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Diversity and distribution of arbuscular mycorrhizal fungi associated with Bambara groundnut (*Vigna subterranea* (L.) Verdcourt) in Benin

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Bambara groundnut, despite its attributes in providing protein to human, is facing soil fertility and degradation problems, always leading to little performances. Arbuscular mycorrhizal fungi (AMF) constitute a microorganism group used by many researchers to improve productivity of crops in poor soils. This study aimed to evaluate the distribution and diversity of AMF associated to Bambara groundnut in different agro-ecological zones in Benin. A survey was conducted through 20 villages chosen based on Bambara groundnut yield, cropping area and its production across five agro-ecological zones (from AEZ 1 to AEZ 5). Soil and root samples were collected to assess spore density and diversity, root colonization levels and soil chemical properties. Results revealed significant difference (p <0.0001) among agro-ecological zones in terms of density of AMF, which varied from 2825 to 5713 spores per 100 g of soil, depending on the AEZ. The highest density was recorded in the cotton zone in the northern Benin. The diversity of AMF also varied, depending on the AEZ. In total, 14 morphotypes belonging to five genera (*Glomus, Gigaspora, Acaulospora, Scutellospora* and *Diversispora*) were identified in the different studied zones with *Glomus* genus the most frequently recorded in all AEZ. Correlation tests among the different parameters have, in general, revealed that, the zones with the low rates of the different parameters had those with the highest frequencies of mycorrhization. It also appears that spore density did not correlate with diversity index, mycorrhization frequencies and intensities, but soil chemical parameters significantly did.

Key words: Bambara groundnut, mycorrhiza, agro-ecological zone, density, Arbuscular mycorrhizal fungi (AMF).

INTRODUCTION

Nutritional proteins are one of the major metabolism needs, which are rarely satisfied in some developing

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countries, especially by Africans. Indeed, in Africa, animal proteins are less available for people due to economic constraints.

Therefore, focusing on plant proteins becomes the preferred way to satisfy protein needs in Africa (Mazahib et al., 2013). Among protein-providing crops, legumes take a large part in supplying safe proteins in food systems by increasing proteins levels for human adequate nutrition (Vertès et al., 2015). Moreover, legumes have ability to fix atmospheric nitrogen, and then transfer it into, and fertilize the soil. Like other legume species, Bambara groundnut (*Vigna subterranea* (L.) Verd.) contains high level of lysine and plays an important nutritional role when it is eaten combined with cereals in rural peoples' diet (Massawe et al., 2005; Bamshaiye et al., 2011). Moreover, Bambara groundnut takes part in increasing phosphorus bio-availability in the soil (Ndiang et al., 2012; Touré et al., 2013; Gbaguidi et al., 2015).

Despite its attributes, Bambara groundnut always remains a neglected and underutilized species often scoring low yields in Benin (Dansi et al., 2012). In fact, the low crop low yields are caused by low cation exchange capacity, low sum of bases, mineral deficiency (nitrogen and mainly assimilable phosphorus) and low organic matter contents of the soil, which are not favourable for increased growth of many crops in Benin soils (Igue et al., 2013). However, Arbuscular mycorrhizal fungi (AMF) constitute a microorganism group mainly having beneficial effects on plant growth (by mobilization of some nutrients) and tolerance to many biotic and abiotic stresses after initializing symbiosis with plant roots (Smith and Read, 2008; Saïdou et al., 2012; Haougui et al., 2013).

Many researchers have reported use of endomycorrhizal symbiosis to improve productivity of many plants (Aboubacar et al., 2013; Usharani et al., 2014; Haro et al., 2015; Do Rego et al., 2015). In Benin, Houngnandan et al. (2009) evaluated indigenous *Glomus* species diversity in the clear forest of *Isoberlina doka* (Craib et Stapf) at Wari-Maro in Benin centre. Tchabi et al. (2008) and Balogoun et al. (2015) also have studied the diversity of endomycorrhizal fungi associated respectively with cotton and cashew tree. These different researches resulted in identification of some species of Glomeromyceta associated with many crops in Benin. To the authors' knowledge, no research was carried out in West Africa, especially in Benin focusing on AMF species and strains associated to Bambara groundnut. Therefore, the aim of this work was to study AMF community present in Bambara groundnut rhizosphere in the different agro-ecological zones in Benin.

