-dotted.

Despite the distinctive characteristics of "Myco Brown-orange" spores comparatively to other isolated spores, no correspondence with the strains described in the literature was found (Figure 4C). Representing 11% of isolated spores, with a shiny appearance; these spores were ovoid and sessile because they bore no attached hyphae.

Abundance and distribution of AMF

Whatever the study site, the number of spores present in 100 g of each soil prospected varied significantly ($P < 0.05$) according to the identified species (Table 5). Most of them have been found in almost all the sites sampled, but their numbers remain variable. *Acaulospora* sp.2 was the most abundant (144 spores / 100 g sol), but was only found in Dogba site; unlike *Glomus* sp. which was present in almost all the sites sampled, but in very low numbers (less than 20 spores / 100 g of soil). *G. hoi, S. gregaria, S. Nigra* and "Myco Brown-orange" spores were the most distributed species (100%), with populations of up to 60 to 80 spores / 100 g of soil. As for other species, they are unequally distributed in half of sampled sites and their numbers are between 4 to 50 spores / 100 g of soil.

Diversity of AMF communities

The diversity of isolated AMF in the study area varied from one site to another, but this remains low. Eleven species of AMC from a total of 1491 spores, unequally distributed in the prospected soils were identified (Table 6). The largest number of species was obtained in the

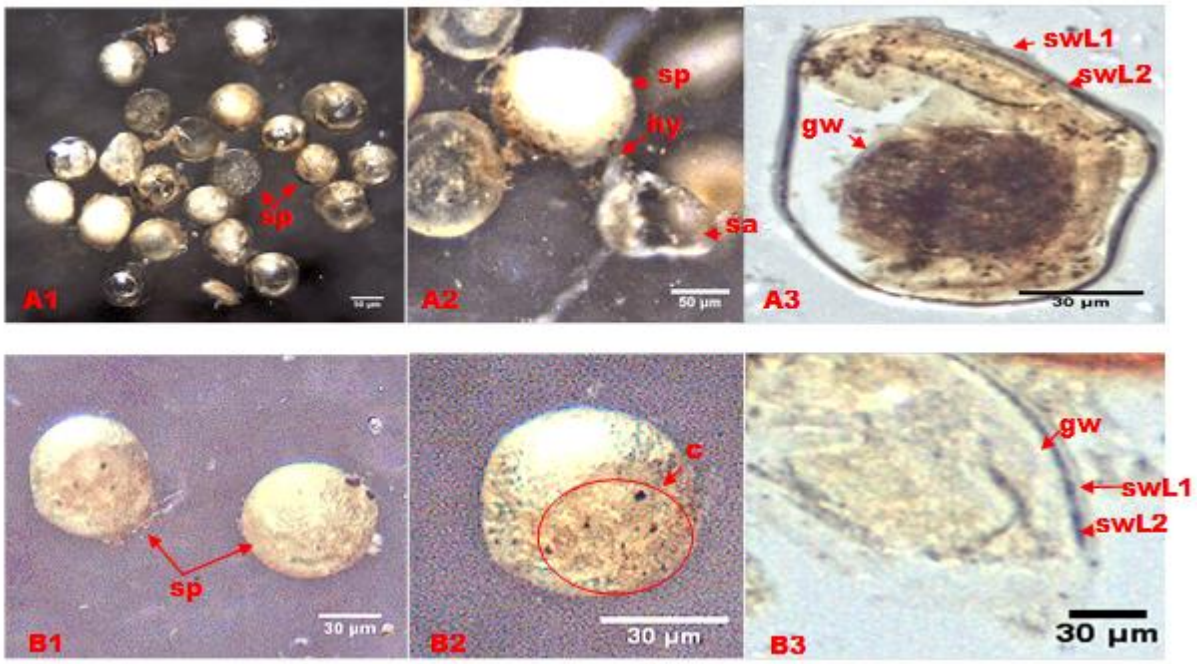

Figure 5. Morphological and anatomical characteristics of *Acaulospora* genus **A:** *Acaulospora* sp.1and **B :** *Acaulospora* sp.2 with: **A1, B1:** isolate spores; **A2, B2:** saccule structure on spore; **A3, B3:** wall structure of spore in PVLG with Melzer. (c) cicatrix; (hy) subtending hypha; (gw) germination wall; (sa) saccule; (sp) whole spore ; (swL1) outer layer of spore wall; (swL2) inner layer of spore wall.

