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Diversity of Arbuscular Mycorrhizal Fungi Associated with Cocoa (*Theobroma cacao* L.) Agroforestry Systems in Togo

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

This study aims to assess the diversity and prevalence of arbuscular mycorrhizal fungi (AMF) associated with cocoa trees in different agroforests of the agroecological zones of cocoa production in Togo. The substrates, consisting of soil samples, were collected from twenty-four (24) cocoa orchards belonging to four types of agroforests (< 10 years, 10 to 20 years, 21 to 30 years, and > 30 years) in the three agroecological subzones of cocoa production (Agou peneplain, piedmonts, and plains and plateaus and mountains) of Togo. These samples were used to trap in pots the AMF spores associated with cocoa trees using sorghum (*Sorghum bicolor*) as a reference trap plant. The culture was maintained for 12 months in a greenhouse after which the densities and diversity of the spores were evaluated. The AMF spores extracted were identified based on morphological criteria. The results showed a great diversity of spores identified in the different agroforests. A total of thirty species of AMF belonging to sixteen genera and nine families (Acaulosporaceae, Ambisporaceae, Archaeosporaceae, Gigasporaceae, Claroideoglomeraceae, Dentiscutataceae, Diversiporaceae, Glomeraceae and Paraglomeraceae) were identified. Furthermore, the study revealed the predominance of the genera Rhizophagus, Acaulospora, and Gigaspora. However, the less frequent genera are represented by Entrophospora, Ambispora, Archaeospora, Cetraspora, Racocetra, Dentiscutata, Diversipora, and Paraglomus. The most frequent species were *Claroideoglomus etunicatum*, *Acaulospora scorbiculata* and *Acaulospora* sp. Results showed that AMF spore density varied from 16 spores/gram of soil in young plantations entering production and old plantations to 37 spores/gram of soil in mature plantations in production on the Agou peneplain. Finally, the highest AMF densities were recorded in agroforests less than 30 years old.
This study has identified a great diversity of AMF associated with cocoa orchards in the different agroforests and some strains could be used as biofertilizers for sustainable cocoa production in Togo.

Keywords: Theobroma cacao L.; agroforests; agroecological subzones; arbuscular mycorrhizal fungi; AMF density.

1. INTRODUCTION

Plant growth depends on interactions with the surrounding environment, particularly the soil and the microorganisms it harbors (Touré et al., 2021). Among these microorganisms, Arbuscular Mycorrhizal Fungi (AMF) is the most widespread group with recognized beneficial effects on plant growth (Smith et al., 2011, Shi et al., 2013). AMFs are microbiota forming mutualistic symbioses between a plant and a fungus at the root level. These microorganisms are present in most ecosystems and are an important component of tropical soil microflora (Cardoso et al., 2006, Tchabi et al., 2008). These telluric fungi play an important role in the functioning of earth ecosystems, due to their ubiquity and direct involvement in essential processes taking place at the soil-plant interface. They enable plants to better adapt to their environment, particularly in ecosystems with a deficit of water and/or nutrients (Amani et al., 2003).

In Togo, cocoa is one of the main agricultural exports and a significant source of income for producers. Over 90% of cocoa production in Togo is carried out by small-scale farmers (Djiwa et al., 2021). These farmers cultivate an average area of 26,356.66 ha for a national production of around 12,674.43 tons of merchantable cocoa per year with a low average yield of 506.83 kg/ha compared with a potential of 1,200 to 3,500 kg/ha) CRA-F 2004). This low orchard productivity can be explained by the degradation of biodiversity, aging orchards, loss of soil fertility, as well as the effects of climate change such as drought, reduced and irregular rainfall, and shorter rainy seasons. These constraints are real handicaps to sustainable and efficient cocoa production. In these unfavorable conditions, AMFs offer an alternative for improving cocoa production, as they can be biological substitutes for synthetic chemical fertilizers and pesticides (Finlay et al., 2008).

Several studies on the molecular identification of AMF (Redecker et al., 2000) have led to a better understanding of the link between AMF communities and various parameters, such as cropping systems and intensification of cropping practices (Jansa et al., 2002, Oehl et al., 2004), soil types, and soil depth (Oehl et al., 2005). However, studies on the diversity and identification of AMF in African ecosystems are

relatively limited Mathimaran et al., 2016, Gnamkoulamba et al., 2018) with a particular rareness in West African forest ecosystems (Zézéet al., 2007, Tchabiet al., 2008) more specifically in cocoa agroforestry systems. AMF can colonize the root cortex cells of over 80% of the earth's plants (Gnamkoulamba et al., 2018). In Togo, cocoa agroforests have been maintained with minimal disturbance for decades. Understanding how these relationships thrive can unlock a deeper appreciation for the intricate web of life they support. This knowledge will enable these AMFs to be conserved in germplasm banks or used in future research into soil defense and restoration. Consequently, assessing the diversity of AMF communities and understanding the factors that determine their distribution is a necessary condition for determining their beneficial effects in cocoa agroforests in Togo. The present study aims to investigate the mycorrhizal status of cocoa agroforests in different production subzones in Togo.

2. MATERIALS AND METHODS

2.1 Presentation of the Study Area

The study was conducted in the forest agroecological zone, which represents the main cocoa production area in Togo. This zone is subdivided into three agro-ecological sub-zones: the Plateaux sub-zone, the Piedmonts and Plains sub-zone, and the Agou peneplain (Fig. 1):

- Mountains and plateaux sub-zone: This sub-zone comprises the plateaux of Kouma, Danyi, Akposso, and Akébou, with an average altitude of 700 m, and the isolated mountains of Agou and Haïto. It enjoys a mountain climate with annual rainfall of 1,200-1,600 mm. Soils are ferritic or ferruginous with concretions. Vegetation consists of partially degraded forests and wooded and shrubby savannahs.
- The piedmonts and plains sub-zone: This is represented to the east by the plains of the Kloto, Kpélé-Akata, and Amou prefectures, and to the west by the Litimé plain (west of the Wawa prefecture). These areas benefit from a mountain climate with annual rainfall of 1300-1600 mm. The soils are thick and ferritic. Vegetation consists of Sudano-Guinean forests alternating with wooded savannahs.
- The Agou peneplain: This corresponds to the vast expanse of ferruginous soils on metamorphic rock that covers the whole of the Agou prefecture, except for the mountainous part. The climate is humid tropical, with annual rainfall of 900-1200 mm. Vegetation consists mainly of wooded or Guinean savannahs and gallery forests, with artificial teak forests in places.

