American Journal of BioScience
2023; 11(1): 1-10
http://www.sciencepublishinggroup.com/j/ajbio
doi: 10.11648/j.ajbio.20231101.11
ISSN: 2330-0159 (Print); ISSN: 2330-0167 (Online)

Morphometric Characterization of Endomycorrhizal Fungi (Glomeraceae and Acaulosporaceae) from the Bouaflé and Niellé Areas in Côte d'Ivoire

Germain Droh[1, *], Kouadio Meliton Djezou[1], Seydou Tuo[1], Mamadou Touré[2], Abou-Bakari Kouassi[1]

[1]Laboratory of Biotechnology, Agriculture and Valorization of Biological Resources, Faculty of Biosciences, University Félix HOUPHOUËT-BOIGNY, Abidjan, Côte d'Ivoire

[2]Laboratory of Ecology and Sustainable Development, University of NANGUI ABROGOUA, Abidjan, Côte d'Ivoire

Email address:

drohge7@yahoo.fr (Germain Droh), djezou_k@yahoo.com (Kouadio Meliton Djezou), tuoseydou4@yahoo.fr (Seydou Tuo), tourexham@yahoo.fr (Mamadou Touré), abou_kouassi@yahoo.fr (Abou-Bakari Kouassi)

[*]Corresponding author

To cite this article:

Germain Droh, Kouadio Meliton Djezou, Seydou Tuo, Mamadou Touré, Abou-Bakari Kouassi. Morphometric Characterization of Endomycorrhizal Fungi (Glomeraceae and Acaulosporaceae) from the Bouaflé and Niellé Areas in Côte d'Ivoire. *American Journal of BioScience*. Vol. 11, No. 1, 2023, pp. 1-10. doi: 10.11648/j.ajbio.20231101.11

Received: October 31, 2022; **Accepted**: December 7, 2022; **Published**: January 10, 2023

Abstract: Mycorrhizal symbioses, which are widespread in various terrestrial ecosystems, constitute a very important research topic for many biologists. Arbuscular mycorrhizal fungi (AMF), belonging to the phylum Glomeromycota, take their name from their characteristic structures: arbuscules. Spores represent the main structures allowing the morphological identification and characterization of AMF. The spores directly extracted from soil samples were identified under an optical microscope. The diversity of AMF in maize rhizospheres in Bouaflé and Niellé areas was highlighted. Thus, 12 genera of AMF divided into 8 families have been identified. Spores of two families of endomycorrhizae (Glomeraceae and Acaulosporaceae) have been described. The spores of the Glomeraceae family represented by 4 genera *Glomus*, *Funneliformis*, *Septoglomus* and *Rhizophagus* are globose to subglobose, 45 to 200 µm in size. The spores have a single wall composed of 3 to 4 parietal layers. The outer layers stain with Melzer's reagent. The suspensory hypha often characterized by the presence of constriction, light brown in color is the extension of layer 2. Morphologically, the spores of Acaulosporaceae represented by a single genus *Acaulospora*, are distinguished by the globose to subglobose shape. The spores are whitish, pale yellow, golden yellow to orange-brown in color and 90 to 200µm in diameter. The subcellular structure of spores consists of a spore wall (outer wall) and two inner walls. The outer and inner walls of the spores consist of three parietal layers. The outermost layers are evanescent, hyaline whitish, orange-brown to pale yellow. The layers of the inner wall are adherent to each other appearing as a single layer. The surface of the spores, populated with projections of different shapes resembling often rudimentary hyphae, show scars and ornamentations. This study gives the essential of the status of AMF and revealed the morphometric and structural characteristics of some AMF.

Keywords: Arbuscular Mycorrhizal Fungi, Glomeraceae, Spore Diversity, Spore Density, Acaulosporaceae

1. Introduction

Mycorrhizal symbioses, which are widespread in various terrestrial ecosystems, constitute a very interesting research subject for many biologists. Mycorrhizal interaction is a long-lasting association between species of mycorrhizal fungi and plant roots [1], characterized by reciprocal nutritional exchanges [2, 3]. The symbiotic structure in this interaction, commonly called mycorrhiza, mixed structure is the place of exchange between the two partners [4]. Mycorrhizal symbiosis is an important innovation in the evolution and adaptation of plants to their terrestrial living environment. It has allowed plants to better adapt to their environment, especially in ecosystems with a water and/or mineral deficit [5]. Mycorrhizal symbiosis exists in several forms such as arbuscular mycorrhizae, ectomycorrhizae,

orchid mycorrhizae and heath mycorrhizae. In nature, arbuscular mycorrhizae are the most widespread symbionts [6]. Arbuscular mycorrhizal fungi (AMF), belonging to the phylum of Glomeromycota, take their name from their characteristic structures, the arbuscules [7]. They are the most common mycorrhizal symbionts and form associations with over 80% of both temperate and tropical terrestrial vascular plants [8]. AMF appeared probably millions of years ago [9, 10]. These micro-organisms are obligate symbionts which, from an anchorage point in the root, develop hyphae allowing them to exploit the soil, profile and infect other roots. They form asexual spores individually or in clusters from the extra-root hyphae in the soil to ensure their progeny [11, 12]. These spores are of different shapes (glomoid or acaulosporoid) and also have walls whose thickness and color vary depending on the species. The size of AMF can vary from a few tens of micrometers to half a millimeter. According to taxonomists and biologists, spores represent the main structures allowing the morphological identification and characterization of AMF [13]. Also, the morphological diversity and density of AMF spores are strongly influenced by various factors such as the age of the spore, the physicochemical characteristics of the soil, pedo-climatic variables, the presence of bacteria and human activities [14, 15].

The study of AMF systematics has developed based mainly on the morphology of spores [14, 15]. The morphological characterization of mycorrhizal fungal spores is an important means for the description of spores. It's based on the description of spores by observing the color, size; parietal structures that are the number of cell wall layers, the thickness of the cell wall and the presence of characteristic structures specific to each taxon, germination shield, hypha or suspensory bulb and sporogenous cells [16-18].

AMF currently represent 5 orders and 14 families within the Glomeromycota phylum [19]. For example, gigasporoid AMF have been rearranged into multiple families and genera due to differences in spore wall structure, germination shield shape and pigmentation, and germ lobe structures [20, 21]. Acaulosporoid and entrophosporoid AMF have also been rearranged recently for differences in spore formation and morphology [22]. Finally, the glomoid AMF, which are more widespread in nature and group together the largest number of species within the Glomeromycota phylum, have been attributed to distinct phylogenetic clades for which spores can be produced alone or in clusters in soil. [23]. Glomoid AMF represents the morphologically most important species. They show a very great difference in the characteristic structures of the spore wall, the pigmentation, the structure of the wall and the type of closure of the hyphae at the base of the spores [23, 24].

However, due to the differences, the morphological characterization of AMF is necessary to highlight all the diversity at the level of arbuscular mycorrhizal fungi. In previous works, the diversity of AMF according to morpho-metric characteristics is highlighted. [13, 18, 19, 25-27].

As part of a complex research project on AMF diversity, fungal spores were extracted from soils directly from the sampling sites. The objective is to describe the arbuscular mycorrhizal fungi belonging to the Glomeraceae and Acaulosporaceae families, isolated from maize rhizospheres on the basis of morphometric and structural analyzes of the spores.