Table 5. Average count of spores present in the soils sampled.

Species	Dargala	Dogba	Doukoula	Laf	Tokombéré	Zidim	Frequence of occurrence
Acaulospora sp.1	0	36±9^{bc}	30±3^{ab}	56±8e	26±4^{b}	0	66
Acaulospora sp.2	0	144±66d	0	0	0	0	16
Gigaspora margarita	0	16±4^{abc}	14±2^{ab}	16±2^{bc}d	0	0	50
Glomus aggregatum	42±6^{a}	15±2^{abc}	0	15±6^{bc}	0	25±10^{a}	66
G. hoi	50±44^{a}	66±2^{abc}	36±29^{b}	48±22e	52±30^{b}	27±12^{a}	100
G. manihotis	58±7^{a}	0	0	107±30f	0	0	50
Glomus sp.	0	8±7^{a}	9±3^{a}	8±5^{a}	8±1^{a}	18±5^{a}	83
Scutellospora cerradensis	27±4^{a}	0	0	13±3^{b}	0	0	50
S. gregaria	13±2^{a}	12±3^{ab}	25±8^{ab}	21±11^{c}d	12±5^{a}	30±5^{a}	100
S. nigra	29±8^{a}	52±8^{c}d	62±20^{c}	23±4d	31±22^{b}	41±22^{a}	100
Myco Brun-orange	7±4^{a}	83±77d	34±27^{b}	4±3^{a}	25±17^{b}	23±12^{a}	100
Total	226±75	432±178	210±92	310±94	154±79	164±66	
P-value	0.1163^{Ns}	0.0008***	0.0013**	<0.0001***	0.0009***	0.4122^{Ns}	

^{Ns}: not significant; *: significant (P < 0.05); **: very significant (P < 0.01); ***: very highly significant (P < 0.001). Values followed by the same letter on the same column are not significantly different according to the Duncan test at the 5% sill.

soil of Laf (10 species), followed by Dogba (9 species), Dargala (7) and Doukoula (7). In addition, the smallest number of species obtained came from the soils of Tokombere and Zidim which contained 6 and 5 species respectively. The values of the Shannon-Wiener index obtained according to the soils sampled were substantially equal, but low (not exceeding 0.82) with a very small variation. In addition, the highest value was obtained at Laf (0.82) and the lowest at Tokombere (0.7). As for the values of the Pielou Equitability Index, they are

Table 6. AMF diversity in sampled sites.

Sites	Rw	N	H'	EQ	S'
Dargala	7	225	0.77	0.74	0.81
Dogba	9	432	0.80	0.76	0.80
Doukoula	7	210	0.78	0.75	0.86
Laf	10	310	0.82	0.79	0.80
Tokombere	6	150	0.71	0.68	0.78
Zidim	5	164	0.76	0.73	0.82
Total	11	1491	/	/	/
Moyenne			0,77	0,74	0,81

Rw: Specific wealth; N: Total number of spores; H ': Shannon-Wiener index; EQ: Pielou Equitability Index; S: Simpson diversity index (1-D).

greater than 0.7 in all the prospected soils. This value is close to 1, indicating an almost equitable distribution of isolated species in the region. Nevertheless, the probability that two randomly selected individuals belong to different species varied from one site to another, but remained close to 1. The value of the lowest Simpson's index is obtained at Tokombere (0.78) and the largest in Doukoula (0.86).

