

Morphological and Structural Diversity of Native Mycorrhizae Communities Associated with *Gossypium hirsutum* L. under Sudano-Sahelian Climate of North Cameroon

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Abstract : This study describes endomycorrhiza that enter into association with *Gossypium hirsutum* L. (cotton) grown in North Cameroon. During the study, twenty seven (27) soil samples were collected from three Divisions of the North Cameroon (Benoue, Mayo-Rey, Mayo-Louti). In each Division, nine (09) composite soils were sampled, thus 03 per locality (village), corresponding to sampling sites. Cotton seeds were grown in field for 04 months. Mycorrhizal parameters, spores density and specific richness were determined following to the appropriate methods. After spore extraction, species description and characterization were obtained through the informations provided by the International Vesicular Mycorrhizal fungi collection (INVAM): http://invam.caf.wv.edu/fungi/taxonomy/species_ID.htm. Results indicate that mycorrhizal parameters varied depending on sampling sites significantly ($p < 0.05$). Mayo-Louti (286.22 ± 11.47 spores/100 g of soil) and Benoue (273.77 ± 83.28 spores/100 g of soil) had higher spores densities than Mayo-Rey (209.55 ± 40.01 spores/100 g of soil). The morphological and structural characterization enabled the description of five endomycorrhizal species, belonging to four genera: *Glomus constrictum*, *Glomus manihotis*, *Acaulospora kentinensis*, *Entrophospora infrequens*, *Rhizophagus intraradices*. *Glomus* is the only genus found in all sampling sites. These findings open opportunities for domestication and application of endomycorrhiza for a sustainable field productivity of cotton in the North Cameroon.

Keywords: North Cameroon, *Gossypium Hirsutum*, Mycorrhizal Parameters, Spores Density, Specific Richness

Introduction

North Cameroon region are located in the Sudano-Sahelian zone, known for the severity of their climatic conditions (Tsozue *et al.*, 2015) and the low level of their soils fertility (Guibert *et al.*, 2008). Several works have shown that the potential contribution of Arbuscular Mycorrhizal Fungi (AMF) to soil can be critical in addressing this type of problems. In poor soils, several plant species are ecologically dependent on mycorrhizal fungi (Gemma *et al.*, 2002). These fungi are a major component of the soil microbial community that have successfully established a symbiotic relationship with two-thirds of plant species (Dechamplain, 2002; Dalpé, 2005; Wang and Qui, 2006). Mycorrhizal fungi allow plants to obtain an extension of its root system (Hamel, 1996) and to

optimize its supply in water and mineral elements, to improve its resistance against stresses including cold and drought (Dalpé, 2003). The benefit effect of this symbiosis is not limited to both partners, but also relates to ecosystem integrity since it improves soil quality (Caravaca *et al.*, 2002). In the Northern Cameroon, cotton is a crop of choice, this plant is the engine of economy in Soudano-Sahelian zone of this country (Abakar *et al.*, 2019).

Gossypium hirsutum L. (cotton) is subtropical, perennial plant belong to family Malvaceae, with 50 wild and cultivated species. Seeds produced by cotton plant are used for a multiproduct base like hulls oil, lint and food for animal as well as in textile manufacturing (Aragao *et al.*, 2005). Its leaves and roots are used in medicine; seeds cake is

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Published at: <http://www.ijsciences.com/pub/issue/2020-01/>

DOI: 10.18483/ijSci.2257; Online ISSN: 2305-3925; Print ISSN: 2410-4477



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used for livestock nutrition and for soil fertilization (Shahin *et al.*, 2018). In Cameroon: the cotton sector employs more than 2500 people and supports more than 2 million people (MINADER, 2013); cotton crop covers about 250 000 ha and it is practiced by about 300 000 farmers. Average area per producer is around 0.8 ha (Ekorong, 2004 cited by Olina *et al.*, 2008); also, it contributes to rehabilitation of approximately 9.000 km of tracks, the granting of credits for foodstuff inputs (about 3 billion FCFA/year) and for livestock development integrated in the farm (30-50 million FCFA/year) (Kadekoy-Tigague *et al.*, 2010).

Recent work on cotton aims to improve durably its growth potential. In this respect, the study of the endomycorrhizal status associated to this plant can obviously be of great importance. Furthermore, ecological studies on the diversity of AMF elsewhere, whether they relate to the morphological characterization of spores, molecular biology techniques or endomycorrhizal inoculants tests in the field have been generally limited to exotic species, with little or no investigations on available indigenous species (Symanczik, 2016; Abakar *et al.*, 2019). To the best of our knowledge, no work

has been carried out on endogenous mycorrhizal associated with cotton in Cameroon. In this context, the main objective of current study was to determine cotton mycorrhizal status in our country. Specifically it consisted to: (1) Carry out soils physicochemical properties from cotton rhizosphere in the North Cameroon; (2) Evaluate the parameters of cotton root mycorrhizal colonization; (3) Study the diversity of mycorrhizal fungi associated with cotton rhizosphere in the North Cameroon. The interest and usefulness of this work was that the endogenous strains of mycorrhizal fungi associated with cotton rhizosphere will constitute a basic data for the formulation of suitable mycorrhizal inoculum for cotton productivity. Thus, our next work will be focused on the study of effects of endogenous strains of mycorrhizal fungi relative to cotton productivity.

3. Materials and Methods

3.1. Physical description of the study sites :

The experiment was conducted in North Cameroon located in agroecological zone I (Sudano sahelian type). Figure 1 illustrates the map showing the study area and the sampling localities.