2. Material and Methods

2.1. Soil Sampling Areas

Soil samples were collected from the areas of Bouaflé (Marahoué) and Niellé (Tchologo) in Côte d'Ivoire chosen according to a pedo-climatic gradient (Table 1). In each of the zones, soil samples were collected from maize rhizosphere of five randomly chosen localities (Figure 1). The physicochemical characteristics of the soil samples from each of the areas determined in previous work are presented in Table 2. The soil samples were taken from a depth of 0-20 cm in the stratum using an auger. Three soil samples were collected at the foot of different maize plants chosen at random. These three samples were then homogenized to form a composite sample. 500 g of soil were sampled, packed in plastic bags and then transported to Abidjan and kept at the Unit of Pedagogy and Research (UPR) in Genetics of University Félix HOUPHOUËT-BOIGNY (UFHB).

Table 1. *Characteristics of the study areas: Bouaflé and Niellé.*

	Bouaflé (Marahoué)	**Niellé (Tchologo)**
Climate	Tropical climate of the Baoulé type characterized by four seasons (2 rainy seasons and 2 dry seasons) and 1300 mm of rain on average per year	Tropical climate of the Sudano-Guinean type characterized by two seasons (1 rainy season and 1 dry season) and an annual rainfall varying between 1000 and 1200 mm
Vegetation	Mesophilic forest of the Guinean domain, which is considered to be the fundamental type of semi-deciduous forest. There is a mosaic of forests and savannah	Sub-Sudanese sector of the Sudanese domain. The vegetation is characterized by dry open forests and wooded, treed and shrubby savannas.
Relief	Plateaus and plains with an average altitude of 250 meters. However there are some elevations including Mount Lotanzia (652 m) of the Baoulé mountain range	Plains and plateaus (Mount Gnangbabka) with altitudes varying from 200 to 500 m, and with a mountain range (Mount Ouamelhoro)
Soil	Moderately desaturated ferralitic clay-sandy dominance characterized by a shallow humus horizon rich in organic matter, weakly acidic and well structured	The soil in this area is predominantly clay and sand.
Hydrography	the Bandama River and many tributaries which dry up in the dry season	the Bandama River and tributaries
Temperature and humidity	The average annual temperature is 26°C. Relative humidity, it averages around 75%	The average annual temperature is 26.4°C (24.7°C - 29.5°C). The monthly average relative humidity varies between 35 and 79%. Insolation values range up to 273.8 hours (month of January).

Source: [29]

Table 2. *Physical and chemical characteristics of sampled area soils.*

Sampled Area	pH	C	N	C/N	P	CEC	Ca^{2+}	Mg^{2+}	K^{+}	Na^{+}
BOUAFLE	6.38±0,3	1,01±0,4	0,10±0,0	10,40±0,8	60,17±16,2	7,20±3,4	1,27±0,5	0,73±0,1	0,10±0,01	0,11±0,08
NIELLE	6,35±0,2	1,60±0,7	0,14±0,0	10,92±0,9	117,30±50,4	12,18±4,7	3,11±1,9	0,63±0,1	0,11±0,01	0,15±0,1

NB: pH = hydrogen potential, C = organic carbon, N = total nitrogen, C/N = "organic carbon to total nitrogen" ratio, P = available phosphorus, CEC = cation exchange capacity, Ca^{2+} = calcium ion, Mg^{2+} = magnesium ion, K^{+} = potassium ion, Na^{+} = sodium ion.
Source: [26]

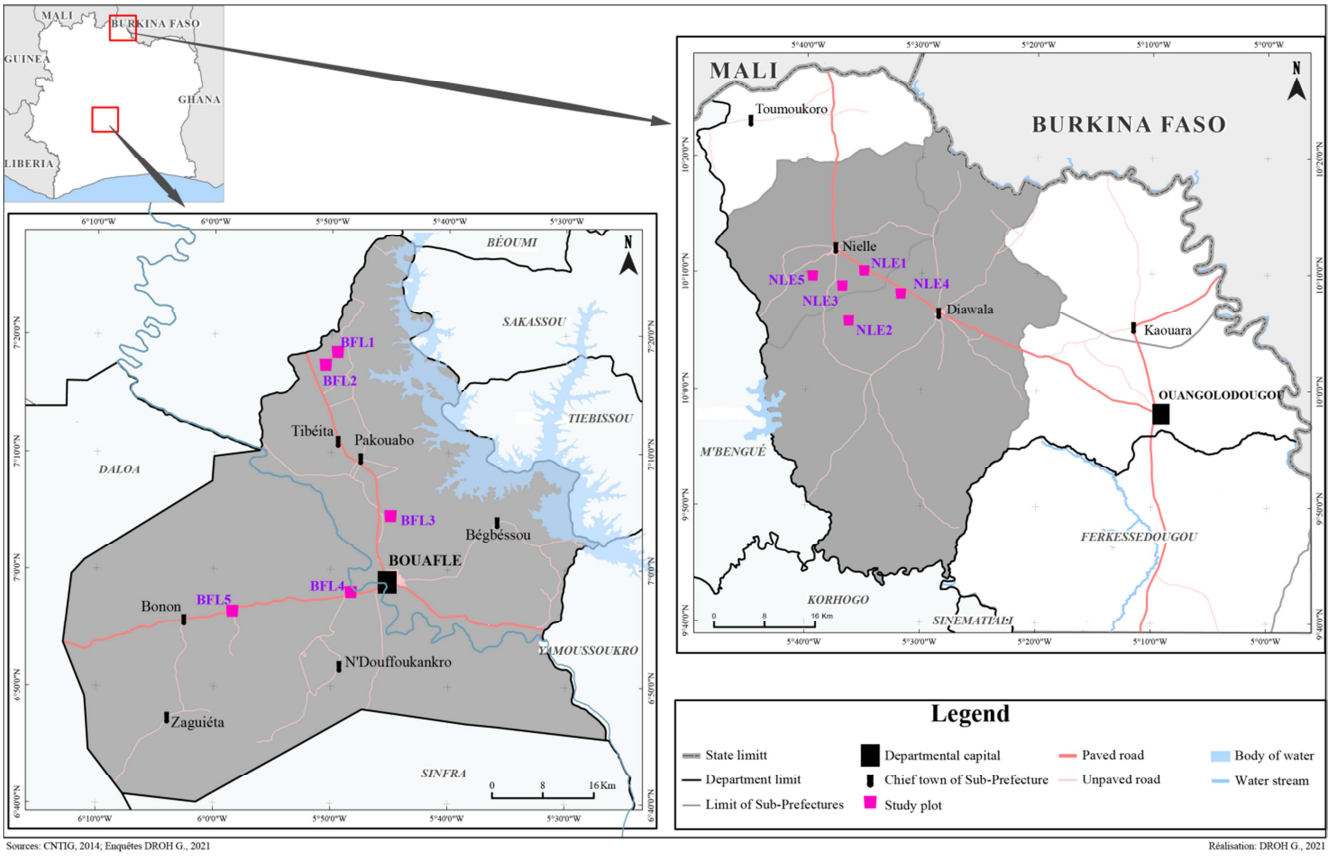

Figure 1. *Location of soil sample collection areas in Ivory Coast.*

2.2. Extraction and Enumeration of AMF Spores

The spores were extracted using the wet sieving method [28]. In a 1 Litre beaker, a 100 g soil sample was suspended to separate fungal propagules from soil particles. After 10 to 30 seconds of settling, the suspension was returned to a series of four sieves superimposed in descending order of mesh diameters 500, 200, 90 and 45 µm. This operation was repeated three times.