Correlation between AMF parameters and physico-chimical properties of soils

Principal component analysis (PCA) was used to verify whether there is a relationship between species richness, total spore count and soil physico-chemical parameters, as well as root mycorrhizal parameters of trapping plants (Figure 6). In this model, the axes 1 and 2 describe respective variations of 32.21 and 30.14%, for a total variation of 62.35%. The first axis expressed the highest percentage variation and was positively correlated with the total number of spores and species richness. This correlation was also positive for the pH_{-H2O}, the silt (L), the organic matter (O.M), the total nitrogen (N), the assimilable potassium (K), as well as the intensity of mycorrhization (M). In relation to species richness, there was a significant correlation and a positive correlation with the total number of spores ($r = 0.82$), silt ($r = 0.81$) and total nitrogen ($r = 0.82$). Moreover, for the number of spores, this correlation was significantly negative ($P < 0.05$) with assimilable phosphorus ($r = -0.88$) and clay ($r = -0.88$).

DISCUSSION

Soils are usually complex and particular environments; the loss of one or more of their properties degrades their ability to produce biomass (Ouallal et al., 2018). In fact, the soils of the Far North region are generally acidic (Olina et al., 2008; Abakar et al., 2019) and the analysis of soil samples carried out in this study confirms this acidity status (pH = 4.98 to 6.5) according to the INRA (1995) pH interpretation standards. They are sandy-loamy in nature with an average predominance of sand. Furthermore, the detailed analysis of the distribution of mineral fractions reveals that the Doukoula soil is relatively rich in clay (56%), unlike the Dargala and Laf soil which are richer in silt (47 and 50% respectively). In fact, soils rich in colloids would be favorable for the formation of mycorrhizae (Ouallal et al., 2018), since they are generally more porous and less fertile, thus giving them good aeration; optimal condition of development of mycorrhizal fungi. However, the chemical parameters of the soils analyzed show that they are moderately rich in organic matter and nitrogen, but their phosphorus levels are still rather low according to the interpretation standards of the Calvet and Villemin (1986) soil analyzes. Since the plots sampled are agricultural areas, this wealth in organo-mineral element could be explained by the continual supply of organic amendment by the farmars. Moreover, the phosphorus level found in the Dogba soil could negatively influence the presence of arbuscular mycorrhizal fungi (AMF). According to Gosling et al. (2006), increased phosphorus significantly reduces fungal colonization, density and spore diversity in the soil.

In the directly sampled soils samples, not all spores are always represented (Zézé et al., 2007). Most of the AMF isolated directly from the sampled soils are poorly diversified and represent only those whose root colonization activity is sufficiently important for sporulation and to produce biomass especially in arid and semi-arid condition (Morton et al., 1993). Therefore, trapping is undertaken to create favorable conditions for the sporulation of all native species found there and to obtain viable and healthy spores for better observation (Straker et al. 2010). Thus, microscopic examination of maize roots (*Z. mays* L.) used as a trap plant revealed the presence of mycorrhizal structures, reflecting the mycotrophic nature of this species on the one hand, and