2.2 Soil Sampling

Soil samples were taken from the three agroecological sub-zones. In each agroecological sub-zone, samples were taken considering four agroforest types that represent the main stages of orchard evolution over time:

- Young orchards entering into production (< 10 years),
- Mature cocoa trees in full production (10 to 20 years),
- Aging cocoa trees in production decline (21 to 30 years)
- Old orchard (>30 years)

In each parcel, five sampling points were chosen, following the diagonals of the parcel. At each sampling point, soil samples were taken under five cocoa trees. Under each tree, a soil sample (250-300 g) was taken from the 50 cm crown around the cocoa tree over a radius of 50 to 100 cm. Samples from each parcel were mixed to form a composite sample for extraction and identification of AMF spores. Across all sites, a total of 24 composite soil samples were collected. Each plantation was geo-referenced using GPS and its age was recorded.

2.3 Trapping and Multiplication of Arbuscular Mycorrhizal Fungi Spores

AMF trapping was carried out in the greenhouse at the Station d'Expérimentations Agronomiques de Lomé (SEAL) over 12 months from August 2020 to July 2021. Thus, sorghum (*Sorghum bicolor*) was used as the reference trap plant because its roots promote greater multiplication of mycorrhizal fungus spores (INRA, 2017). For each soil sample collected, two 5-liter pots were used, for a total of 48 pots for all samples. In each pot, 4 kg of substrate consisting of sea sand (1/3) and arable soil (2/3) collected at the SEAL were used. The substrate was sterilized at 120°C for 2 hours. Initially, 3 kg of substrate was introduced into each pot. Next, 200 g of soil was inoculated into the substrate, onto which sorghum was sown at a rate of three grains/pot,

and then covered with the remaining 1 kg of composite substrate. Before sowing, the sorghum grains were sterilized with 90° alcohol for 1 minute and rinsed with distilled water. Replanting was carried out each time the plant reached senescence. These plants were watered daily throughout the development phase.

2.4 Extraction and Morphological Identification of AMF Spores

Spores were extracted at the Laboratoire des Sciences Agronomiques et Biologiques Appliquées (LaSABA), University of Kara, using the wet sieving technique described by Gerdemann and Nicolson (Gerdemann et al., 1963). 50 g of soil was suspended in water and placed on the top of a series of three sieves superimposed from bottom to top according to the increasing value of their mesh opening (32µm, 90µm, and 500µm). The soil was subjected to this series of sieves under a jet of tap water, and the contents of the last two sieves (90 and 32µm) were poured into 50 ml flasks and centrifuged in a gradient of 70% sucrose solution (Oehl et al. 2003) at 2,000 rpm for 5 min. The supernatant from each treatment was collected with a volumetric pipette and returned to the smallest sieve (32µm), then rinsed to remove residual sucrose solution and poured into pillboxes. The spores and spore clusters stored in the pillboxes were transferred to Petri dishes with grid bottoms before being observed and counted with a binocular magnifying glass (G = X40).