The sieve with a mesh diameter of 500 µm was used to eliminate the waste that is the root fragments as well as the large particles of sand. The selected contents of the sieves with a diameter of 200, 90 and 45 µm mesh were distributed separately in centrifugation tubes containing a 50% sucrose solution. The mixture was centrifuged at 2000 rpm for 10 min. The supernatants of the three tubes were collected separately in the 200, 90 and 45 µm sieves. The spores contained in each sieve were rinsed with tap water to remove the sucrose and collected in a Petri dish 9.5 cm in diameter, the bottom of which is lined with squared filter paper and moistened with NaCl. AMF spore density is estimated for each area in spores per gram of soil.

2.3. Identification and Morphological Analysis of AMF Spores

AMF spores extracted from each soil sample were mounted on a microscope slide in two permanent mounting media: 1) polyvinyl glycerol (PVLG) without prior staining and 2) PVLG stained with Melzer's reagent. The spores were then observed under a Leica version 3.4.0 microscope (Gx40). Identification and analysis of AMF spores were performed based on the characteristics of general appearance (size and color), parietal structures (number of walls, number of parietal layers, cell wall thickness) and the characteristic structures specific to each genus (germination shield, sporiferous saccule, suspensory bulb (hypha), sporogenous cell). The spores observed were identified, described and analyzed using the recommended manual, the *International Culture Collection of Vesicular – Arbuscular Mycorrhizal Fungi* [30].

2.4. Species Richness and Diversity Indices

Biological diversity indices, classic indicators in ecology, were calculated on the basis of the numbers of AMF spores obtained according to the morphometric and structural characteristics. It is:

1) Specific richness (*N*): refers to the total number of AMF species observed in a soil sample.
2) The Shannon index: allows diversity to be expressed by taking into account the total number of species and the abundance of individuals within a species.

$$H'H' = -\sum_{i=1}^{s} pi.\ln(pi)$$

$p_i = \frac{ni}{N}$, where ni is the number of individuals of species i, N is the total number of individuals.

3) Simpson's index is used to calculate the probability that two randomly selected individuals in a given environment are of the same species.

$$D = 1 - \sum_{S}^{1} \frac{ni(ni-1)}{N(N-1)}$$

n_i: Number of individuals of a given AMF species.
N: Total number of individuals.
S: Total number of AMF species.

4) The Piélou index, also called the equal-distribution index, represents the ratio of H' to the theoretical maximum index in the stand (Hmax).

$$R = \frac{H\prime}{H\prime max} = \frac{H\prime}{log2\ (S)}$$

These indices were calculated in order to understand the structuring of AMF communities observed in each of the areas.

2.5. Statistical Analysis

Statistical analyzes were performed with RStudio software (2022.07.1+554 "Spotted Wakerobin" Release for Windows). The density and diversity data were subjected to an analysis of variance with one classification criterion (sample area). These analyzes made it possible to highlight the existence or not of significant differences between the study areas. The Student-Newmann-Keuls and Turkey tests at the 5% threshold were used to compare the values of the diversity data.

3. Results

3.1. AMF Spore Density

The results of the spore observations after wet sieving show that the two study sites contain spores in the soils taken directly from the maize fields. The average densities obtained by locality are 2.64±0.31 spores/g of soil for the locality of Bouaflé and 1.96±0.27 spores/g of soil for Niellé (Table 3). The analysis of variance reveals that these densities of the two localities differ significantly at the alpha risk of 5% with p-value=0.0201. Mycorrhizal spores appear to be heterogeneously distributed throughout the study area.

Furthermore, positive correlations were observed between spore densities and carbon, nitrogen and magnesium contents. On the other hand, spore densities were negatively correlated with phosphorus, calcium, potassium and sodium contents. A negative and significant correlation was observed between spore densities and pH (Table 4).

Table 3. *Comparison of CMA spore densities of sampling areas.*

Sampling areas	Spore density (spores per gram of soil)	Fisher test	
		F	p-value
BOUAFLE	2.64 ± 0.31a	2.811093	0.0201
NIELLE	1.96 ± 0.27 ab		

Spore density values followed by the same letter are not significantly different at 5% level

Table 4. *Correlation between AMF spore density and soil chemical factors.*

	pH	C	N	C/N	P	CEC	Ca^{2+}	Mg^{2+}	K^{+}	Na^{+}
Spore density	-0,3**	$0,1^{ns}$	$0,1^{ns}$	$0,2^{ns}$	$-0,1^{ns}$	$-0,05^{ns}$	$-0,1^{ns}$	$0,09^{ns}$	$-0,1^{ns}$	$-0,2^{ns}$

ns: not significant; **: significant correlation at 5% threshold

3.2. AMF Spore Diversity

The assessment of diversity takes into account specific richness, which is the total number of species observed and diversity indices. The identification under a microscope made it possible to highlight 10 genera of AMF in the soil samples of Niellé and 8 genera of AMF in those of Bouaflé (Figure 2).

Most genera were found in all collection areas. The analysis of variances of the diversity indices observed in the soil samples of the zones showed no significant difference. Similarly, the analysis of species richness showed no significant difference between the two sampling localities (p-value>0.05). Table 4 indicates that the soil of the rhizospheres of Niellé presents the greatest diversity with an average index of Shannon $H'_{Niellé}$=1.47±0.13 while the samples of soils of Bouaflé only present 1.21±0.09. The gender distribution is less equitable in the soil samples from the two study localities with Piélou indices (r) of 0.30±0.02 and 0.36±0.03 respectively for Niellé and Bouaflé.

However, the Simpson indices (D) have values very far from 1 (D is between 0.28±0.04 and 0.35±0.01). These values indicate that fungal diversity is very low in the rhizosphere of the study areas (Table 5).