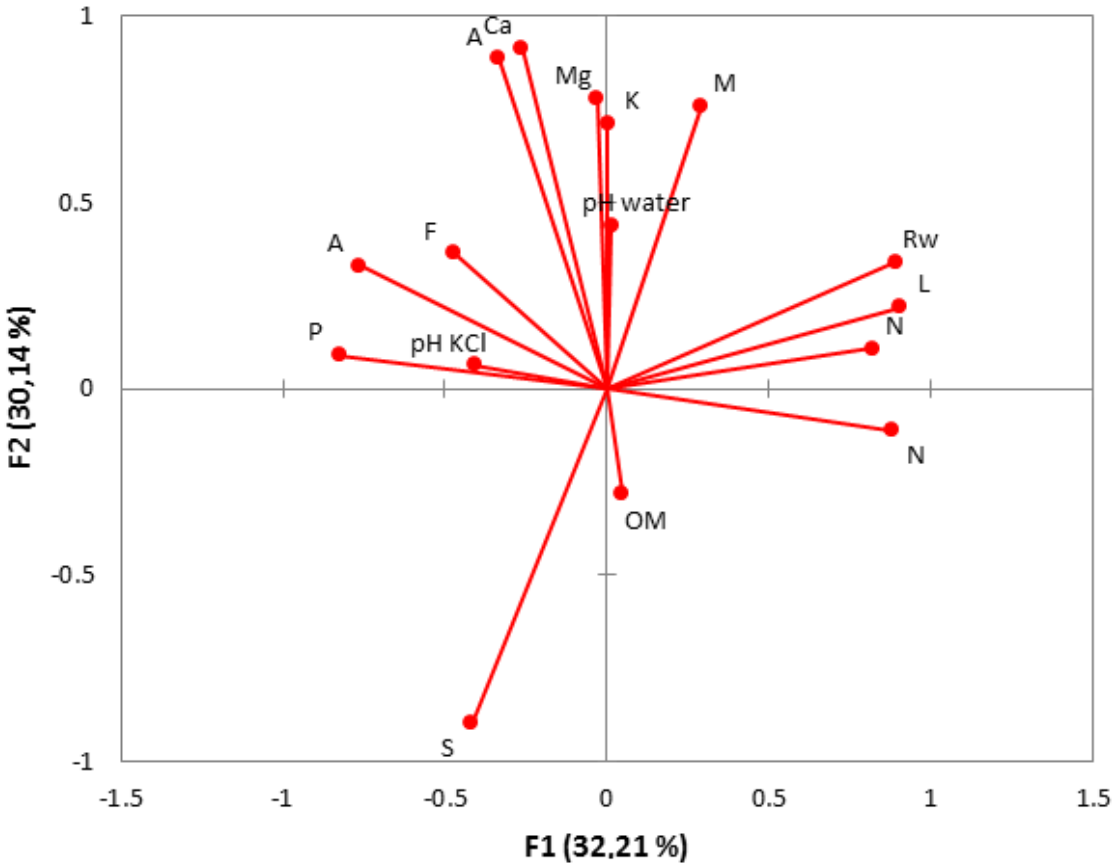

Figure 6. Graphical correlation between AMF parameters and physico-chimical properties of investigated soils; with: A: argile; L: slit; OM: organic matter; S: Sand; Rw: Specific wealth; N: Total number of spores.(axis F1 and F2: 62.35%)

the effective presence of AMF in the different soils. The main mycorrhizal structure observed in the totality of the root samples was of the type "arum" according to Gallaud's classification (1905) cited by Sidhoum (2011). In fact, this arbuscular mycorrhiza is dominant in most cultivated plants and is characterized by a rapid expansion of the fungus, facilitating the transfer of nutrients into the plant (Dickson et al., 2007). Despite the fact that the quantified mycorrhizal frequencies were very high, the ANOVA found no change in soil factor for all evaluated mycorrhizal parameters. Although mycorrhizal intensities and arbuscular levels are moderately low, they largely exceed those obtained by Tobolbai et al. (2018), who obtained mycorrhization intensities ranging from 1 to 14% only in maize cultivation in Diamaré (Far North Cameroon), as well as those obtained by Bossou et al. (2019) on maize cultivation in the Benin cotton zone (around 2.47%).

The abundance of isolated AMF spores showed variability among the sampled sites. This abundance was higher in the Dogba site (432 spores per 100 g soil) and lower in the Tokombere site (154 spores per 100 g soil). These numbers are comparable to those obtained by Tobolbai et al. (2018) on maize cultivation in the Far-North Cameroon region; as well as those obtained by Begoude et al. (2016) in the southern and eastern regions of Cameroon and by Haougui et al. (2013) in Niger's market gardening zone. But these remained very low compared to those obtained by Bossou et al. (2019) in the cotton zone of Benin with 12501 spores per 100 g of soil; as well as those obtained by Temegne et al. (2017) on the rhizosphere of peanut in the region of Central Cameroon. Indeed, all the sites sampled were characterized by a low level of soil fertility and the cultural practices were essentially based on a strong use of chemical amendments which leads to a strong reduction in root colonization, as well as the density of spores in the soil. In addition, the variation in AMF spore abundance among different sites could be due to vegetation, host specificity between AMF and plants, and the sporulation ability that is specific to each species from AMF (Husband et al., 2002).