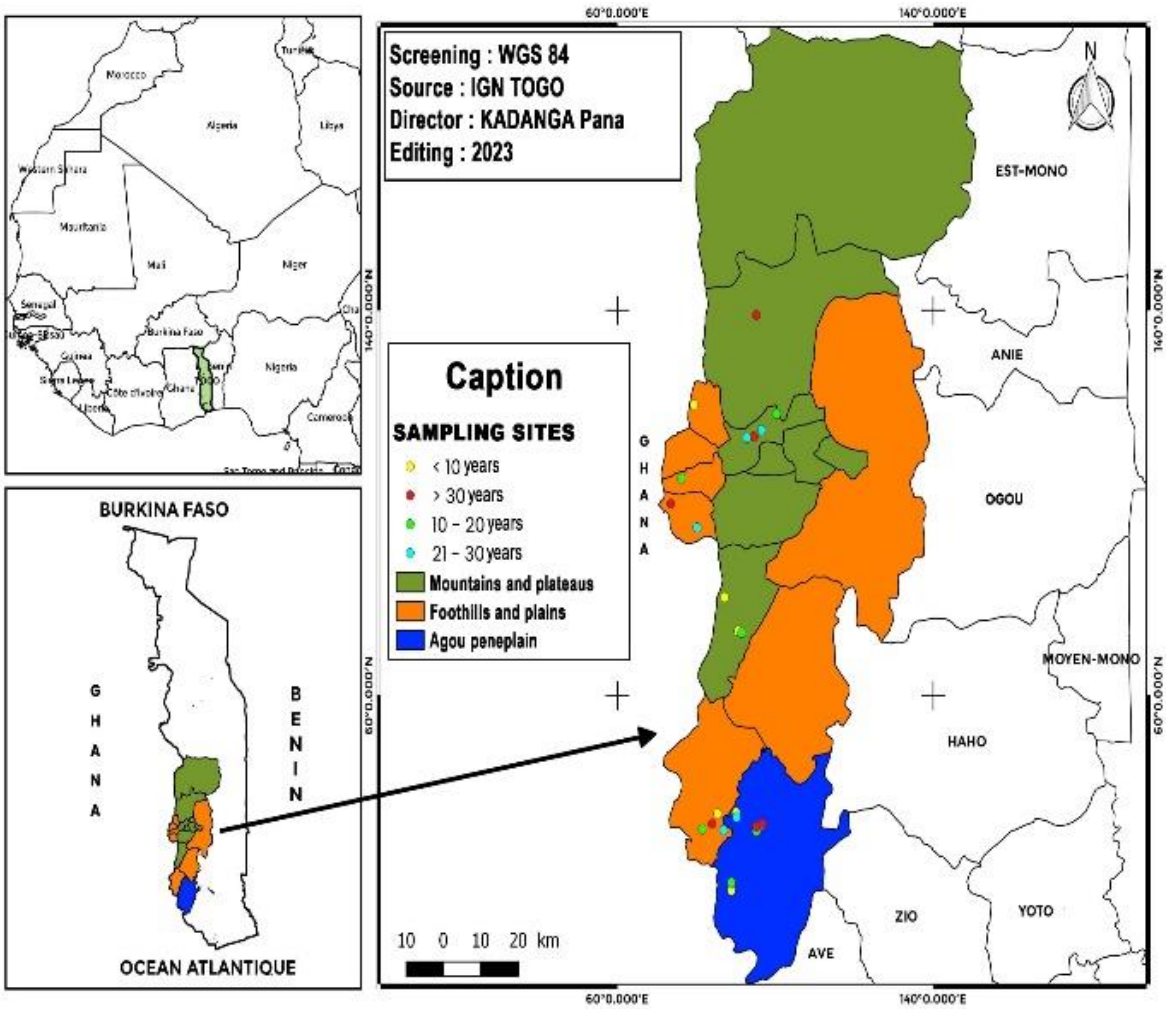

Fig. 1. Soil sampling locations

For microscopic identification, healthy spores were mounted on glass slides and stained with glycerol mixed with Melzer's reagent (1:1 vol/vol, Brundrett et al., 1994). The spores were examined under a binocular microscope (G = X400) and identified based on the description and identification manual by Schenck et Pérez (1990), INVAM (International Culture Collection of Vesicular Arbuscular Mycorrhizal Fungi (http://invam.caf.wvu.edu/cultures/cultsearch.ht) and scientific publications on AMF classification (Palenzuela et al., 2008, Redecker et al., 2013, Błaszkowski et al., 2017, Crossay et al., 2018, Anand et al., 2022).

2.5 Assessment of AMF Taxonomic Diversity

Taxonomic diversity was assessed by calculating the following indices:

- Species richness: refers to the number of species present in a given environment.
- Shannon-Weaver index (H') (Krebs, 1985):

$$H' = -\sum_{i=1}^{S} pi.log_2(pi)$$

Pi is the percentage abundance of an AMF genus (pi = ni/N), ni represents the number of individuals counted for the genus, N is the total number of individuals counted for all genera, and S is the total number of genera identified. The Shannon index is used to characterize species diversity in a community and ranges from 0 to Ln(S).

- Piélou equitability index (E) (Pielou, 1977) : E = H'/ln(S)

This index quantifies the regularity of species distribution within the community. Piélou's equitability index varies between 0 and 1.

2.6 Statistical Analysis

Data-t-on spore density, species richness, and AMF community diversity indices (Shannon-Weaver and Piélou equitability indices) were analyzed by analysis of variance (ANOVA) using SPSS (Statistical Package for Social Sciences) software version 25.0. The different means were discriminated and compared using the Student-Newmann-Keuls (SNK) test at the 5% threshold. Before analysis, mean AMF spore densities were normalized using the log10 (x + 1) transformation (where x is the mean spore density).

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 AMF diversity in soils under cocoa trees in different agroforests in the three agroecological subzones

A total of 30 AMF species, belonging to 16 genera and 9 families, were identified in soil samples under cocoa trees (Table 1). The Rhizophagus genus was the most represented, with five species, followed by Acaulospora (four species), Gigaspora (three species), Scutellospora, Claroideoglomus, Funneliformis, Glomus, and Septoglomus (two species each) and finally the other genera, each represented by a single species (Table 1).

3.1.2 Specific richness of AMF in soils under cocoa trees in different agroforests in the three agroecological subzones

Irrespective of the agroecological sub-zone considered, the analyses revealed no significant differences ($p > 0.05$) in specific richness between the different agroforests (Fig. 3). However, in the Agou peneplain, the highest species richness was recorded in mature plantations in production (16 species). On the other hand, in the piedmonts and plains, the highest number of species was recorded in young plantations entering production (14 species), while in the plateaux and mountains, the highest number of species was recorded in plantations between 10 and 30 years old (10 species) (Fig. 4).

Concerning the different AMF species, the most frequent are represented by *Claroideoglomus etunicatum* (24%), *Acaulospora scorbiculata* (23%), and *Acaulospora* sp (23%) (Table 2). The highest spores density was recorded for *C. etunicatum* (4 spores/gram of soil), followed by *E. infrequens*, *G. microaggregatum,* and *R. intraradices* (3 spores/gram of soil each).

3.1.3 Specific richness of AMF in soils under cocoa trees according to the different agro-ecological sub-zones

In the Agou peneplain, eleven (*A. corbiculata, A. excavata, Acaulospora sp, E. infrequens, G. gigantea, C. etunicatum, Diversipora sp, F. mosseae, F. monosporus, G. microaggregatum, and R. intraradices*) of the thirty species listed were found in soils under cocoa trees of all four agroforest types. However, only one species (*Dentiscutata sp*) was not found in this agroecological subzone (Table 3).

Table 1. Families, genera, and number of AMF species found in cocoa agroforests and their average frequencies

Families	Genera	Number of species
Acaulosporaceae	*Acaulospora*	4
	Entrophospora	1
Ambisporaceae	*Ambispora*	1
Archaeosporaceae	*Archaeospora*	1
Gigasporaceae	*Cetraspora*	1
	Gigaspora	3
	Scutellospora	2
	Racocetra	1
Claroideoglomeraceae	*Claroideoglomus*	2
Dentiscutataceae	*Dentiscutata*	1
Diversiporaceae	*Diversipora*	1
Glomeraceae	*Funneliformis*	2
	Glomus	2
	Rhizophagus	5
	Septoglomus	2
Paraglomeraceae	*Paraglomus*	1
Total number of species		30

Table 2. Spores frequencies and densities of AMF species identified in cocoa orchards

AMF species	Frequency (%)	Density (Number of spores/gram of soil)
A. scorbiculata	23	2
A. excavata	11	1
A. colombiana	13	1
***Acaulospora* sp**	23	2
***Ambispora* sp**	4	1
***Archaeospora* sp**	4	1
***Cetraspora* sp**	20	1
C. etunicatum	24	4
C. claroideum	12	1
***Dentiscutata* sp**	3	1
***Diversipora* sp**	17	1
E. infrequens	19	3
F. mosseae	8	1
F. monosporus	15	2
G. gigantea	10	1
G. candida	6	1
G. Rosa	3	1
G. clavisporum	4	2
G. microaggregatum	16	3
***Paraglomus* sp**	6	2
R. aggregatus	14	2
R. clarus	8	1
R. intraradices	15	3
R. manihotis	6	1
R. sinuosus	13	2
S. calospora	3	2
***Scutellospora* sp**	3	2
S. deserticola	4	1
***Septoglomus* sp**	4	1
***Racocetra* sp**	3	1