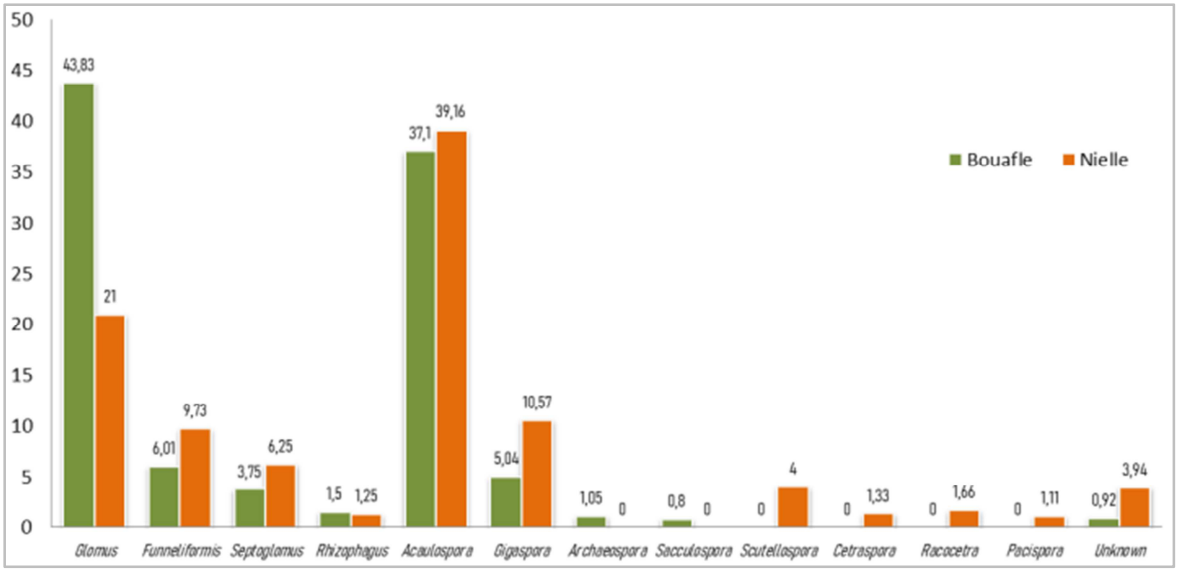

Figure 2. *Frequency of species observed from the two sampling areas.*

Table 5. *CMA diversity indices of sampling areas.*

Sampling area	Shannon index (H')	Simpson's Index (D)	Pielou index (R)	Species richness
BOUAFLE	1,21±0,09	0,34±0,02	0,30±0,02	4,6±0,4
NIELLE	1,47±0,13	0,28±0,04	0,36±0,03	5,6±0,6
p-value	0,45297^{ns}	0,18859^{ns}	0,45297^{ns}	0,5322ns

ns = non-significant difference; the analyzes are carried out at the risk α =5%.

The abundance of AMF seems to be influenced by the physicochemical characteristics of the sampling areas. Positive correlations were observed between the abundance of *Glomus sp* and pH as well as magnesium and potassium contents. The abundances of *Funneliformis* sp and *Pacispora* sp were positively correlated with pH and physicochemical characteristics and negatively with magnesium and sodium contents. The abundances of *Septoglomus* sp, *Acaulospora* sp and *Gigaspora* sp appeared to be negatively correlated with pH and potassium content.

On the other hand, the abundances of *Scutellospora* sp and *Cetraspora* sp were negatively correlated with pH and carbon and magnesium contents. The abundance of *Rhizophagus* sp is positively correlated with pH, potassium and sodium contents and negatively with other contents (Table 6).

3.3. Morphometric and Structural Study of AMF

AMF spores identified have different characteristics. Within the same family or the same genus, the species observed differ for several parameters.

3.3.1. Glomeraceae

(i). Glomus

The AMF spores belonging to the genus *Glomus* identified differ according to the sampling areas. In Bouaflé area, the spores observed are globose, sometimes ovoid, orange-yellow, orange-brown, light brown to dark brown and ranging in size from 45 to 200 µm.

Table 6. *Correlation between AMF species abundance and soil chemical characteristics.*

AMF species	pH	C	N	C/N	P	CEC	Ca^{2+}	Mg^{2+}	K^{+}	Na^{+}
Glomus sp	0,8	-0,4	-0,9	0,6	-0,4	-0,4	-0,4	0,6	0,6	-0,6
Funneliformis sp	0,4	0,0	0,3	0,2	0,8	0,8	0,8	0,2	-0,2	-0,2
Septoglomus sp	-0,4	0,8	0,9	0,0	0,8	0,8	0,8	0,0	-0,9	0,0
Rhizophagus sp	0,5	-1,0	-0,5	-0,5	-0,5	-0,5	-0,5	-0,5	0,9	0,5
Acaulospora sp	-0,8	0,4	0,3	-0,4	-0,4	-0,4	-0,4	-0,4	-0,3	0,4
Gigaspora sp	-0,4	0,8	0,9	0,0	0,8	0,8	0,8	0,0	-0,9	0,0
Scutellospora sp	-1,0	-1,0	0,0	-1,0	-0,5	-0,5	-0,5	-1,0	0,9	1,0
Cetraspora sp	-0,5	-0,5	0,9	-0,5	0,5	0,5	0,5	-0,5	0,0	0,5
Pacispora sp	1,0	1,0	0,0	1,0	0,5	0,5	0,5	1,0	-0,9	-1,0

They are present in the soil in a solitary state or in aggregates and develop at the end of the hyphae. The observation of spores stained with Melzer shows that they have a single spore wall composed of two, three or four different parietal layers. These observations showed identified spores are mature.

Spore wall layer 1 (C1PS) forming the spore surface is mucilaginous, evanescent hyaline. It reacts best to Melzer's reagent staining brown, yellowish brown, pale yellow and red. Layer 1 is sometimes difficult to observe because it deteriorates, with the age of the spores, the washing of the spores during the extraction or because of bacterial activities.

Layer 2 of the spore wall (C2PS), light brown to dark brown in color, smooth and permanent, is thicker than layer 1. It is stratified with two to three layers, consisting of very thin lamellae.

Layer 3 of the spore wall (C3PS), flexible, thin or thick, is colored brown or purplish red in Melzer's reagent. It is attached to layers 1 and 2 and has laminations.

Layer 4 of the spore wall (C4PS), pale yellow to golden yellow, is composed of several laminated sublayers. This formation is observed in mature spores and is absent in young spores.

The spore suspensory hypha is uniquely pale yellow to golden yellow in color, straight or curved in a funnel shape and slightly constricted at the base of the spores. The suspensory hypha is sometimes characterized by the presence of constriction at the point of attachment of the spore. The layers of the underlying hyphal wall are a continuation of layers 1 and 3 or 1 and 2 of the spore wall depending on the species (Figure 3).

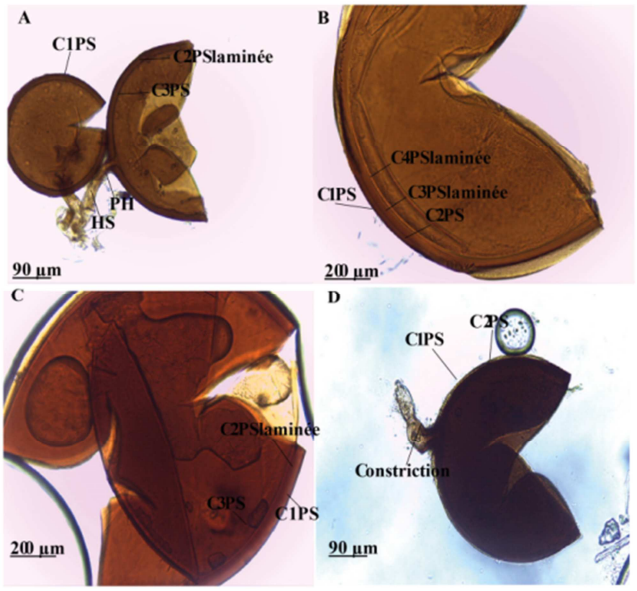

Figure 3. *AMF spores of the genus Glomus observed under an optical microscope (G40x) in PVLG.*

Glomus sp1 (A); *Glomus* sp2 (B); *Glomus* sp3 (C); *Glomus* sp4 (D), (C1PS: layer1 of the wall; C2PS: layer2 of the wall; C3PS: layer3 of the wall; C4PS: layer4 of the wall; HS: suspensory hypha; PH: hyphal wall).