MATERIALS AND METHODS

Study areas

Earlier researches showed that Bambara groundnut was grown in Benin with more production areas localized in the northern part of the country (Gbaguidi et al., 2016). Statistical data on Bambara groundnut production also showed that it is cultivated in six agro ecological zones in Benin, namely the Far North Benin (AEZ 1), Cotton region of North Benin (AEZ 2), Food crop region of South Borgou (AEZ 3), West zone of Atacora (AEZ 4), Cotton region of the centre (AEZ 5) and Bar lands (AEZ 6) (Figure 1). However, the production of the crop is not so much important across all sixth AEZ and therefore the study covered only five AEZs.

Survey in the field

Based on Bambara groundnut sowing date in the different areas, the survey was carried out in September 2018 in order to be sure to find Bambara groundnut in the field and especially at flowering phase. Two regions were selected per agro-ecological area. These regions were taken on the base of Bambara groundnut yield, cultural surface affected to this species and its production in these regions the last five years. The online database of FAO (Country Stat) has been used for obtaining this information on Bambara groundnut in each region and it helps for sampling place choice. Each sampling site was georeferenced with GPS.

Laboratory work

Density and diversity assessment of AMF, assessment of mycorrhization level and soil chemical characteristics determination were done at the Laboratory of Soil Microbiology and Microbial Ecology (LMSEM) of the Faculty of Agronomic Sciences (FSA) in the University of Abomey-Calavi.

Soil and root sampling from the fields

Per sampling site, composite samples have been made. Four soil samples were collected (20 soil samples) at four different points using a custom-made corer at 20-cm depth. All of these samples were mixed together and the mixture was used to obtain a composite sample. At each soil sampling site, four plants were also been pulled up for root sampling.

Spore extraction, counting and morphological identification

AMF spores were extracted using the wet sieving and centrifugation method of Gerdmann and Nicholson (1963). One hundred grams of composite soil sample from Bambara groundnut rhizosphere was weighed, mixed in water, stirred thoroughly, let decant before being sieved through serial sieves with different mesh sizes (2 mm, 250 µm, 150 µm, 63 µm and 50 µm). This process was repeated four times. The sediments from the different sieves were collected in tubes and finally centrifuged after adding sucrose solutions (5mL of 20 and 60% w:v) at 4000 rounds/min for 4min at 4ºC (Oehl et al., 2003). After this process, suspended spores were collected and counted under a binocluar magnifying glass using gridded Petri dish (5cm of diameter) to make the counting easier. Based on morphological criteria (colour, presence or absence of suspensor bulb and hyphae, etc.), some AMF morphotypes were identified, counted and grouped into genera by using identification and description keys from the International Collection of Vesicular and Arbuscular Mycorrhizal fungi (INVAM, http//www.invam.caf.wdu.edu). Spore density was expressed as numbers of AMF spores per 100 g of dry soil. AMF spore density in each sample was obtained by summing abundances of all species recorded in the sample.

AMF diversity assessment

Biological diversity indexes; that is, Shannon diversity Index (H')