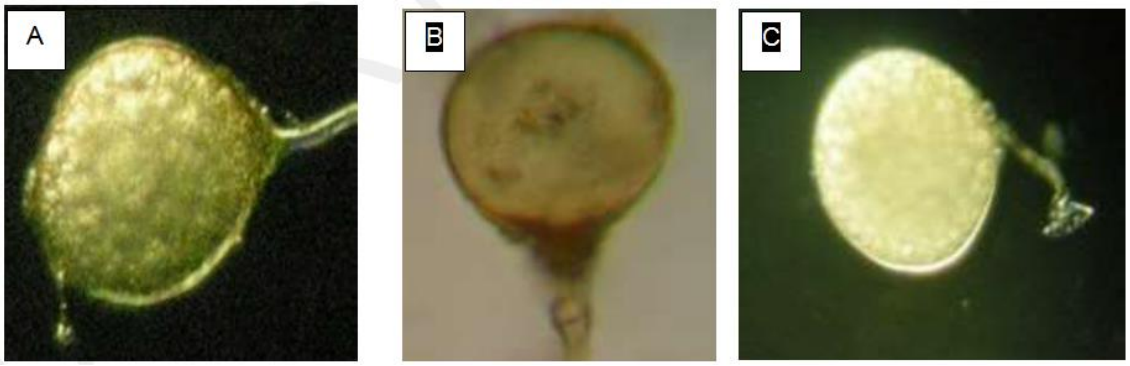

Fig. 2. Most frequent AMF spores in samples
A = Rhizophagus spp; B = Gigaspora spp and C = Acaulospora spp

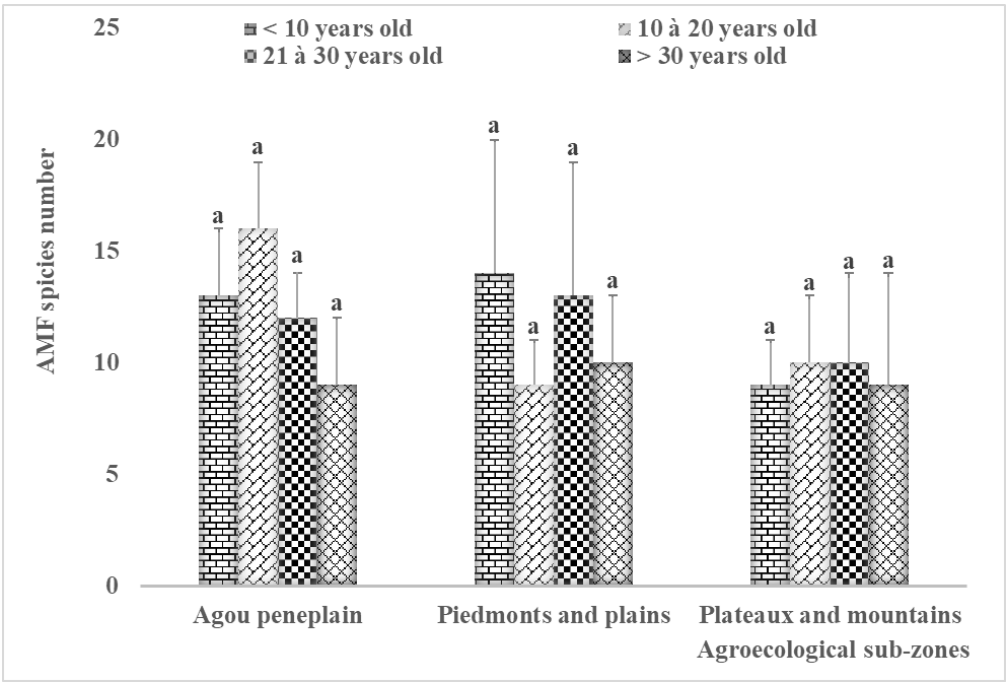

Fig. 3. Specific richness of AMF in soils under cocoa trees from different agroforests in the three agroecological subzones. Significant differences are indicated by different letters (Student-Newman-Keuls test at 5% threshold)

In the piedmonts and plains, nine species (A. *scorbiculata, A. colombiana, Acaulospora sp, G. gigantea, C. etunicatum, Diversipora sp, G. microaggregatum, R. aggregatus, and R. intraradices*) were found in the soils of all four agroforest types. Two species (*G. rosa and S. calospora*) were not found in the piedmonts and plains (Table 4).

Finally, in the plateaux and mountain agroecological sub-zone, six species (*A. scorbiculata, Acaulospora sp, E. infrequens, G. gigantea, C. etunicatum and G. microaggregatum*) were found in the soils of all four agroforests. However, five species (*Archaeospora sp, S. calospora, Scutellospora sp, Racocetra sp, Paraglomus sp*) were not found in the plateaus and mountains (Table 5).

3.1.4 AMF community diversity indices

AMF community diversity was assessed through species richness, Shannon-Weaver Index (H'), and Piélou Equitability (E) indices for the different agroforests and agroecological subzones. Statistical analyses showed no significant differences ($p > 0.05$) in species richness, Shannon-Weaver Index (H'), and Piélou

Table 3. Specific richness of AMF in soils under cocoa trees in agroforests on the Agou peneplain

AMF species	**< 10 years old**	**10 à 20 years old**	**21 à 30 years old**	**> 30 years old**
Acaulosporaceae				
A. scorbiculata	X	X	X	X
A. excavata	X	X	X	X
A. colombiana	X	X		X
Acaulospora sp	X	X	X	X
E. infrequens	X	X	X	X
Ambisporaceae				
Ambispora sp	X	X	-	-
Archaeosporaceae				
Archaeospora sp	X	X	-	-
Gigasporaceae				
Cetraspora sp	**-**	-	X	X
G. gigantea	X	X	X	X
G. candida		X	-	-
G. rosa	X	X	-	-
S. calospora	X	-	-	-
Scutellospora sp	**-**	X	-	-
Racocetra sp	**-**	X	-	-
Claroideoglomeraceae				
C. etunicatum	X	X	X	X
C. claroideum	X	X	X	-
Dentiscutataceae				
Dentiscutata sp	**-**	-	-	-
Diversiporaceae				
Diversipora sp	X	X	X	X
Glomeraceae				
F. mosseae	X	X	X	X
F. monosporus	X	X	X	X
G. clavisporum				
G. microaggregatum	X	X	X	X
R. aggregatus	X	X	X	-
R. clarus	X	X		
R. intraradices	X	X	X	X
R. manihotis	X			
R. sinuosus	X	X	X	-
S. deserticola				
Septoglomus sp	**-**	X	-	-
Paraglomeraceae				
Paraglomus sp	X	X	-	-
Total	**22**	**24**	**15**	**13**