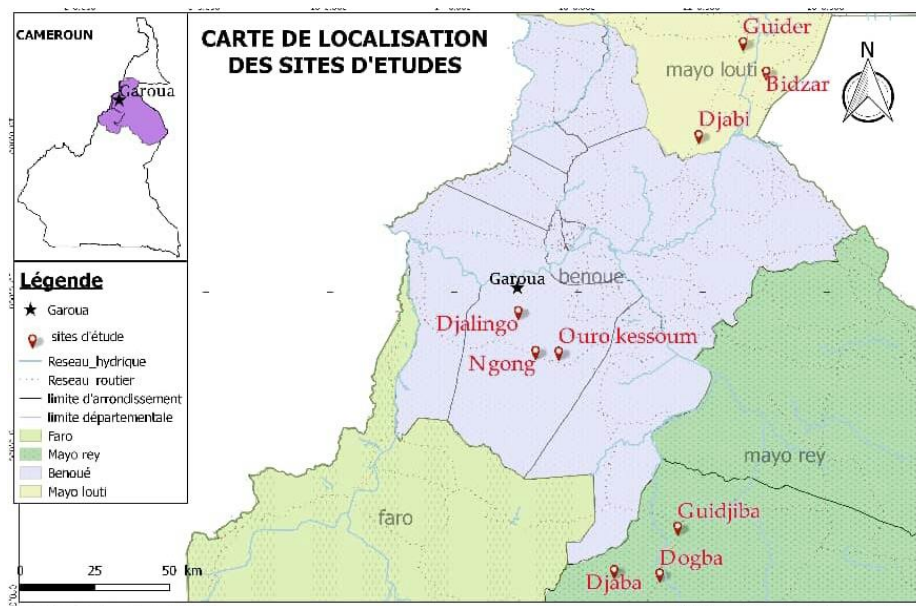


Figure 1. Map of North Cameroon and sampling sites
Sampling sites are in red color

3.2. Soil sampling :

Soils were sampled at 25 cm depth (INRA, 2017) after the surface was cleared from plant debris and other large particles. Sampling was carried out in

three villages (localities) in each of the three Divisions of our study area as summarized in table 1.

Table 1. Description of soil sampling sites

Region	Divisions	Localities	Altitude (m)	Longitude (°)	Latitude (°)
North Cameroon	Benoue	Djalingo	244	13°27'08''E	9°12'45''N
		Ngong	320	13°30'31''E	9°00'05''N
		Ouro-Kessoum	241	13°37'35''E	9°00'27''N
	Mayo-Louti	Bidzar	394	14°07'48''E	9°55'23''N
		Djabi	268	13°48'40''E	9°37'23''N
		Guider	401	13°56'36''E	9°55'32''N
	Mayo-Rey	Djaba	387	13°54'51''E	8°34'51''N
		Dogba	398	13°68'08''E	8°33'48''N
		Guidjiba	393	13.73'56''E	8°47'63''N

3.3. Determination of the physico-chemical properties of soils

Soil samples were taken according to the Zig-zag method of Barker (1985). The soils were analyzed at the Laboratory of Soil Analysis and Environmental Chemistry Research Unit of the University of Dschang (Cameroon). Soils physicochemical properties (granulometry, pH, cation exchange capacity, soil contents in nutrients elements such as nitrogen, phosphorus and potassium) were evaluated according to standard methods.

3.4. Trapping of endomycorrhizal spores from collected soil samples

Trapping of spore was carried out according to the method described by Brundrett *et al.*, (1996) modified as follows: *Gossypium hirsutum* was sown in the field in each locality. IRMA Q302 variety was used. Cotton crop were left at natural watering rainfall capacity for four months (may to august 2018) (figure 2). The roots of plants at flowering and rhizospheric soils were sampled for laboratory analysis. The roots in particular were preserved in the refrigerator at 4°C. Fine harvested cotton roots were thinned according to Phillips and Hayman (1970) method to highlight endomycorrhizal infestation structures. Cotton roots were: (1) carefully washed, the youngest taken and cut to 1-2 cm in length; (2) put into a test tube with 10% potassium hydroxide, and heated in a water bath at 90 °C for 30 minutes to clear the roots; (3) the potash was discarded, filtered through a sieve, before neutralization by rinsing with acidified water; (4) neutralized roots were kept into cotton blue in a water bath for 15 minutes, filtered again through a sieve, and rinsed with distilled water; (5) some of these roots were mounted in water for direct observations, while other were mounted in glycerine for later observations. The mycorrhizal parameters such as mycorrhizal frequency, mycorrhizal intensity, cotton roots arbuscular content were determined according to Trouvelot *et al.* (1986). These mycorrhizal parameters were calculated automatically using "Mycocalc" software.



Figure 2 : Cotton plants in Djalingo locality at 40 days after sowing

3.5. Extraction of endomycorrhizal spores from the rhizospheric *Gossypium hirsutum* soils

Endomycorrhizal spores were extracted according to the wet extraction method described by Gerdemann and Nicolson (1963) modified by the follows steps: (a) suspension of soil sample (100 g) in water; (b) mechanical stirring of soil for 15 min (repeated thrice); (c) passing the soil through a series of sieves of size corresponding to the range of spores sizes of between [25 - 400 µm]; (d) creating a density gradient by centrifugation; (e) filtering through a 25 µm sieve for spores collection.