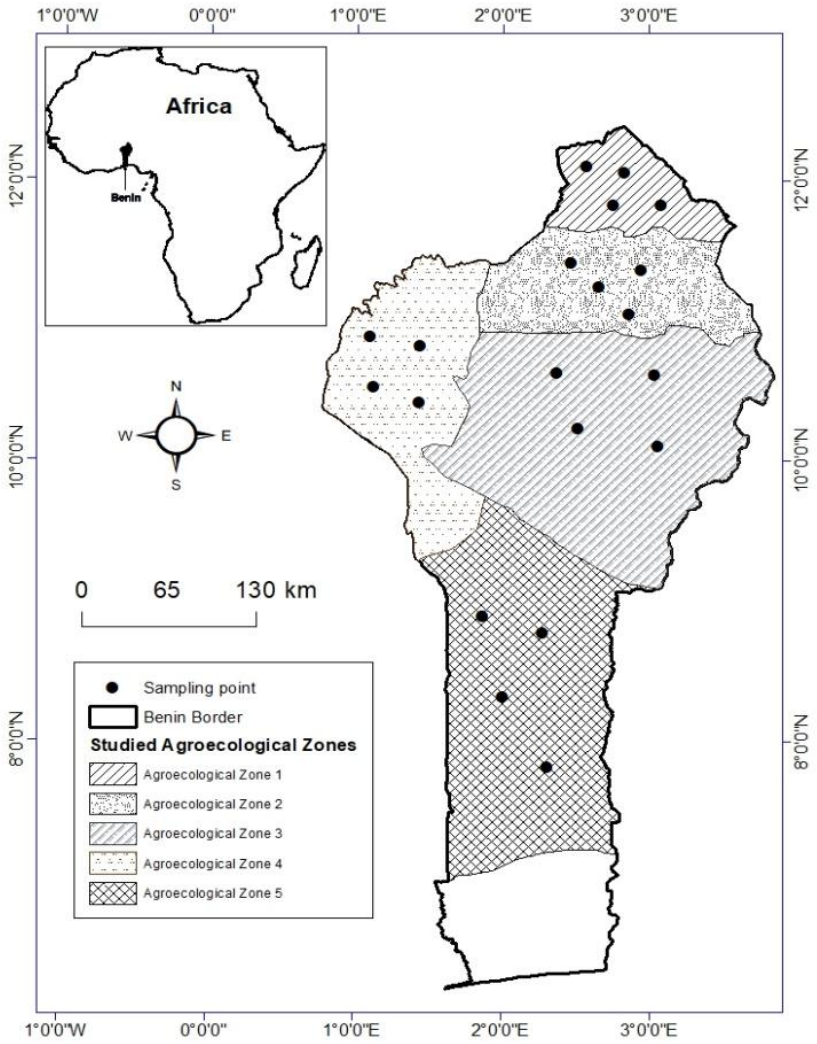

Figure 1. Benin geographic map with different sampling places.

(Shannon and Weaver, 1962), Simpson diversity Index (1-D) (Simpson, 1949) and Hill diversity index (1-Hill) were calculated for each agro ecological zone to evaluate AMF diversity.

Assessment of mycorrhization

Fresh roots of Bambara groundnut collected during the survey were used. After staining with trypan blue dye using the method described by Phillips and Hayman (1970), 20 fragments of fine Bambara groundnut roots were cut and placed on a glass slide with cover slip, and were observed with an optical microscope (XSP-BM-2CA, AliExpress) to observe different structures of AMF from different samples. Assessment of AMF colonization was performed using intersection method described by Trouvelot et al. (1986). The mycorrhization rates were assessed by two parameters of arbuscular mycorrhizal colonization namely: mycorrhization frequency and mycorrhization intensity.

Soil chemical analysis

From each Bambara groundnut field soil collected during the survey was subsampled (20), air dried and sieved for determination of chemical properties including the pH by potentiometric method in water (soil: water ratio 1:2.5) using a digital pH meter, organic carbon (C) by the Walkley–Black (1934) method, total N by Kjeldahl method and assimilable P by Bray I method.

Statistical analysis

One-way Analysis of Variance (ANOVA) was performed to assess the effects of agro-ecological zone (AEZ) on AMF spore density and diversity parameters, but also soil chemical parameters. When significant differences ($P < 0.05$) were found, post hoc comparisons of means among AEZ were made using a Student-Newman–Keuls' test. This ANOVA was performed with non-transformed data after ensuring conformity of these data with ANOVA assumptions. Furthermore, a Factorial Component Analysis (FCA) was performed in order to characterize different zones with identified spore genera. For choosing axes, the two principal's components must present more than 50% of the total information. Additionally, a Pearson correlation test was performed among all of the studied parameters in order to determine the relationships between them. All statistical

Table 1. Total AMF spore density and specific density by colour.