X : Présent ; - : Absent

Table 4. Specific richness of AMF in soils under cocoa trees in piedmont and plain agroforests

AMF species	**< 10 years old**	**10 à 20 years old**	**21 à 30 years old**	**> 30 years old**
Acaulosporaceae				
A. scorbiculata	X	X	X	X
A. excavata	-	X	X	-
A.colombiana	X	X	X	X
Acaulospora sp	X	X	X	X

AMF species	< 10 years old	10 à 20 years old	21 à 30 years old	> 30 years old
E. infrequens	X	X	X	-
Ambisporaceae				
Ambispora sp	X	-	-	-
Archaeosporaceae				
Archaeospora sp	X	-	-	-
Gigasporaceae				
Cetraspora sp	-	X	X	X
G. gigantea	X	X	X	X
G. candida	-	-	-	X
G. rosa	-	-	-	-
S. calospora	-	-	-	-
Scutellospora sp	X	-	X	X
Racocetra sp	-	-	X	X
Claroideoglomeraceae				
C. etunicatum	X	X	X	X
C. claroideum	X	X	-	-
Dentiscutataceae				
Dentiscutata sp	X	X	X	-
Diversiporaceae				
Diversipora sp	X	X	X	X
Glomeraceae				
F. mosseae	X	-	-	-
F. monosporus	X	X	X	-
G. clavisporum				X
G. microaggregatum	X	X	X	X
R. aggregatus	X	X	X	X
R. clarus	X	-	-	X
R. intraradices	X	X	X	X
R. manihotis	X	-	-	-
R. sinuosus	X	-	X	-
S. deserticola	-	-	X	X
Septoglomus sp	-	-	X	X
Paraglomeraceae				
Paraglomus sp	X	-	-	X
Total	**21**	**15**	**19**	**18**

X : Présent ; - : Absent

Table 5. Specific richness of AMF in soils under cocoa trees in plateau and mountain agroforests

AMF species	< 10 years old	10 à 20 years old	21 à 30 years old	> 30 years old
Acaulosporaceae				
A. scorbiculata	X	X	X	X
A. excavata	X	-	X	-
A. colombiana			X	X
Acaulospora sp	X	X	X	X
E. infrequens	X	X	X	X
Ambisporaceae				
Ambispora sp	-	-	-	X
Archaeosporaceae				
Archaeospora sp	-	-	-	-
Gigasporaceae				
Cetraspora sp	-	X	X	X

AMF species	< 10 years old	10 à 20 years old	21 à 30 years old	> 30 years old
G. gigantea	X	X	X	X
G. candida	-	-	-	X
G. Rosa	-	-	-	X
S. calospora	-	-	-	-
Scutellospora sp	-	-	-	-
Racocetra sp	-	-	-	-
Claroideoglomeraceae				
C. etunicatum	X	X	X	X
C. claroideum	X	X	-	X
Dentiscutataceae				
Dentiscutata sp	-	-	-	X
Diversiporaceae				
Diversipora sp	-	X	X	X
Glomeraceae				
F. mosseae	X	X	-	X
F. monosporus	X	-	X	X
G. clavisporum	X	-		X
G. microaggregatum	X	X	X	X
R. aggregatus	X	X	X	-
R. clarus	X	X	-	-
R. intraradices	X	-	X	-
R. manihotis	-	-	-	-
R. sinuosus	X	X	X	-
S. deserticola	-	X	X	-
Septoglomus sp	-	X	-	-
Paraglomeraceae				
Paraglomus sp	-	-	-	-
Total	**15**	**15**	**15**	**17**

X : Présent - : Absent

Table 6. Specific richness, Schannon-Weaver, and Piélou equitability indices of AMF communities in soils under cocoa trees in different agroforests

Agroforest type	Specific richness	Shannon-Weaver index (H')	Piélou equitability index (E)
< 10 years old	19 ± 4 a	2,66 ± 0,03 a	0,78 ± 0,01 a
10 à 20 years old	18 ± 4 a	2,61 ± 0,21 a	0,77 ± 0,06 a
21 à 30 years old	16 ± 2 a	2,51 ± 0,06 a	0,74 ± 0,12 a
> 30 years old	16 ± 2 a	2,38 ± 0,40 a	0,70 ± 0,07 a
p-value	0,439	0,486	0,511

Data are reported as means and standard deviations for the four agroforests. Means with the same letter are statistically identical at the 5% level

Table 7. Specific richness, Schannon-Weaver, and Piélou equitability indices of AMF communities in soils under cocoa trees in the three agroecological sub-zones

Agroecological sub-zones	Specific richness	Shannon-Weaver index (H')	Piélou equitability index (E)
Agou peneplain	18 ± 5 a	2,53 ± 0,30 a	0,75 ± 0,09 a
Piedmonts and plains	18 ± 3 a	2,61 ± 0,18 a	0,77 ± 0,05 a
Plateaux and mountains	16 ± 1 a	2,47 ± 0,22 a	0,73 ± 0,07 a
p-value	0,633	0,717	0,710

Data are reported as averages and standard deviations for the three agro-ecological production sub-zones. Means with the same letter are statistically identical at the 5% threshold