3.6. Morphological and structural characterization of endomycorrhizal spores in cotton root

For the identification of Arbuscular Mycorrhizal Fungi (AMF), the extracted spores were grouped by morphotype under criteria such as size, shape and color. Two groups of spores from each morphotype were mounted between slide and coverslip, thus one in PVGL (Polyvinyl-Lactic Acid-Glycerol), and the other in the PVGL-Melzer Reagent mixture (1:1/v:v) (Koske and Tessier, 1983). The morphotypes determination of the genus was made based on the classifications described by Morton and Benny (1990). The original descriptions of species, as well as the descriptions provided on the website of the International Vesicular Mycorrhizal fungi collection (INVAM): <http://invam.caf.wv.edu/fungi/taxonomy/speciesID.htm> were used as the reference during the identification process. Morphological characters of

spores were compared with those of standard specimens and the reference strains. Several parameters were used to characterize AMF spores and were evaluated based on the formula proposed by Sghir *et al.*, (2013). The species richness refers to the total number of different morphotypes recorded in a 100 g soil sample, and was expressed by: $R (\%) = N/100 \text{ g}$, where N is the number of different specimens. The specific density indicates the number of spores recovered in 100 g soil sample, and was expressed as: $D (\%) = N/100 \text{ g}$, where N is the number of spores. The diversity of endomycorrhizal species in all the sites was calculated using Shannon-Weaver diversity index (H) (Shannon, 1948). The Shannon index is given by the formula below: $-H = -\sum p_i \ln p_i$, where $p_i = S/N$, S is the total number of individuals of one species, N is the total number of all individuals in the sample and \ln = logarithm to base e. The proportion of species relative to total number of species (p_i) was calculated, and multiplied by natural logarithm of this proportion ($\ln p_i$). The results were summed across the species, and multiplied by -1.

3.7. Data analysis

Data were subjected to variance analysis followed by the Duncan multiple range tests when any significant effect was observed. The statistical software "Statgraphics plus" was used for this propose. Excel 2010 software was used for data entry and graphing.

4. Results and discussion

4.1. Physicochemical characteristics of composite soils of the sampling sites

Composite soil samples from different Division of the North Cameroon had varying physicochemical properties (Table 2). The Granulometry study shown that the most sandy soils was from Benoue ($75 \pm 10.44\%$), while the highest soil clay contents was recorded in Mayo-Louti ($17.66 \pm 15, 88\%$) and the soils of Mayo-Rey have the highest silt content ($28.00 \pm 10.78\%$). Soil pH varied from 4.30 ± 0.20 to 7.82 ± 0.00 , respectively for Ngong in Benoue Division and Djaba in Mayo-Rey Division. Globally soil of Benoue Division is acid ($\text{pH} = 5.00 \pm 0.60$) while that of Mayo-Rey ($\text{pH} = 7.75 \pm 0.06$) is basic and Mayo-Louti's soil is neutral ($\text{pH} = 6.61 \pm 1.00$). The lowest soil phosphorus contents was from Mayo Rey and it ranged from $1.77 \pm 0.06 \text{ ppm}$ to $3.02 \pm 0.02 \text{ ppm}$, respectively for Djaba and Dogba localities. Soils from Benoue and Mayo-Louti have presented the highest phosphorus contents and it ranged from $7.21 \pm 0.32 \text{ ppm}$ to 20.38 ± 0.28 respectively for Bidzar and Guider localities. However, soil of Ngong in the Benoue Division had a phosphorus content almost twice lower (4.53 ± 0.12) compared to those of other localities of this Division. Globally, soil of Mayo-

Louti had a phosphorus content ($13.65 \pm 6.58 \text{ ppm}$) almost twice as high as Benoue soil ($6.95 \pm 2.12 \text{ ppm}$) and almost six fold higher than that of Mayo-Rey Division ($2.41 \pm 0.62 \text{ ppm}$). Regarding soils nitrogen contents, the highest values of this parameter are found in Mayo-Louti ($0.55 \pm 0.32 \text{ g/kg}$) while the lowest was observed in Mayo-Rey ($0.31 \pm 0.04 \text{ g/kg}$). Soil potassium content is higher in Mayo-Rey ($0.32 \pm 0.05 \text{ meq/100g of DM}$) while the lowest value was observed in Benoue ($0.20 \pm 0.10 \text{ meq / 100g}$). The soils of North Cameroon was low in organic matter: soil carbon content ranged from $0.46 \pm 0.1\%$ to $1.94 \pm 0.17\%$ and organic matter content ranged from $0.8 \pm 0.1\%$ to $3.34 \pm 0.43\%$ respectively for Djaba and Bidzar. However these soils had high mineral elements content. Soil phosphorus content varied from 1.77 ± 0.06 to $20.38 \pm 0.28 \text{ mg/kg}$ respectively for Djaba in Mayo-Rey and for Guider in Mayo-Louti. It was observed in this study that soil pH varied from 4.30 ± 0.20 for Ngong in Benoue Division to 7.82 ± 0.00 for Djaba in Mayo-Rey Division, this result corroborate partially data found in litterature.. Indeed, Tobolbai *et al.* (2018) did studies on the morphological and structural diversity of native endomycorrhizae associated with maize in Northern Cameroon and observed acid soils for Benoue (4.98), Mayo-Louti (5.30) and Mayo-Rey (5.02). Olina *et al.* (2008) revealed that average soil pH of Far North Cameroon is 6. In addition Mbonigaba *et al.* (2009) reported that tropical soils are acidic. In the current study, soil contents in organic matter and mineral elements vary depending on study sites, thus suggesting that these localities would influence mycorrhizal parameters as well as the density and diversity of mycorrhizal fungi associated with plants rhizosphere. Indeed, Shukla *et al.* (2009) and Voko *et al.* (2013) reported that the distribution, abundance and viability of endogenous AMF species results from soil physicochemical properties. In addition, several authors (Diouf *et al.*, 2013 ; Tobolbai *et al.*, 2018) indicated that the specific richness of the Arbuscular Mycorrhizal Fungi (AMF) spores increases with the clay. In this respect, Guider locality would present the highest specific richness of AMF spores, but this needs to be investigated.