Moreover, the analysis of morpho-anatomical characteristics of isolated spores in our study revealed

the presence of ten species belonging to three families Acaulosporaceae (2), Gigasporaceae (4) and Glomaceae (4), unequally distributed in two orders, that of Diversisporales and that of Glomerales (Redecker et al., 2013). This species richness is much higher than that obtained for maize cultivation in the same study area by Tobolbai et al. (2018), but remained comparable to that obtained by Bossou *et al.* (2019) in the Benin cotton zone. On the other hand, it remained low compared to those obtained in natural environments, such as in the forest zone of Cameroon (Musoko et al., 1994; Onguéné et al., 2002, Ngonkeu et al., 2013; Tchinmegni et al., 2016). Indeed, Tchabi et al. (2008) found that the species richness of AMF in natural forests is higher than in agricultural plots. This low abundance and specific wealth obtained may be due to the different agricultural practices carried out in the study area, because the cotton growing in this part of Cameroon is characterized by a strong use of mineral fertilizers, as well as by a continual turnover of soil by animal traction. Peyret-Guzzon (2014) has shown that cultural practices such as soil turnaround and especially chemical fertilization cause significant and considerable changes in the composition of AMF communities in soils.

Of the four genera identified, the description of *Glomus* species was facilitated by their cosmopolitan character, but also by their mode of anchoring in the soil. In this taxonomic genus, most species have been isolated in the form of compact (*G. aggregatum*) and free (*G. hoi*) sporocarps. This predominance has been reported in several studies in plots grown in Cameroon (Mbogne et al., 2015; Begoude et al., 2016; Temegne et al., 2017; Tobolbaï et al., 2018), in West Africa (Tchabi et al., 2008; Haougui et al., 2013; Johnson et al., 2013; Voko et al., 2013; Nandjui, 2015; Bossou et al., 2019), and in Eastern and Southern Africa (Jefwa et al., 2009; Straker et al., 2010). Indeed, several authors have associated the dominance of *Glomus* spp. by their rapid multiplication capacity and their better ability to adapt to the most hostile environmental conditions such as drought, extreme pH and other environmental stresses (Blaszkowski et al., 2002). Among the species identified in this genus, *Glomus manihotis*, also known as *Rhizophagus manihotis* (Schüßler and Walker, 2010) or *Rhizoglomus manihotis* (Sieverding et al., 2014), was the most abundant species in the study area despite its low distribution (50%). In sub-Saharan Africa, very few authors have reported its presence in the rhizosphere of cultivated plants, but the work carried out by Tobolbaï et al. (2018) have revealed its presence in the far north of Cameroon. Unlike the latter, *G. hoi*, still known under the name of *Simiglomus hoi* (Oehl et al., 2011), was encountered in all the sites surveyed. Like the previous species, its presence in the cultivated soils was only very little reported, because it is morphologically similar to *Glomus deserticola* present in many ecosystems (Talbi et al., 2014). In addition, the genera *Acaulospsora*, *Gigaspora* and *Scutellospora* have also been identified in culture medium in Cameroon by Ngonkeu et al. (2013), Mbogne et al. (2015) and Temegne et al. (2017). Three species were identified in the *Scutellospora* genus, with *Sctellospora ceradensis* comparable to those isolated by Straker et al. (2010) in the rhizosphere soils of cassava cultivation in South Africa; *S. gregaria* by Diallo (1998) and Belay et al. (2013) in Sudano-Sahelian area of Senegal and Ethiopia, respectively and *S. nigra* which has been identified in the soil of some plantations in Kenya by Jefwa et al. (2009). Concerning *Gigaspora*, only a poorly distributed species (50%) was isolated from the sites surveyed (*Gigaspora margarita*). Indeed, this genus is strongly represented in arid or semi-arid tropical zones (Diallo, 1998) and *G. margarita* for its part is a species highly represented in acid soils in Cameroon; it would promote the tolerance of plants sensitive to soil acidity (Ngonkeu et al., 2013). In addition, previous work on maize cultivation in the same study area by Tobolbaï et al. (2018) mentioned the absence of species belonging to *G. margarita*, but it has been identified in the cassava rhizosphere cultivated in the eastern and southern regions of Cameroon and peanuts grown in the Central Cameroon region by Temegne et al. (2017) then joining the assertion of Bossou et al. (2019) that the species richness of AMF soil varied according to the type of crop.