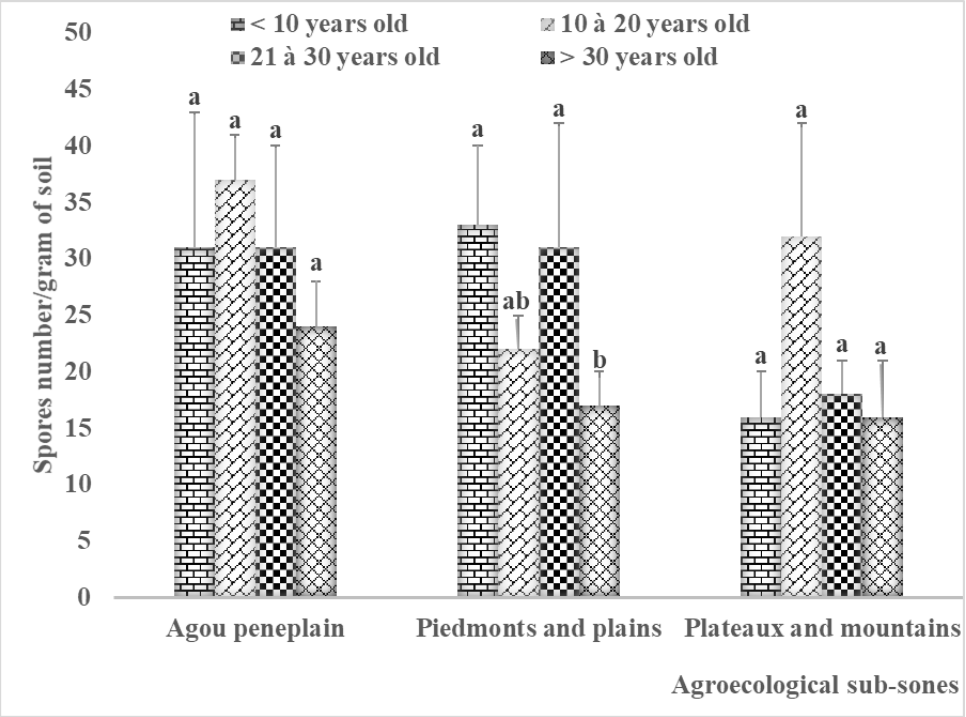

Fig. 4. AMF spore density in soils under cocoa trees in different agroforests of the Agou peneplain, piedmonts and plains, plateaus, and mountains. Significant differences are indicated by different letters (Student-Newman-Keuls test at 5% threshold)

equitability (E) indices for either agroforests or agroecological subzones. However, for agroforests, the lowest values of these parameters were recorded in old plantations (Table 6). Similarly, for agroecological sub-zones, plateaus and mountains recorded the lowest values (Table 7).

3.1.5 AMF spore density

After trapping, spores' density varied from 16 spores/gram of soil in young plantations entering production and old plantations to 37 spores/gram of soil in mature plantations in production on the Agou peneplain. The results of statistical analysis showed no significant difference ($p > 0.05$) between the spore densities of the different agroforests of Agou peneplain and plateau and mountains. However, a significant difference ($p < 0.05$) was observed between the spore densities of agroforests in the piedmonts and plains. In the Agou peneplain, as well as in the piedmonts and plains, the highest spore densities were observed in agroforests less than 30 years old. On the other hand, in the plateaux and mountains, spore densities were highest in plantations between 10 and 30 years old.

3.2 Discussion

This study has revealed the diversity of AMF in cocoa agroforests in different production sub-zones. The identification of AMF associated with cocoa trees enabled us to list a total of 30 species belonging to 16 genera and 9 families. The most predominant genera were Rhizophagus (five species), Acaulospora (four species), and Gigaspora (three species), belonging to the Glomeraceae, Acaulosporaceae, and Gigasporaceae families respectively. The most frequent species are *Claroideoglomus etunicatum* (24%), *Acaulospora scorbiculata,* and *Acaulospora* sp (23%). The presence or absence of certain AMF species in the different agroecological sub-zones shows a probable influence of climatic differences and soil characteristics between agroecological sub-zones (Thomson et al., 2015, Amani et al., 2023). Amani et al., (2023) identified a total of 72 AMF morphotypes belonging to 10 families, 17 genera, and 49 AMF species, with a predominance of the genus Glomus in cocoa orchards in Côte d'Ivoire. Our results are similar to those of (Rojas et al., 2010) who identified 21 AMF morphotypes in the rhizosphere of cocoa trees in the department of San Martin, Peru.

Llanos-Gómez (2023) identified 57 different morphotypes, classified into 9 genera with a higher abundance of the genera Glomus, Acaulospora, and Gigaspora in soils under cocoa trees in the Amazon region of Peru. The studies of (Luis-Alaya et al., 2023) revealed the presence of the families Paraglomeraceae, Glomeraceae, Claroideoglomeraceae, and Acaulosporaceae in Tarapoto plantations, while (Cuenca et al., 1996) observed the predominance of the Acauloporaceae, Glomeraceae, and Gigasporaceae families in Venezuelan cocoa orchards. In cocoa orchards in the Yamoussoukro region of Côte d'Ivoire, (Zako et al., 2012) identified nine AMF species belonging to the Glomus, Acaulospora, and Gigaspora genera. In Ecuador, in conservative cocoa crops, the genera Glomus, Acaulospora, Ambispora, Pacispora, Diversispora, Scutellospora, Racocetra, Entrophospora, Gigaspora, Intraspora, Paraglomus and Archaeospora were identified, while in semi-conservative cocoa crops, the genera Glomus, Acaulospora, Ambispora, Pacispora, Diversispora, Scutellospora, Racocetra, Entrophospora, Gigaspora, Intraspora, Paraglomus and Archaeospora were identified (Pacheco et al., 2022). The predominance of the genera Rhizophagus, Acaulospora, and Gigaspora in cocoa agroforests shows their better adaptation to a wide range of conditions, even the most hostile, such as drought, salinity, and other environmental stresses (Houngnandan et al., 2009). Furthermore, _ (Amani et al., 2023) suggested that species of the genus Glomus (Rhizophagus) and Acaulospora propagate preferentially by spores resistant to harsh conditions, while other genera such as Scutellospora and Gigaspora propagate with other types of propagules such as hyphae and extra-root mycelial fragments.

Concerning the diversity indices of AMF communities in cocoa plantations, it emerges that the values obtained in the present study are similar to those obtained by (Cuenca et al., 1996) in Venezuelan cocoa plantations. On the other hand, the specific richnesses reported in this study in agroforests in the three agroecological subzones are low compared with those obtained by (Amoa et al., 2017) in plantain plantations in three agroecological zones (Abengourou, Azaguié, and Bouaflé) in Côte d'Ivoire (around 28.50 to 253.50). Furthermore, (Amoa et al., 2017) obtained Shannon and Piélou index values similar to those obtained in this study in these plantain plantations.