Table 2. Soils physico-chemical properties

Parameters	Djalingo	Ngong	Ouro-Kessoum	Bidzar	Djabi	Guider	Djaba	Dogba	Guidjiba
pH	5.40±0.26 ^{bc}	4.30±0.20 ^a	5.30±0.10 ^b	6.60±0.36 ^d	5.70±0.34 ^c	7.71±0.00 ^a	7.82±0.00 ^a	7.69±0.04 ^a	7.76±0.05 ^a
N (g/kg)	0.28±0.07 ^{ab}	0.36±0.12 ^d	0.29±0.02 ^{abc}	0.88±0.32 ^f	0.53±0.16 ^a	0.24±0.28 ^a	0.31±0.06 ^{bcd}	0.50±0.02 ^a	0.35±0.45 ^{cd}
P (ppm)	7.82±0.07 ^f	4.53±0.12 ^d	8.51±0.02 ^e	7.21±0.32 ^e	13.36±0.16 ^b	20.38±0.28 ⁱ	1.77±0.06 ^a	3.02±0.02 ^c	2.44±0.45 ^b
Ca (méq/100g)	17.50±1.35 ^a	36.2±6.00 ^{bcd}	21.30±2.23 ^a	43.70±4.61 ^d	31.50±0.62 ^b	35.36±1.13 ^{bc}	17.68±1.20 ^a	65.58±10.00 ^a	42.16±3.17 ^{cd}
Mg (mg/100g)	101.30±2.45 ^b	180.00±12.50 ^d	165.00±20.00 ^d	127.10±7.21 ^c	135.00±7.00 ^c	107.44±6.23 ^b	46.24±4.71 ^a	35.36±2.35 ^a	44.88±4.08 ^a
K (meq/100g)	0.14±0.02 ^a	0.14±0.00 ^a	0.32±0.03 ^c	0.14±0.01 ^a	0.32±0.02 ^c	0.43±0.05 ^a	0.27±0.01 ^b	0.32±0.02 ^c	0.38±0.01 ^d
OM (mg/g)	2.05±0.16 ^{bc}	2.91±0.16 ^{de}	2.26±0.23 ^c	3.34±0.43 ^e	2.48±0.10 ^{cd}	0.86±0.13 ^a	0.80±0.10 ^a	1.94±0.64 ^{bc}	1.53±0.54 ^b
OC (mg/g)	1.19±0.13 ^{bc}	1.69±0.37 ^{de}	1.31±0.26 ^c	1.94±0.17 ^e	1.44±0.12 ^{cd}	0.50±0.00 ^a	0.46±0.10 ^a	1.13±0.06 ^{bc}	0.89±0.14 ^b
Clay (g/100 g)	7.00±0.00 ^{ab}	7.00±0.00 ^{ab}	6.00±0.00 ^a	8.00±0.00 ^{bc}	9.00±0.00 ^c	36.00±1.00 ^e	16.00±2.00 ^a	18.00±2.00 ^f	11.00±1.00 ^d
Silt (g/100 g)	24.00±0.00 ^d	6.00±0.00 ^a	24.00±0.00 ^d	44.00±0.00 ^a	12.00±0.00 ^b	17.00±2.00 ^c	24.00±4.00 ^d	21.00±3.46 ^d	41.00±2.00 ^a
Sand (g/100 g)	68.00±0.00 ^d	87.00±0.00 ^f	70.00±0.00 ^d	48.00±0.00 ^b	79.00±0.00 ^a	44.67±3.50 ^a	58.00±3.00 ^c	58.00±3.00 ^c	46.00±1.00 ^{ab}
CEC (mEq/100g)	5.73±0.57 ^a	7.21±1.66 ^{ab}	12.48±0.79 ^c	24.74±4.20 ^{ef}	11.12±2.48 ^{bc}	21.45±1.91 ^{de}	23.45±1.64 ^{ef}	27.28±1.55 ^f	19.15±4.09 ^d

DM : Dry matter ; CEC : Cation Exchange Capacity ; OC : Organic carbon, OM : Organic matter
Values of line affected by the same letter are not significantly different (p<0.01)

4.2. Mycorrhizal parameters

Globally, The analysis of variance (ANOVA) revealed that there was a significant incidence (p<0.05) of sampling sites relative to mycorrhizal parameters such as mycorrhizal intensity colonization, roots arbuscular content.

4.2.1. Mycorrhizal frequency

Mycorrhizal frequency varied from 16.66 ± 5.51% for Bidzar to 25.45 ± 6.70% for Djabi in Mayo-Louti Division. In Benoue Division it is higher in Ngong (36.66 ± 4.60%) than in Ouro-kessoum (26.66 ± 4.03%) while in Mayo- Rey Division it varied from 32.66 ± 5.05% for Dogba to 34.16 ± 7.00% for Guidjiba. Generally, mycorrhizal frequency was higher in Mayo-Rey (34.60 ± 0.82%) and Benoue (31.10 ± 5.09%) than in Mayo-Louti (21.75 ± 4.55%)