(ii). Septoglomus

AMF spores of the genus *Septoglomus* observed are globose brown to dark brown in color and vary in size from 45 to 90 µm in diameter. They are present in aggregates or alone on hyphae. Observation of spores stained with Melzer's reagent revealed that they have a single wall composed of three different parietal layers.

Layer 1 of the spore wall (C1PS) is mucilaginous, hyaline, thin lighter golden yellow in color. This layer usually deteriorates as the spore's age or because of bacterial activities. Layer 2 of the spore wall (C2PS), thicker than layer 1, is brown to dark brown in color. Layer 3 of the spore wall (C3PS), is membrane, very thin and difficult to observe. The spore wall is made up of microcavities due to bacterial activity.

The suspensory hypha is a single hyaline to golden yellow constricting in the form of a funnel at the base of the spores. The hyphal wall is formed by the extension of layers 1 and 2. The base of the hypha is closed by a septum formed by the spore wall 3 (Figure 4).

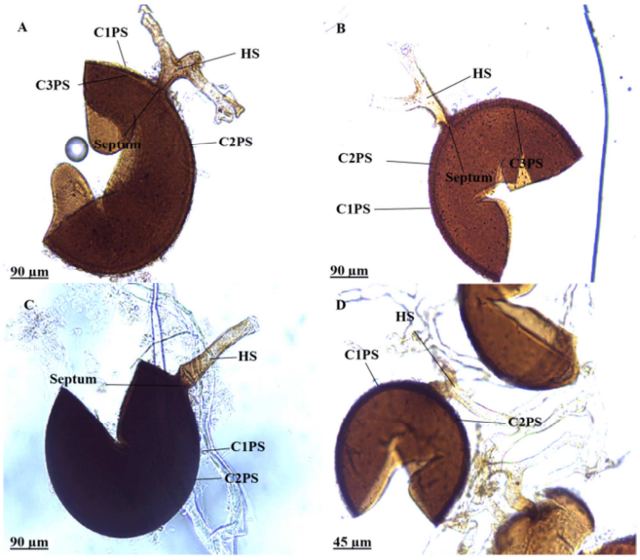

Figure 4. *AMF spores of the genus Septoglomus observed under an optical microscope (G40x) in PVLG.*

Septoglomus sp1 (A), *Septoglomus* sp2 (B), *Septoglomus* sp3 (C), *Septoglomus* sp4 (D). (C1PS: wall layer1; C2PS: wall layer2; C3PS: wall layer3; HS: suspensory hypha)

(iii). Funneliformis

AMF belonging to the genus *Funneliformis* produce glomoid, individual spores of yellow, brown to dark brown color and size 90 µm in diameter. Some AMF spores are orange-brown in color. The surface of the spores exhibit irregular shapes of the ornamentations of nuclei. The spores observed develop at the end of the hyphae. They have a single wall composed of two to three different parietal layers. The colorless layer 1 of the spore wall (C1PS) disappears with the age of the spores. Layer 1 of some spores is hyaline to sub hyaline, evanescent to semi-persistent.

Layer 2 of the spore wall (C2PS), thicker than layer 1, is yellow, light brown to dark brown. On the other hand, other AMF spores are sub hyaline.

Layer 3 of the spore wall (C3PS) is membrane and very difficult to observe. Some stumps orange-brown to dark orange-brown in color, thinly stratified and crowded with concave circular to ovoid pits (Figure 5).

The funnel-shaped spore suspensory hypha is highly pigmented; most pronounced from the base of the spore and gradually becomes hyaline to golden yellow. The spores are closed by a strong and straight septum at their base at the level of the suspensory hypha (Figure 5).

3.3.2. Acaulosporaceae

Acaulospora

Spores belonging to the genus *Acaulospora* are formed in isolation in the soil. The spores observed are globose to sub globose, whitish, pale yellow, golden yellow to orange-brown and 90 to 200 µm in diameter. The subcellular structure of the spores consists of a spore wall (outer wall) and two inner walls. The outer spore wall consists of three different parietal layers. Layer 1 of the spore wall (C1PS), evanescent, hyaline, whitish to pale yellow, is thin. Layer 2 of the spore wall (C2PS), thicker than layer 1, is pale yellow, golden yellow to orange-brown. This layer has laminations, the number of which varies with age. Layer 3 of the spore wall (C3PS), membrane, orange-brown to pale yellow is generally difficult to observe. This layer is inflexible when it separates from layer 2 of the spore wall.

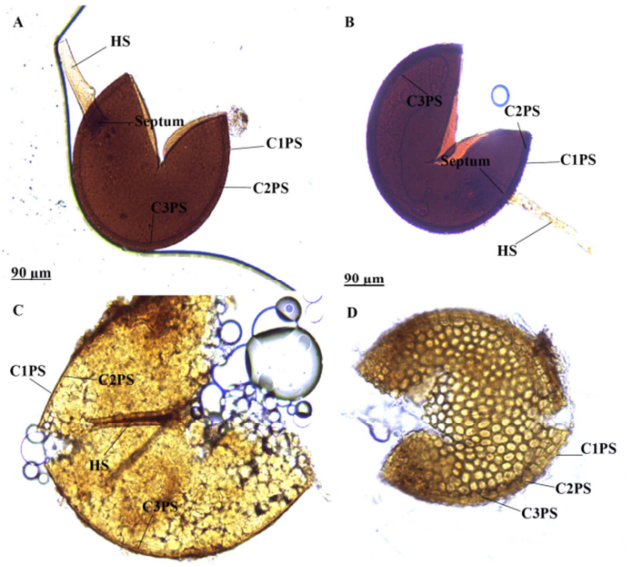

Figure 5. *AMF spores of the genus Funneliformis observed under an optical microscope (G40x) in PVLG.*

Funneliformis sp1 (A), *Funneliformis* sp2 (B), *Funneliformis* sp3 (C), *Funneliformis* sp4 (D). (C1PS: wall layer1; C2PS: wall layer2; C3PS: wall layer3; HS: suspensory hypha, PS: spore wall).

Spore wall and the inner walls are separated by a middle wall. This median wall is hyaline, orange-brown to pale yellow.

The inner wall 1 (Pi1) of the spores, hyaline, orange-brown to pale yellow, consists of two to three different layers (C1Pi1, C2Pi1, C3Pi1) which separate easily from the spore wall. These layers separate slightly in some AMF spores of the genus *Acaulospora*, but they may adhere to each other and appear as a single layer that is difficult to observe.

The inner wall 2 (Pi2) of the hyaline, orange-brown to pale yellow spores is flexible and consists of two to three different layers (C1Pi2, C2Pi2, C3Pi2).

The surface of the spores observed is densely populated with projections of various shapes resembling often rudimentary hyphae. AMF spores of the genus *Acaulospora* are characterized by the presence of scars and ornamentations (Figure 6).

4. Discussion

Soils are complex and particular environments composed of a great diversity of microorganisms.