Studied factor	Modality	Density (number of spores/100g of soil)	Spores density by colour			
			Black	Brown	White	Yellow
AEZ	AEZ 1	5175.94 ± 46.68^{b}	3144±56.26^{a}	777.5±7.37^{b}	1142.94±13.82^{c}	111.5±0.71^{a}
	AEZ 2	5713.00 ± 34.48^{a}	3020.25±65^{a}	1180.06±9.52^{a}	1400.25±18.9^{b}	112.31±1.04^{a}
	AEZ 3	5169.81 ± 21.36^{b}	2855.19±4.61^{b}	333.94±9.96^{c}	1945.75±9.12^{a}	34.94±0.51^{d}
	AEZ 4	2825.63 ± 27.85^{d}	1861.56±35.34^{d}	316.25±3.37^{c}	543.81±4.17^{e}	104±2.61^{b}
	AEZ 5	3248.13 ± 53.16^{c}	2347.94±74.35^{c}	223.94±2.07^{d}	611.31±10.74^{d}	64.94±1.5^{c}

AEZ: Agro-ecological zone. Means with the same letters are not significantly different (*P*>0.05) based on Student Newman-Keuls test.

Table 2. Specific AMF spore density by size.

Studied factor	Modality	Spores density by size			
		250 µm	150 µm	63 µm	50 µm
AEZ	AEZ 1	154.38±1.71^{b}	463.44±9.87^{c}	2641.19±45.59^{c}	1913.94±41.83^{a}
	AEZ 2	269.81±4.95^{a}	805.25±17.28^{a}	3169±17.01^{b}	1468.81±26.43^{b}
	AEZ 3	161.94±3.84^{b}	536.38±9.85^{b}	3533.69±26.97^{a}	937.81±8.87^{c}
	AEZ 4	107.81±0.74^{c}	361.94±4.56^{d}	1575.56±38.97^{d}	780.31±12.59^{d}
	AEZ 5	104.13±1.14^{c}	534.13±12.23^{b}	1638.5±18.27^{d}	971.38±33.58^{c}

AEZ: Agro-ecological zone. Means with the same letters are not significantly different (*P*>0.05) on the basis of Student Newman-Keuls test. Different letters represent SNK groups ranking.

analyses were carried out using SAS software version 9.2.

RESULTS

Density of spores associated to Bambara groundnut in different agro-ecological zones

Analysis of variance results showed that all of the recorded mean spore densities were significantly different (p <0.001) among surveyed AEZs (Table 1). In different Bambara rhizosphere soil samples, important spore densities were recorded. The highest spore density (5713 ± 35 spores per 100 g of soil) was recorded in AEZ 2 while AEZ 4 has recorded the lowest spore density (2826 ± 28 spores per 100g of soil). Thus, the ANOVA results showed that the mean spore density recorded was significantly different (p <0.001) among different surveyed AEZs. Additionally, when identification criteria were considered, it appeared that black spores were more frequent compared to other coloured spores; and the AEZ 1 recorded the highest black spore density (3144 ± 56spores per 100g of soil) followed by AEZ 2 (3021 ± 62 spores per 100g of soil) (Table 1). Based on spore size, it generally appeared that spores with small size were most abundant. It was noticed that spore size of 63µm showed the highest spore density compared to other spore sizes (250µm, 150µm and 50µm); and the AEZ 3 had recorded the highest spore density of 63µm (3534 ± 27spores per 100g of soil) followed by the AEZ 2 (3169 ± 17 spores per 100g of soil (Table 2).

AMF community composition

In the Bambara rhizosphere studied, 14 mycorrhizal fungal morphotypes have been identified according to morphological criteria (colour, presence or absence of suspensor bulb and hyphae, etc.). After identification of these morphotypes based on the INVAM identification key, results indicated that the AMF belonged to five genera (*Glomus, Gigaspora, Acaulospora, Scutellospora and Diversispora*), four families (Glomeraceae, Acaulosporaceae, Diversisporaceae and Gigasporaceae) and two (02) orders (Diversisporales and Glomerales), all in Glomeromycetes Phylum. Relative abundance of these genera broadly among AEZs showed that *Glomus* species are the most abundant genera recorded in all surveyed AEZs, and were represented by 66.82% of all recorded genera. Other genera found in surveyed AEZs were *Gigaspora, Scutellospora, Acaulospora* and *Diversispora* genera scoring 18.46, 8.25, 4.89 and 1.58%, respectively (Figure 2). Furthermore, analysis of this fungal distribution in the different AEZs indicated that species belonging to *Glomus* and *Diversispora* genera are mostly recorded in AEZ 1 and AEZ 4, while species