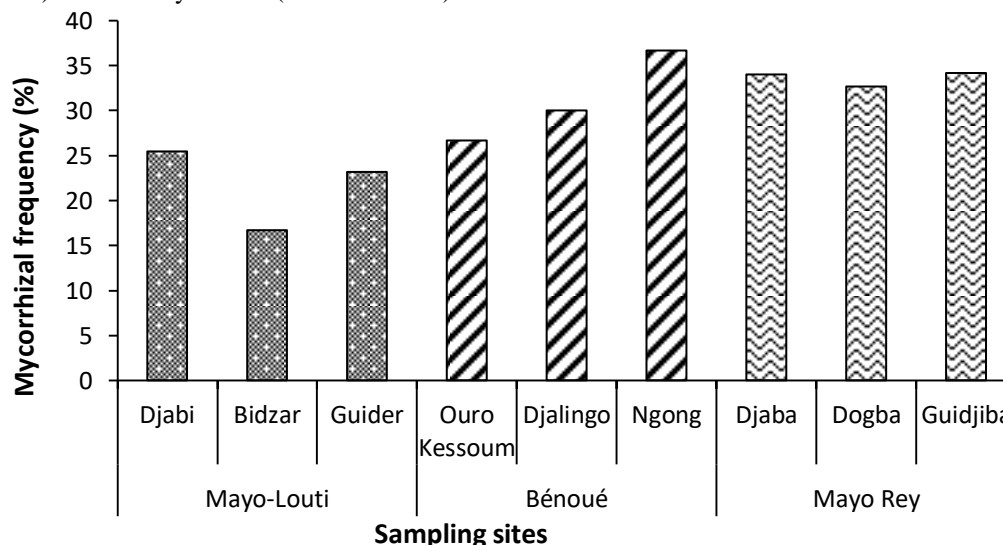


Figure 3: Variation in mycorrhizal frequency depending on sampling sites

4.2.2. Mycorrhizal intensity colonization

In Mayo-Louti, the highest mycorrhizal intensity colonization was observed in Djabi (1.02 ± 0.88%) while the smallest value of this parameter was from Bidzar (0.17 ± 0.05%). Guider had presented an intermediate value (0.7 ± 0.62%). In the Benoue Division, cotton mycorrhizal intensity in Ngong was 19.83 ± 7.57%. Mycorrhizal intensity was almost 04 fold higher than that of Djalingo (5.33 ± 2.02%) and

(figure 3). The values of mycorrhizal frequency obtained in the current study are higher than data reported by Tobolbai *et al.* (2018). These authors reported that roots mycorrhizal frequency of mays was 3.66% for Benoue, 4.33% for Mayo-Rey and 4.66% for Mayo-Louti. The higher value of cotton mycorrhizal frequency compared to that of mays would be justified by that mycorrhizal fungi would have a better association with cotton than maize, but this need to be investigated. The mycorrhizal frequency confirms the existence of a mycorrhizal symbiosis through the number of roots infested by the spores of the arbuscular mycorrhizal fungi and marks the beginning of the symbiotic phase. In this respect, mycorrhizal symbiosis exists with cotton regardless of North Cameroon locality.

08 fold higher than that of Ouro-Kessoum (2.5 ± 3.92%). In Mayo-Rey Division, mycorrhizal intensity is higher in Djaba (18.06 ± 7.78%) and lower in Dogba (12.61 ± 1.44%), it presented intermediate value in Guidjiba (16.46 ± 0.91%). Globally, mycorrhizal intensity was 15.71 ± 2.80%, 9.22 ± 9.29% and 0.63 ± 0.42% respectively in Mayo-Rey, Benoue and Mayo-Louti (figure 4). The mycorrhizal intensity shows the degree of plants roots

colonization with spores of arbuscular mycorrhizal fungi and provides information about the symbiotic phase evolution. In Northern Cameroon, the cotton

roots were all infested with arbuscular mycorrhizal fungi, but this colonization varied depending on sampling sites.

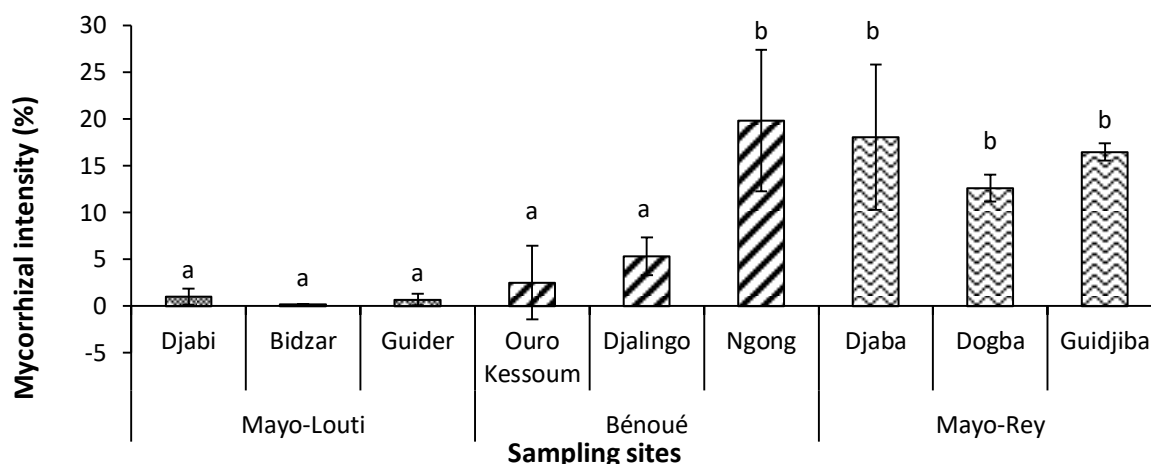


Figure 4: Variation on mycorrhizal intensity of cotton depending on sampling sites

Bars affected by the same letter are not different significantly ($p < 0.05$) for each Division

4.2.3. Arbuscular content

In Mayo-Louti Division, arbuscular content was higher in Djabi locality ($0.5 \pm 0.88\%$), followed by Guider ($0.2 \pm 0.75\%$) and Bidzar ($0.01 \pm 0.00\%$). In Benoue Division, the arbuscular content in Ngong locality ($14.3 \pm 8.21\%$) was 12 fold higher than that of Djalingo ($1.2 \pm 1.01\%$) and 20 fold higher than that of Ouro-kessoum ($0.64 \pm 1.08\%$). In Mayo-Rey Division, the arbuscular content was higher in Djava ($11.37 \pm 7.58\%$) and Dogba ($11.12 \pm 1.12\%$) localities compared to Guidjiba ($10.95 \pm 2.86\%$). Globally, the highest value of arbuscular content ($11.14 \pm 0.21\%$) was from Mayo-Rey Division and the smallest value ($0.23 \pm 0.24\%$) was from Mayo-

Louti while intermediate value (5.38 ± 7.73) of this parameter was observed in Benoue (figure 5). Values obtained relative to the roots arbuscular content in the current study are lower than that of Sidhoum (2011), who studied the diversity of arbuscular mycorrhizas associated to olive tree and found that it varied from 12 to 33.67%. The arbuscular content shows the level of mycorrhizal structures differentiation in order to assess the efficiency and type of mycorrhizal symbiosis. There was a positive and significant correlation between mycorrhizal frequency and mycorrhizal intensity ($r = 0.9$; $P < 0.001$), mycorrhizal intensity and cotton roots arbuscular content ($r = 0.977$; $P < 0.0001$).