Analysis of soil samples from maize plantations was carried out with the aim of understanding the distribution and density of arbuscular mycorrhizal (AMF) fungi as well as the relationship between soil physicochemical parameters, diversity and abundance of AMF. Isolation of AMF spores showed variability in density between sampling areas. The average density of spores observed in the soils of the sampling areas (2.64±0.31 spores per gram of soil in Bouaflé and 1.96±0.27 spores per gram of soil in Niellé) can be explained both by the characteristics of the litter, in particular the biomass and the physicochemical characteristics of the soils considered. The physicochemical properties of the soil are known to affect AMF population and therefore the spore density [19]. In the present study, the soils were slightly acidic (pH = 6.3), which could be a factor influencing the density of AMF spores. Some authors have reported that soil pH can influence the proliferation of AMF spores in the rhizosphere of crop areas, and that acidic or slightly acidic soils harbor a low number of AMF fungal propagules [31]. This influence of pH on spore density has also been observed in ginger rhizospheres from northern India [32].

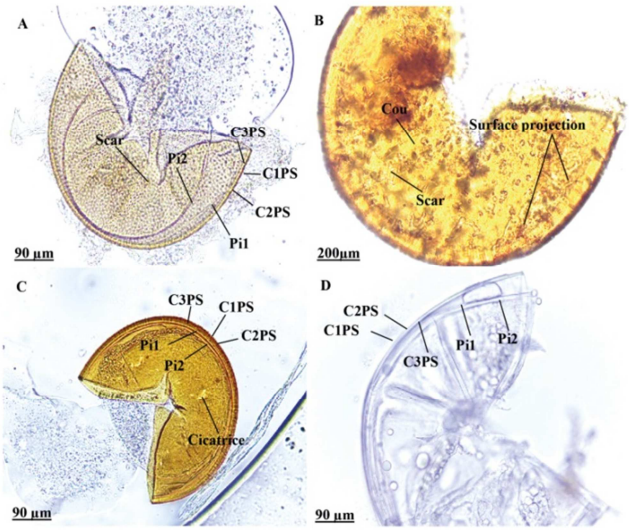

Figure 6. *AMF spores of the genus Acaulospora observed under an optical microscope (G40x) in PVLG.*

Acaulospora sp1 (A), Acaulospora sp2 (B), Acaulospora sp3 (C), Acaulospora sp4 (D). (C1PS: layer1 of the wall; C2PS: layer2 of the wall, C3PS: layer3 of the wall, Pi1: inner wall 1, Pi2: inner wall 2).

Mineral element contents and climatic conditions appeared as factors influencing the density of AMF spores [33, 34]. AMF spore density was significantly and negatively correlated with the pH of sampled soils. Negative correlations were demonstrated between the density of AMF spores and the capacity for exchangeable cations as well as the contents of phosphorus, sodium, calcium and potassium. This trend has also been reported by the work of Bossou in Benin [35]. The negative correlation could be explained by the fact that during the establishment of the mycorrhizal symbiosis with the roots of the plants, several AMFs are inhibited when the mineral contents of the soil are high, the latter attach themselves to the mycelium of the AMFs, and they only reach the root of the plant in small quantities [36].

Moreover, positive correlations were observed between the density of AMF spores and the contents of carbon, magnesium and nitrogen.

The low densities of AMF spores recorded in the soils of Ferké and Niellé can be explained by the presence of saprophytic organisms such as endophytic fungi of the genus *Neotyphodium*. These fungi parasitize the spores of AMF [37, 38].

The satisfactory sampling effort revealed on the basis of the morphometric and structural characteristics of the spores seventeen genera of AMF. This specific richness is much higher than that observed without the work of certain Ivorian authors. These authors have revealed over the past five years, five genera of AMF in the rhizospheres of maize (*Zea mays*) and groundnut (*Arachis hypogaea*) [39], 7 genera in cocoa rhizospheres (*Theobroma cacao*) [40, 41], eleven genera in the litter of the forest-savanna area [34] and 17 genera in the maize rhizosphere (*Zea mays*) [26]. It is also superior to that obtained by the work of Bossou [35] who revealed 4 genera of CMA in the rhizosphere of maize (*Zea mays*) in Benin and by sesame [42] whose work showed six genera of AMF in the rhizospheres of the olive tree (*Olea europaea*) in Morocco. However, Bouaflé area has the lowest specific richness. This diversity of AMF genera could be explained by the fact that soil sample collection areas were expanded. On this basis, the diversity indices are acceptable regardless of the area chosen. Thus, an average Shannon index of 1.47 is observed in Niellé, 1.21 in Bouaflé. These values indicate that AMF communities are more or less diversified depending on the study areas. In addition, the lowest values of the index show that there was not much variation in an area and this is in agreement with the results of the work of several authors [43-45].

Values of the Simpson index very far from 1 (D between 0.28±0.04 and 0.35±0.01) particularly in the areas of Niellé in addition to the specific richness, confirms that fungal diversity is low in maize rhizospheres in the areas studied.

Like the density of spores, the abundance of AMF species appeared to be impacted by the physicochemical characteristics of the soil. Positive and negative correlations were observed between the physicochemical characteristics of the soil and the AMF. This observation is highlighted by the work of other authors [33, 34, 46].

This work led to the identification of two major families, the Glomeraceae represented by the genera *Glomus*, *Septoglomus*, *Funneliformis* and the Acaulosporaceae represented by a single genus *Acaulospora*. However, spores of both families are easily distinguished from spores of all other known Glomeromycota families due to the type of spore formation, spore color, and spore wall structure. The genus *Glomus* comprises orange-yellow, orange-brown, light brown to dark brown spores ranging in size from 45 to 200 µm in diameter, underlying hyphae curved in the form of a funnel and constricted at the base of the spores and a single wall composed of three to four parietal layers [47]. The genus *Septoglomus* comprises brown to dark brown globose spores ranging in size from 45 to 90 µm in diameter, single hyaline to golden-yellow suspensory hyphae constricting in a funnel shape at the base of the spores, and a single wall composed of three different parietal layers [13]. The genus *Funneliformis* comprises spores which can be easily distinguished from all other species of the Glomeraceae by the combination of color, size of the spore structure and hyphae. The spores are yellow, brown, and orange-brown to dark brown in color, 90 µm in diameter. They also show more pronounced pigmented funnel-shaped underlying hyphae, a curved septum at the base, and irregular, more evenly distributed pit ornamentation. The spores have a single wall composed of three different parietal layers [27].

The genus *Acaulospora* consists of whitish, pale yellow, golden yellow to orange-brown spores, 90 to 200 µm in diameter. The surface of the spores is characterized by the presence of an ornamental scar and also populated by projections. AMF spores of the genus *Acaulospora* have two walls; an outer wall and an inner wall each consisting of three layers [48, 49].

It is appropriate to remember that there is a relationship between the physicochemical characteristics of the soil and the arbuscular mycorrhizal fungi (AMF).

5. Conclusion

This study is initiated in order to evaluate the diversity of arbuscular mycorrhizal fungi (AMF) in maize rhizospheres and to highlight the relationship between the physicochemical characteristics of the soil and the AMF. Considering the fact that the soils were sampled following the pedo-climatic gradient, one could more logically expect a diversity of AMF communities. However, the density of AMF spores differed between sampling areas. The highest average density estimated at 2.64±0.31 spores per gram of soil was recorded in the Bouaflé sampling areas. Positive and negative correlations were observed between the physicochemical characteristics of the soil and the total density of AMF spores. These results would make it possible to speculate on the existence of the influence of the physicochemical characteristics of the soil on the density of AMF spores. Morphological studies of AMF spores have revealed 12 genera of AMF. The Niellé area records the highest specific richness. The physicochemical parameters of the soil also influenced the diversity of AMF genera. There is a strong relationship between the

physicochemical parameters of the soil and the diversity of AMF genera. The Shannon, Simpson and Pielou diversity indices showed no significant difference and varied little according to the sampling areas. This analysis reveals a low fungal diversity in the maize rhizospheres of the areas studied.