Results on AMF spore density revealed that the number of spores varied from 16 spores/gram of soil in young plantations entering production and old plantations to 37 spores/gram of soil in mature plantations in production on the Agou peneplain. These values are higher than those reported by (Cuenca et al., 1996), which are 4 spores/gram of soil in plantations less than 20 years old and 2 spores/gram of soil in old plantations (> 30 years) in Venezuela. In Côte d'Ivoire, (Amani et al., 2023) showed that in cocoa plantations, the abundance of arbuscular mycorrhizal fungi spores ranged from 10.53 to 31.24 spores/gram of soil, with an average of 18.30 spores/gram of soil. Snoeck et al., (2010) found similar spore densities (16 to 36 spores/gram soil) to those reported in our study in cocoa agroforests in Cameroon. Luis-Alaya et al., (2023) showed that in soils under cocoa trees in the San Martin region of Peru, spore density varied from 13.8 spores/100 grams of soil in 10-year-old plantations to 131.8 spores/100 grams of soil in 3-year-old plantations. Vestberg et al., (2015) found that spore density varied between 280 and 610 spores in 100/gram of dry soil in agricultural systems. In conservative and semi-conservative cocoa plantations, (Pacheco et al., 2022) recorded densities of 300 to 400 spores in 100/g dry soil. The difference between our results and those quoted above could be due to the cultivation practices and edaphoclimatic conditions of the plantations, as well as the particular characteristics of the cultivation and management of the plantations (Luis-Alaya et al., 2023).

4. CONCLUSION

This is the first study to assess the species diversity of arbuscular mycorrhizal fungi in cocoa plantations in Togo. The identification of AMF associated with cocoa trees within the framework of this study enabled us to list a total of 30 species divided into 16 genera and 9 families. The most common species are *Claroideoglomus etunicatum, Acaulospora scorbiculata and Acaulospora* sp*.* The high diversity of AMF recorded in cocoa plantations opens the door to further in-depth studies on the importance of this fungal group in cocoa cultivation.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Authors hereby declare that no generative Artificial Intelligence technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

Amani, C. Y. F., M'bo K. A., Cherif, M., Koné, D., & Kouamé, C. (2023). Diversité des champignons mycorhiziens à arbuscule associés aux cacaoyers (*Theobroma cacao* L.) en Côte d'Ivoire. *European Scientific Journal, 19*(27), 179. https://doi.org/10.19044/esj.2023.v19n27p179.

Amoa, A. J., Fotso, B., & Zeze, A. (2017). Potentialities of native arbuscular mycorrhizal fungi strain to improve the quality of macropropagated seedlings of plantain cv Orishele and FHIA. *Research Journal of Agricultural Science, 7*(1), 9-14.

Anand, K., Pandey, G. K., Kaur, T., Pericak, O., Olson, C., Mohan, R., Akansha, K., Yadav, A., Devi, R., Kour, D., Rai, A. K., Kumar, M., & Yadav, A. N. (2022). Arbuscular mycorrhizal fungi as potential biofertilizers for agricultural sustainability. *Journal of Applied Biology and Biotechnology, 10*(Suppl 1), 90-107. https://doi.org/10.7324/JABB.2022.10s111

Berruti, A., Lumini, E., Balestrini, R., & Bianciotto, V. (2016). Arbuscular mycorrhizal fungi as natural biofertilizers: Let's benefit from past successes. *Frontiers in Microbiology, 6*, 1559.

Błaszkowski, J., Kozłowska, A., & Crossay, T. (2017). A new family, *Pervetustaceae* with a new genus, *Pervetustus*, and *P. simplex* sp. nov. (Paraglomerales), and a new genus, *Innospora* with *I. majewskii* comb. nov. (Paraglomeraceae) in the Glomeromycotina. *Nova Hedwigia*. https://doi.org/10.1127/nova_hedwigia/2017/0419

Cardoso, I. M., & Kuyper, T. W. (2006). Mycorrhizas and tropical soil fertility. *Agriculture, Ecosystems & Environment, 116*, 72-84. https://doi.org/10.1016/j.agee.2006.03.011

CRA-F. (2004). Centre de recherche agronomique de la zone forestière. *Point sur la recherche cacaoyère au Togo*. Kpalimé.

Crossay, T., Cilia, A., Cavaloc, Y., Amir, H., & Redecker, D. (2018). Four new species of arbuscular mycorrhizal fungi (Glomeromycota) associated with endemic plants from ultramafic soils of New Caledonia. *Mycological Progress*, 1–16.

Cuenca, G., & Meneses, E. (1996). Diversity patterns of arbuscular mycorrhizal fungi associated with cacao in Venezuela. *Plant and Soil, 183*, 315–322. https://doi.org/10.1007/BF00011447

Djiwa, O., Pereki, H., & Guelly, K. A. (2021). Ethnocultural perceptions of ecosystem services provided by cocoa-based agroforests in Togo. *Biotechnology, Agronomy, Society and Environment, 25*(3), 208-222.

Finlay, R. D. (2008). Ecological aspects of mycorrhizal symbiosis: With special emphasis on the functional diversity of interactions involving the extraradical mycelium. *Journal of Experimental Botany, 59*(5), 1115–1126.

Gerdemann, J. W., & Nicolson, T. H. (1963). Spores of mycorrhizal endogone species extracted from soil by wet sieving and decanting. *Fungal Biology, 46*, 235-244.

Gnamkoulamba, A., Tounou, A. K., Tchabi, A., Agboka, K., Adjévi, A. K. M., & Batawila, K. (2018). Prevalence and diversity of arbuscular mycorrhizal fungi spores in rice cultivation under different rice cropping systems in five agro-ecological zones in Togo. *Journal of Applied Biosciences, 126*, 12647-12664. https://dx.doi.org/10.4314/jab.v126i1.3

Houngnandan, P., Yemadje, R. G. H., Kane, A., Boeckx, P., & Van Cleemput, O. (2009). Indigenous glomales of the *Isoberlinia doka* (Craib and Stapf) open forest in Wari-Maro in central Benin. *Tropical and Subtropical Agroecosystems, 27*(2), 83-87.

Jansa, J., Mozafar, A., Anken, T., Ruh, R., Sanders, I. R., & Frossard, E. (2002). Diversity and structure of AMF communities as affected by tillage in a temperate soil. *Mycorrhiza, 12*, 225–234.

Lekberg, Y., Roger, T., Rohr, J. R., Aldrich-Wolfe, L., & Morton, J. B. (2007). Role of niche restrictions and dispersal in the composition of arbuscular mycorrhizal fungal communities. *Journal of Ecology, 95*, 95–105. https://doi.org/10.1111/j.1365-2745.2006.01193.x

Llanos-Gómez, K. J., Silva-Manco, M. J., Sanchez-Santillan, T., Arce-Inga, M., & Leiva-Espinoza, S. T. (2023). Morphological identification of arbuscular mycorrhizal fungi in cocoa plantations in the Amazon region, Peru. *Manglar, 20*(1), 7-14.