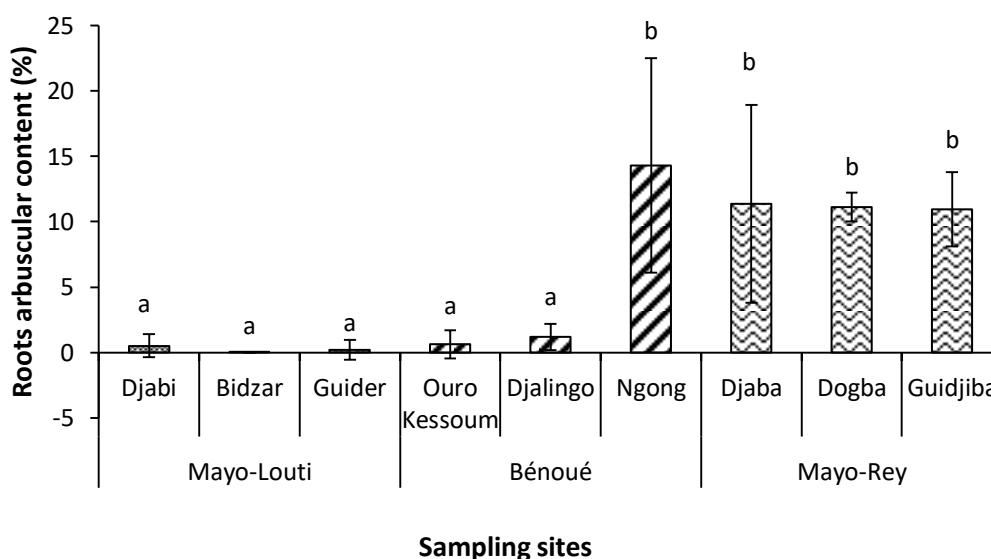


Figure 5: Variation of the roots arbuscular content of *Gossypium hirsutum* (L.) depending on sampling sites
Bars affected by the same letter are not different significantly ($p < 0.05$) for each Division