This work has improved knowledge on the morphometric and structural characteristics of AMF of the Glomeraceae and Acaulosporaceae families.

Other studies, integrating soil bacterial communities, litter quality and known to influence AMF, should be conducted to strengthen the explanation of the distribution of AMF in the different rhizospheres of Côte d'Ivoire.

Acknowledgements

This work was undertaken within the framework of the project called “Promotion of biocompost associated with arbuscular mycorrhizal fungi (AMF) in the production of maize (*Zea mays*) in Côte d'Ivoire”. This project was funding by the Competitive Fund for Sustainable Agricultural Innovation (FCIAD) under the reference of contract N° 18 62 /FIRCA/INADES/FDCI-FCIAD/2018.

The authors would like to thank INADES, Womblegnon Jean-Marc Stéphane, Drissa, N'guessan, and Silué for their technical assistance throughout several stages of the research.

References

[1] De la Fuente Cantó C., Simonin M., King E., et al. (2020). An extended root phenotype: the rhizosphere, its formation and impacts on plant fitness. *Plant J*. 103 (3): 951-964. doi: 10.1111/tpj.14781.

[2] Sanders I. R., Rodriguez A.. (2016); Aligning molecular studies of mycorrhizal fungal diversity with ecologically important levels of diversity in ecosystems. *ISME J*. 10 (12): 2780-2786. doi: 10.1038/ismej.2016.73.

[3] Tedersoo L., Bahram M., Zobel M. (2020); How mycorrhizal associations drive plant population and community biology. *Science*. 367 (6480). doi: 10.1126/science.aba1223.

[4] Smith S. E., Read D. J. (2008). *Mycorrhizal Symbiosis*. Vol 137. Academic Press is an imprint of Elsevier; doi: 10.1097/00010694-198403000-00011.

[5] Gerz M., Guillermo Bueno C., Ozinga W. A., Zobel M., Moora M. (2018); Niche differentiation and expansion of plant species are associated with mycorrhizal symbiosis. *J Ecol*. 106 (1): 254-264. doi: 10.1111/1365-2745.12873.

[6] Brundrett M. C., Tedersoo L. (2018); Evolutionary history of mycorrhizal symbioses and global host plant diversity. *New Phytol*. 220 (4): 1108-1115. doi: 10.1111/nph.14976.

[7] Schüβler A., Schwarzott D., Walker C. (2001); A new fungal phylum, the Glomeromycota: phylogeny and evolution. *Mycol Res*. 105 (12): 1413-1421. doi: 10.1017/S0953756201005196.

[8] Fortin J. A., Becard G., Declerck S, et al. (2002); Arbuscular mycorrhiza on root-organ cultures. *Can J Bot*. 80 (1): 1-20..

[9] Redecker D., Morton J. B., Bruns T. D. (2000); Ancestral lineages of arbuscular mycorrhizal fungi (Glomales). *Mol Phylogenet Evol*. 14 (2): 276-284. doi: 10.1006/mpev.1999.0713.

[10] Delaux P. M. (2017); Comparative phylogenomics of symbiotic associations. *New Phytol*. 213 (1): 89-94. doi: 10.1111/nph.14161.

[11] Brito I., Goss M. J., de Carvalho M., Chatagnier O., van Tuinen D. (2012); Impact of tillage system on arbuscular mycorrhiza fungal communities in the soil under Mediterranean conditions. *Soil Tillage Res*. 121: 63-67. doi: 10.1016/j.still.2012.01.012.

[12] Krishna K. R. (2005). *Mycorrhizas : A Molecular Analysis*. Science Publishers, Inc.; doi: 10.1201/978148228028.

[13] Crossay T. (2018): Caractérisation taxonomique des champignons mycorhiziens à arbuscules natifs des sols ultramafiques de Nouvelle-Calédonie ; analyse de leur synergie permettant l’adaptation des plantes à ces milieux extrêmes. Published online 1-319.

[14] Strullu D. G., Plenchette C. (1991); Les mycorhizes en horticulture. *PHM Rev Hortic*. 352: 50-55.

[15] Ouallal I., Elyacoubi H., Atmane R. (2019). Diversite naturelle des champignons endomicorhiziens associes aux arganeraies et potentiel mycorhizogene de leur rhizosphere. (January).

[16] Dalpé Y. (1995): Systématique des endomycorhizes à arbuscules : de la mycopaléontologie à la biochimie. In: : Fortin, J. A. CC et YP, ed. *La Symbiose Mycorhizienne.* Éditions O.; 1-20.

[17] Sanders I. R. (2004); Intraspecific genetic variation in arbuscular mycorrhizal fungi and its consequences for molecular biology, ecology, and development of inoculum. *Can J Bot*. 82: 1057-1062.

[18] McNeill J. (2007). International Code of Botanical Nomenclature (Vienna Code). In: *The Seventeenth International Botanical Congress Vienna, Austria, July 2005*. Vol 568.; Accessed October 27, 2022. https://cir.nii.ac.jp/crid/1571698600852109440.

[19] Oehl F., Jansa J., Ineichen K. (2011); Champignons mycohiziens arbusculaires, bioindicateurs dans les sols agricoles suisses. ... *Agron suisse*. 2: 304-311. Accessed April 15, 2014. http://dialnet.unirioja.es/servlet/articulo?codigo=3725311&orden=363193&info=link

[20] Da Silva G. A., Maia L. C., Oehl F. (2012); Phylogenetic systematics of the Gigasporales. *Mycotaxon*. 122 (4): 207-220. doi: 10.5248/122.207.

[21] Pontes J. S. De, Sánchez-castro I., Alves G., Oehl F. (2013); Scutellospora alterata, a new gigasporalean species from the semi-arid Caatinga biome in Northeastern Brazil. *Mycotaxon*. 125 (3): 169-181.

[22] Willis A., Błaszkowski J., Prabhu T., et al. (2016); Sacculospora felinovii, a novel arbuscular mycorrhizal fungal species (Glomeromycota) from dunes on the west coast of India. *Mycol Prog 2016 157*. 15 (7): 791-798. doi: 10.1007/S11557-016-1208-6.

[23] Błaszkowski J., Chwat G. (2013); Septoglomus deserticola emended and new combinations in the emended definition of the family Diversisporaceae. *Acta Mycol*. 48 (1): 89-103. doi: 10.5586/am.2013.011.

[24] Sieverding E., Da Silva G. A., Berndt R., Oehl F. (2014); Rhizoglomus, a new genus of the Glomeraceae. *Mycotaxon*. 129 (2): 373-386. doi: 10.5248/129.373.

[25] Zeramdini N. (2009). Étude du polymorphisme intra- et inter-spécifique du gène β-tubuline chez des espèces de champignons mycorhiziens à arbuscules en vue de développer des marqueurs moléculaires. *Mémoire Master en Sci Biol*. Université de Montréal. Published online 2009."