However, the identification and interpretation of the structural composition of spores has not been easy for some AMF, like *Acaulospora* spp. and *Glomus* sp. The observation and structural description of their spores was not sufficient to match those described in the literature. These could constitute fungal isolates specific to the study area, especially for *Acaulospora* sp.2 which is only found in one site and at high density.

The analysis of the diversity of isolated AMF in the study area, varied from one site to another, but this remained low throughout the region. Although the Shonnon-Wiener index evaluated in this study (0.77) was larger than that obtained by Tobolbai et al. (2018) in the Far-North Cameroon (0.45); these remained very low compared to that obtained by Bossou et al. (2019) in the Benin cotton zone (2.12); as well as compared to that obtained by Temegne et al. (2017) in humid zone in Cameroon (1.94). This low diversity of AMF in this zone could be due mainly to different cultural practices such as mineral fertilization or fungicide input, as well as to the harsh climate. Several studies have shown that intensive use of arable land, as well as cropping systems, strongly influenced soil AMF diversity and presence (Oehl et al., 2003). Indeed, Marschner et al. (2003), Vestberg et al. (2005), Gosling et al. (2006) and Borriello et al. (2012) have shown that these cultural practices led to significant changes in the composition of AMF communities. The principal component analysis conducted in this study confirmed these results, while showing how these factors influenced the presence and activity of AMF in the soil.

A significant correlation among species richness, total

number of spores, silt, total nitrogen assimilable phosphorus and clay. But this was negative among the total number of spores, the assimilable phosphorus and the rate of clay; as well as between the total nitrogen and the arbuscular content of the roots. Similar results have also been reported by Mohammad et al. (2003) and Nehila (2016). In the cultivated fields, the number of spores seems to reach a maximum under conditions where the phosphate applications, necessary for the maximum growth of plants were the least (Mekahlia, 2014). According to Juniper and Abbott (1993), high levels of phosphorus in soil prevent some AMF from providing substantial benefits to host plants and may affect the distribution and density of these fungi. This has also been confirmed by Balzergue et al. (2011) who showed that phosphorus is able to almost completely inhibit mycorhization at a very early stage, even before attachment of the fungus to the surface of the root epidermis.

Conclusion

The present study was carried out with the aim of highlighting the diversity of arbuscular mycorrhizal fungi (AMF) associated with the rhizosphere of cotton grown in the Far North Cameroon region. Through the results obtained, it appears that this rhizosphere has a specific diversity of native AMF; but it remains weak. Analysis of corn root fragments used here as trap plant was densely mycorrhized, but with low arbuscular content. Of the 11 spores species isolated from the different sites surveyed (6 sites), 10 of them were associated with the genera *Acaulospora, Gigaspora, Glomus* and *Scutéllospora*, with a dominant species of the genus *Glomus*. One type of spore was not identified in this study; this could thus show that there may be strains of some fungal specific to this area. These results could thus induce a controlled production of local fungal inoculum, adapted to the edapho-climatic conditions of the region and indirectly boosted the agricultural production.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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