4.3. Spores of mycorrhizal fungi associated with Cotton rhizosphere: Density and identified species

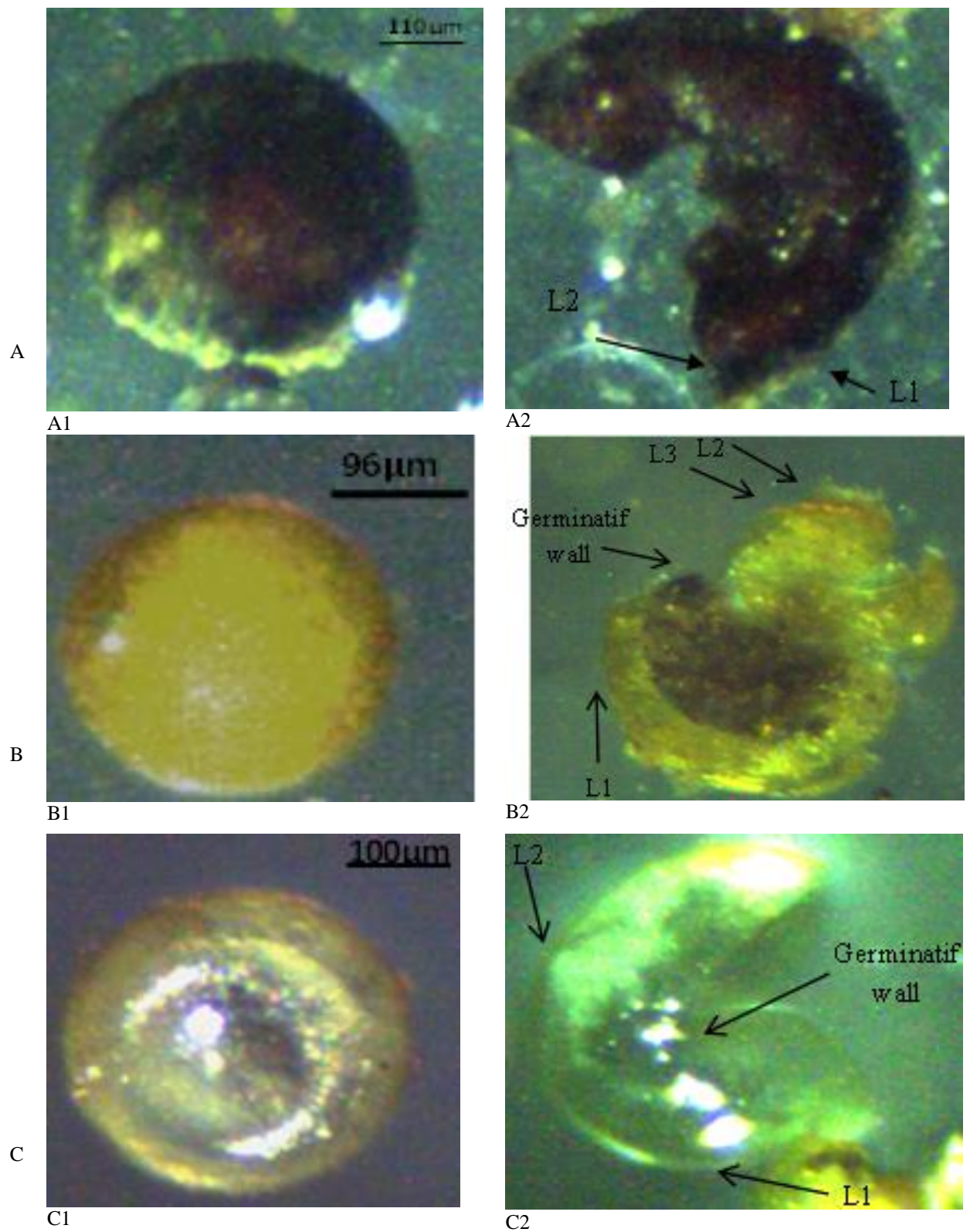
There was a significant ($p < 0.05$) difference between the densities of spores associated with cotton rhizosphere relative to localities. However localities did not affect significantly species richness. In Mayo-Louti Division, the highest densities was observed in Bidzar (295.33 ± 84.89 spores/100 g) and Djabi (290.00 ± 46.50 spores/100 g) locality and the smallest value was from Guider (273.33 ± 10.11 spores/100 g). In Benoue Division, spores density in Ouro-Kessoum (333.66 ± 93.66 spores/100 g) is 02 fold higher than that from Djalingo (178.66 ± 21.19 spores/100 g) and 1.08 fold higher than that from Ngong (309.00 ± 19.28 spores/100 g). In Mayo-Rey Division, Guidjiba locality had the lowest density (164.00 ± 84.89 spores/100 g); it is 1.45 fold lower than that of Djaba (239.00 ± 59.80 spores/100 g) and 1.37 fold lower than that of Dogba (225.66 ± 67.24 spores/100 g). Globally, Mayo-Louti (286.22 ± 11.47 spores/100 g) and Benoue (273.77 ± 83.28 spores/100 g) had higher densities than Mayo-Rey (209.55 ± 40.01 spores/100 g). In general, Ouro-kessoum locality in Benoue Division had the highest specific richness and five (05) species of mycorrhizae were identified in this locality. *Glomus* is the only genus found in all sampling sites. Benoue, Mayo-Rey and Mayo-Louti had respectively 05, 04 and 03 listed species. The values of Shannon index (H') of all the localities (Table 3) varied between 0.31 for Djalingo to 0.61 for Ouro-kessoum, thus suggesting that all the three Divisions had a very low diversity of AMF spores. However, Pielou's equitability index varied from 0.65 to 0.99 respectively for Djalingo and Ngong localities, thus suggesting that identified species in a locality are fairly distributed. In this study five (05) types of spores distributed in four (04) genera could be identified (Figure 6): *Glomus* (2 species), *Acaulospora* (1 species), *Rhizophagus* (1 species), *Entrophospora* (1 species). It was observed in this study that spore densities varied from 164.00 ± 84.89 to 333.66 ± 93.66 spores/100g. These values corroborate Zézé *et al.* (2007) who reported that spores densities ranged from 152.50 to 379.00 spores/100g in different types of forests in Côte

d'Ivoire. In addition, Haougui *et al.* (2013) found that the average spore's density from four marked gardenning sites in the Maradi region of Niger was 284.8 spores/100 g of soil. However, Tobolbai *et al.* (2018) reported that spores density from mays rhizosphere was 532 spores/100 g in Benoue, 304 spores/100g in Mayo-Rey and 121 spores / 100 g in Mayo-Louti. The pH of soil would affect the dissemination an life cycle of spores of mycorrhizal fungi in each locality. It appears in this study that the highest spore's density values are found in localities which acidic pH and the lowest were from localities which basic pH (Table 2), thus corroborate Cius (2017) who reported that acidic or neutral pH better promotes the multiplication of AMF spores. Spores density allows identifying the soil that is most favorable for the multiplication of AMF spores. After direct counting, the isolation of these spores made it possible to identify and describe them. Results obtained on specific richness partially corroborate Tobolbai *et al.* (2018) on mays roots in North Cameroon who found that specific richness varied from 2 and 4 species, but these authors did not found both species *Acaulospora kentinensis* and *Entrophospora infrequens* in mays rhizosphere. However, Voko *et al.* (2013) found that specific richness of cassava rhizosphere in Côte d'Ivoire ranged from 20 to 32 species. This would be explained by that AMF are specific to a host plant. In this study the presence of *Glomus* genus in all sampling sites does not surprise us: soil pH has an influence on species diversity Bansal *et al.* (2012), *Glomus* genus adapts in all soil pH values with optimum pH varying from 6 to 8. In addition, several authors (Azcón-Aguilar, 2003; Mathimaran *et al.* 2005; Abbas *et al.*, 2006) reported that *Glomus* genus has a greater geographic distribution in the world especially in the agricultural environment and specifically in the semi-arid zones. In this work, we expected the locality of Guider to have the most specific richness due to its clay-rich soil, but the highest value of this parameter was rather from the soil of Ouro-Kessoum, thus suggesting that the diversity of AMF at different sites was influenced not only by soil clay content, but also by other soil compositions.