[26] Droh G., Djezou K. M., Kouassi K. A. B., Kouassi A.-B., Tiecoura K. (2022); Diversity of Arbuscular Mycorrhizal Fungi Spores in Maize (Zea mays L.) Plantations in Côte d ' Ivoire. *Am J Agric For*. 10 (5): 170-180. doi: 10.11648/j.ajaf.20221005.14.

[27] Corazon-Guivin M. A., Cerna-Mendoza A., Guerrero-Abad J. C., et al. (2019); Nanoglomus plukenetiae, a new fungus from Peru, and a key to small-spored Glomeraceae species, including three new genera in the "Dominikia complex/clades." *Mycol Prog*. 18 (12): 1395-1409. doi: 10.1007/S11557-019-01522-1.

[28] MINADER. (2021). *Etude de Faisabilité Pour La Mise En Place de Parcs Agroindustriels, de Centres d'agrégation et de Services Du Projet de Développement Du Pôle Agro Industriel Dans Le Nord de La Côte d'Ivoire - (2 PAI-NORD CI)*. Ferkessedougou, *Côte d'Ivoire*.

[29] Gerdemann J. W., Nicolson T. H. (1963); Spores of mycorrhizal Endogone species extracted from soil by wet sieving and decanting. *Trans Br Mycol Soc*. 46 (2): 235-244. doi: 10.1016/S0007-1536(63)80079-0.

[30] INVAM, (2021). "International culture collection of VA Mycorrhizal fungi," Consulté le 15 Juillet 2021. http/www.invam.caf.wvu.edu.

[31] Rajeshkumar P. P., Hosagoudar V. B., Gopakumar B. (2013); Mycorrhizal association of Ochlandra travancorica in Kerala, India. *J Threat Taxa*. 5 (2): 3673-3677. doi: 10.11609/jott.o3235.3673-77.

[32] Pandey R. R., Loushambam S., Srivastava A. K.. (2020); Arbuscular Mycorrhizal and Dark Septate Endophyte Fungal Associations in Two Dominant Ginger Species of Northeast India. *Proc Natl Acad Sci India Sect B Biol Sci*. 90 (4): 885-894. doi: 10.1007/s40011-019-01159-w.

[33] Ouallal I., Abbas Y., Ech-Cheddadi S., et al. (2018); Diversity of endomycorrhizal fungi on argan tree roots and potential for mycorrhizal development in the soil rhizosphere of argan stands in southwestern Morocco. *Bois Forets des Trop*. 338 (4): 73-86. doi: 10.19182/bft2018.338.a31678.

[34] Touré G.-P. T., Nandjui J., Koné A. W., et al. (2020); Diversité des champignons mycorhiziens à arbuscules et interactions avec le système sol-litière dans un écotone forêt-savane, Côte d'Ivoire. *Étude Gest des Sols*. 28: 2021. http://www.afes.fr/publications.

[35] Bossou L. R., Houngnandan H. B., Adandonon A., Zoundji C. (2019); Diversité des champignons mycorhiziens arbusculaires associés à la culture du maïs (Zea mays L.) au Bénin. *Int J Biol Chem Sci*. 13 (2): 597-609. doi: https://dx.doi.org/10.4314/ijbcs.v13i2.2.

[36] Redon P. (2009). Rôle de champignons mycorhiziens à arbuscules dans le transfert du cadmium (Cd) du sol à la luzerne (Medicago truncatula). Doctorate thesis, University Henri Poincaré, Nancy I.

[37] Antunes P. M., Miller J., Carvalho L. M., Klironomos J. N., Newman J. A.. (2008); Even after death the endophytic fungus of Schedonorus phoenix reduces the arbuscular mycorrhizas of other plants. *Funct Ecol*. 22 (5): 912-918. doi: 10.1111/j.1365-2435.2008.01432.x.

[38] Purin S., Rillig M. C. (2008); Parasitism of arbuscular mycorrhizal fungi: Reviewing the evidence. *FEMS Microbiol Lett*. 279 (1): 8-14. doi: 10.1111/j.1574-6968.2007.01007.x.

[39] Koffi G. A., Dibi E. A. D. B., Anon H. A., et al. (2021); Diversité des champignons mycorhiziens arbusculaires associés à la culture du maïs et de l'arachide au nord de la Côte d'Ivoire. *Rev Int des Biosci*. 18 (3): 240-250.

[40] Droh G. (2017). Diversité génétique des champignons mycorhiziens a arbuscules associes au cacaoyer (théobroma cacao L.) de côte d'ivoire : cas de la rhizosphere des cacaoyeres des régions du gôh, de la nawa et de san-pédro. Published online. Doctorate thesis, Université Félix Houphouët Boigny.

[41] Rincón C., Droh G., Villard L., et al. (2021); Hierarchical spatial sampling reveals factors influencing arbuscular mycorrhizal fungus diversity in Côte d'Ivoire cocoa plantations. *Mycorrhiza*. 31 (3): 289-300. doi: 10.1007/s00572-020-01019-w.

[42] Semane F., Chliyeh M., Kachkouch W., et al. (2018); Follow-up of a Composite Endomycorrhizal Inoculum in the Rhizosphere of Olive Plants, Analysis after 42 Months of Culture. *Annu Res Rev Biol*. 22 (2): 1-18. doi: 10.9734/arrb/2018/38604.

[43] Ambili K., Thomas G. V., Gopal M., Gupta A. (2017); Influence of crop combinations and soil factors on diversity and association of arbuscular mycorrhizal fungi in arecanut based cropping systems. *J Plant Crop*. 45 (1): 20-32. doi: 10.19071/jpc.2017.v45.i1.3234.

[44] Bouabdelli Z., Belhadj S., Smail-Saadoun N., et al. (2018); Influence de l'aridité sur la variation de la colonisation mycorhizienne arbusculaire chez cinq populations naturelles algériennes du Pistachier de l'Atlas (Pistacia atlantica Desf.). *Rev d'Ecologie, Terre Vie*. 2018; 73 (June): 330-344.

[45] Gaonkar S., Rodrigues B. F. (2020); Diversity of arbuscular mycorrhizal (AM) fungi in mangroves of Chorao Island, Goa, India. *Wetl Ecol Manag*. 28 (5): 765-778. doi: 10.1007/s11273-020-09747-8.

[46] Rivaton D. (2016). Étude des champignons mycorhiziens arbusculaires des sols en systèmes de grandes cultures biologiques sans élevage : application à la nutrition phosphatée. Mémoire de fin de cycle d'Ingénieur, AGROCAMPUS OUEST, France.

[47] Blaszkowski J., Czerniawska B. (2008); Glomus eburneum and Scutellospora fulgida, species of arbuscular mycorrhizal fungi (Glomeromycota) new for Europe. *aCta Mycol*. 43 (1): 57-65.

[48] Sharma S., Parkash V., Aggarwal A. (2008); Glomales I: A monograph of Glomus spp. (Glomaceae) in the sunflower rhizosphere of Haryana, India. *Helia*. 31 (49): 13-17. doi: 10.2298/HEL0849013S.

[49] Palenzuela J., Azcón-Aguilar C., Barea J.-M., da Silva G., Oehl F. (2013); Acaulospora pustulata and Acaulospora tortuosa, two new species in the Glomeromycota from Sierra Nevada National Park (southern Spain). *Nov Hedwigia*. 97 (3-4): 305-319. doi: 10.1127/0029-5035/2013/0129.