Luis-Alaya, B., Toro, M., Calsina, R., Ogata-Gutiérrez, K., Gil-Polo, A., Ormeño-Orrillo, E., & Zúñiga-Dávila, D. (2023). Evaluation of the presence of arbuscular mycorrhizae and cadmium content in the plants and soils of cocoa plantations in San Martin, Peru. *Diversity, 15*, 246. https://doi.org/10.3390/d15020246

Mathimaran, N., Ruh, R., Jama, B., Verchot, L., Frossard, E., & Jansa, J. (2007). Impact of agricultural management on arbuscular mycorrhizal fungal communities in Kenyan Ferralsol. *Agriculture, Ecosystems and Environment, 119*, 22-32.

Oehl, F., & Sieverding, E. (2004). *Pacispora*, a new vesicular-arbuscular mycorrhizal fungal genus in the Glomeromycetes. *Journal of Applied Botany and Food Quality, 78*, 72–82.

Oehl, F., Sieverding, E., Ineichen, K., Mäder, P., Boller, T., & Wiemken, A. (2003). Impact of land use intensity on the species diversity of arbuscular mycorrhizal fungi in agroecosystems of Central Europe. *Applied and Environmental Microbiology, 69*, 2816-2824.

Oehl, F., Sieverding, E., Ineichen, K., Ris, E.-A., Boller, T., & Wiemken, A. (2005). Community structure of arbuscular mycorrhizal fungi at different soil depths in extensively and intensively managed agroecosystems. *New Phytologist, 165*, 273-283.

Pacheco Flores de Valgaz, A., Naranjo-Morán, J., Reyes Román, G., Oviedo-Anchundia, J., Ratti Torres, M., & Barcos-Arias, M. (2022). Discovering the diversity of arbuscular mycorrhizal fungi associated with two cultivation practices of *Theobroma cacao*. *Diversity, 14*, 651.

Palenzuela, J., Ferrol, N., Boller, T., Azcón-Aguilar, C., & Oehl, F. (2008). *Otospora bareai*, a new fungal species in the Glomeromycetes from a dolomitic shrubland in Sierra de Baza National Park (Granada, Spain). *Mycologia, 100*, 296-305. https://doi.org/10.1080/15572536.2008.11832484

Redecker, D., Morton, J. B., & Bruns, T. D. (2000). Molecular phylogeny of the arbuscular mycorrhizal fungi *Glomus sinuosum* and *Sclerocystis coremioides*. *Mycologia, 92*, 282-285.

Redecker, D., Schuler, A., Stockinger, H., Stumer, S., Morton, J., & Walker, C. (2013). An evidence-based consensus for the classification of arbuscular mycorrhizal fungi (Glomeromycota). *Mycorrhiza, 23*, 515-531. https://doi.org/10.1007/s00572-013-0486-y

Rojas, J. C. (2010). Arbuscular mycorrhizal fungi in the rhizosphere of promising cacao genotypes (*Theobroma cacao* L.) under traditional and under-forest systems in the San Martín Region (Thesis, Agricultural Engineering Degree). National University of San Martín.

Schenck, N. C., & Pérez, Y. (1990). *Manual for the identification of VA mycorrhizal fungi*. Synergistic Publications.

Shi, Y., Davis, K. J., Duffy, C. J., & Yu, X. (2013). Development of a coupled land surface hydrologic model and evaluation at a critical zone observatory. *Journal of Hydrometeorology, 14*, 1401—1420. https://doi.org/10.1175/JHM-D-12-0145.1

Smith, S. E., & Smith, F. A. (2011). Roles of arbuscular mycorrhizas in plant nutrition and growth: New paradigms from cellular to ecosystems scales. *Annual Review of Plant Biology, 63*, 227–250.

Snoeck, D., Abolo, D., & Jagoret, P. (2010). Temporal changes in VAM fungi in the cocoa agroforestry systems of central Cameroon. *Agroforestry Systems, 78*, 323–328.

Tchabi, A., Coyne, D., Hountondji, F., Lawouin, L., Wiemken, A., & Oehl, F. (2008). Arbuscular mycorrhizal fungal communities in sub-Saharan Savannas of Benin, West Africa, as affected by agricultural land use intensity and ecological zone. *Mycorrhiza, 18*(4), 181-195. https://doi.org/10.1007/s00572-008-0171-8

Thomson, B. C., Tisserant, E., Plassart, P., Uroz, S., Griffiths, R. I., Hannula, S. E., Buée, M., Mougel, C., Ranjard, L., Van Veen, J. A., Martin, F., Bailey, M. J., & Lemanceau, P. (2015). Soil conditions and land use intensification effects on soil microbial communities across a range of European field sites. *Soil Biology and Biochemistry, 88*, 403–413. https://doi.org/10.1016/j.soilbio.2015.06.012

Touré, G.-P. T., Nandjui, J., Koné, A. W., Kouadjo, A. G. Z., Ebou, A., Tiho, S., & Zézé, A. (2021). Diversity of arbuscular mycorrhizal fungi and interactions with the soil-litter system in a forest-savanna ecotone, Côte d'Ivoire. *Etude et Gestion des Sols, 28*, 93-104.

Vestberg, M. V., & Assefa, F. (2015). Diversity and abundance of arbuscular mycorrhizal fungi across different land use types in a humid lowland area of Ethiopia. *Tropical and Subtropical Agroecosystems, 18*.

Zako, B. I. M. S., Tié, B. T., Zirihi, G. N., Kouadjo, Z. C. G., Fossou, K. R., & Adolphe, Z. (2012). Arbuscular mycorrhizal fungi associated with *Theobroma cacao* L. in the region of Yamoussoukro (Côte d'Ivoire). *African Journal of Agricultural Research, 7*(6), 993-1001. https://doi.org/10.5897/AJAR11.2057

Zézé, A., Ouattara, B., Brou, C. Y., Van Tuinen, D., Diallo-Attah, H., & Sangaré, A. (2007). Distribution and abundance of spores of arbuscular endomycorrhizal fungi in different types of Téné forest in Côte d'Ivoire. *Agronomie Africaine, 19*(2), 103-111. https://doi.org/10.4314/aga.v19i2.1710

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