Table 3. Diversity and abundance of arbuscular mycorrhizal fungi species depending on sampling sites

Divisions	Localities	<i>A. kentinensis</i>	<i>E. infrequens</i>	<i>G. constrictum</i>	<i>G. manihotis</i>	<i>R. intraradices</i>	D	S	H'	R
Mayo-Louti	Bidzar	++	-	***	***	-	295,33±84,89 ^{cd}	3	0,42	0,88
	Djabi	++	-	***	***	-	290,00±46,50 ^{cd}	3	0,43	0,90
	Guider	++	-	***	***	-	273,33±10,11 ^{bcd}	3	0,41	0,86
Benoue	Djalingo	++	-	+++	+++	-	178,66±21,19 ^{ab}	3	0,31	0,65
	Ngong		+++	***	+++	+++	309,00±19,28 ^{cd}	4	0,6	0,90
	Ouro-Kessoum	+++	++	***	+++	+	333,66±93,66 ^d	5	0,61	0,99
Mayo-Rey	Djaba	++	-	***	+++	+	239,00±59,80 ^{abcd}	4	0,44	0,73
	Dogba		-	***	+++	+	225,66±67,24 ^{abc}	3	0,33	0,7
	Guidjiba	+	-	+++	+++	-	164,00±37,72 ^a	3	0,42	0,90

-: absent; +: weakly abundant (20); ++: Averagely abundant (21-40); +++: abundant (41-100); ***: highly abundant (> 100) ; D: Spores density; S: species richnes, H' : Shannon index; R: Pielou's equitability index. Values of column affected by the same letter are not significantly different ($p < 0.05\%$).



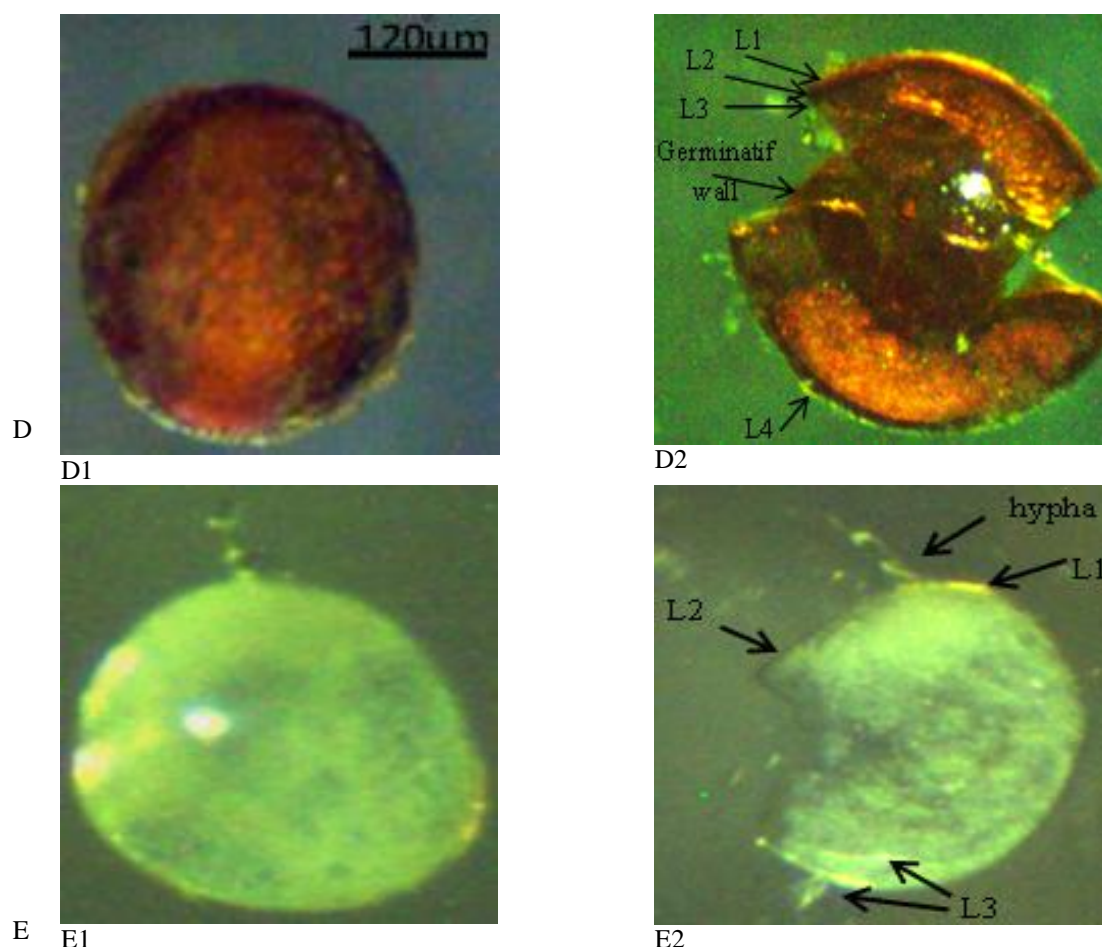


Figure 6 : Morphological and structural diversity of isolated spores.

A : *Glomus constrictum* ; B : *Glomus manihotis* ; C : *Acaulospora kentinensis* ; D : *Entrophospora infrequens* ; E : *Rhizophagus intraradices*.

5. Conclusion

In this study, *Gossypium hirsutum* L. (cotton) was found to be dependent on endomycorrhizal symbiosis in the North Cameroon. Five Arbuscular Mycorrhizal Fungi (AMF) species were involved in this symbiosis in the Mayo-Rey, Benoue and Mayo-Louty Division of North Cameroon which are: *Glomus constrictum*, *Glomus manihotis*, *Acaulospora kentinensis*, *Entrophospora infrequens*, *Rhizophagus intraradices*. The strain *Glomus constrictum* was dominant, while *E. infrequens* was the less frequently encountered specimen. The identification of these endogenous endomycorrhizal spores structures in soils is a potential opportunity for production of endomycorrhizal inoculants to improve cotton productivity in this part of the country. Since, AMF affect not only a single plant species but also populations and plant species composition, these inoculants could be applied to other crop plants.

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