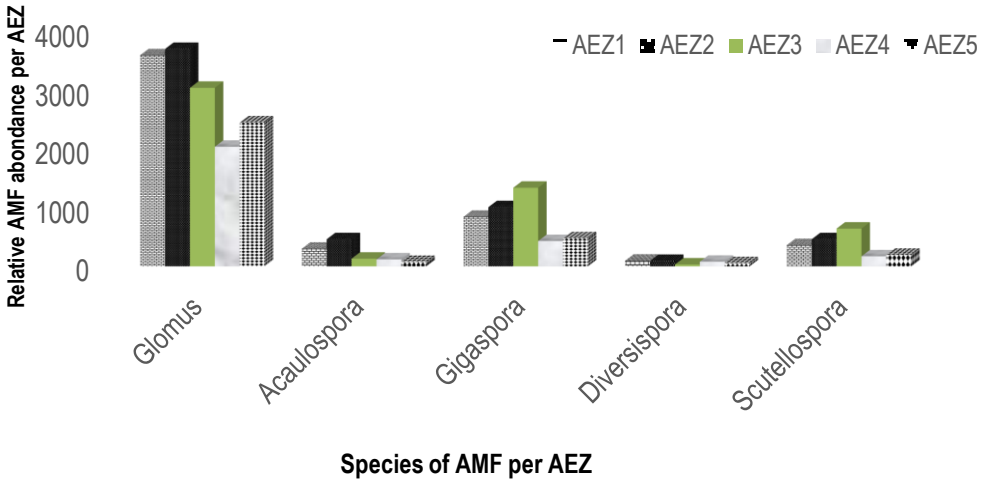

Figure 2. Relative AMF genera abundance in all surveyed zones in Benin.

of *Gigaspora* and *Scutellospora* genera were most common in AEZ 3 (Figure 3). All identified genera were found in AEZ 2 and AEZ 5.

AMF diversity

Statistical analysis showed that for all diversity indexes, there were significant differences ($p<0.0001$) among AEZs (Table 3). Shannon index (H') indicated that the AMF species were most diverse in AEZ 4 based on its highest index value (1.46) as compared to AEZ 3, which recorded the least species diversity (Table 3). When Simpson index (1-D) is considered, it appeared that AEZ 2 showed the most diverse species with the highest Simpson index (0.677) and AEZ 5 showed the least species diversity (0.533). This is true because H' index considered small populations, whereas the 1-D index considered larger populations. It was indicated earlier that AEZ 4 was a less abundant zone compared to AEZ 2, which was the most abundant zone in term of spores. The Hill index (1-Hill) also showed that all of surveyed zones are mainly diverse, like both previous diversity indexes (Table 3).

Assessment of natural mycorrhization level

As shown in Figure 4, there was a significant ($p<0.0001$) difference amongst the studied AEZs in terms of the mycorrhization frequency. The highest frequency (45.63%) was recorded in AEZ 1, compared to other AEZs. There was no significant difference amongst other AEZs and the lowest rate of mycorrhization frequency was recorded in AEZ 4 (17.25%). In terms of mycorrhization intensity, there were significant differences amongst AEZs; and the AEZ 3 recorded the significantly highest value (8.06%), while the lowest rate was from AEZ 4 (2.39%).

Chemical characteristics of soil from surveyed agro-ecological zones

The results showed significant differences ($p<0.001$) amongst the different AEZs based on studied soil parameters (Table 4).

In general, soils from different surveyed zones have relatively low pH (between 5 - 6.5 in all of the surveyed zones), poor in nitrogen (%N < 0.075% in all of surveyed zones) and phosphorus (P < 40 ppm in all of surveyed zones) according to Baize (2000) classification guideline. Furthermore, AEZ 2 had the recorded highest level of studied parameters; that is, Carbon (0.63%), Nitrogen (0.063%) and Phosphorus (29.50 ppm) but was third in pH level as compared to other zones (Table 4). Lowest levels of chemical parameters of soil were mostly recorded in AEZ 1.

Assessment of relationships between all of studied parameters

In AEZ 1, there was a significant positive correlation between Nitrogen and parameters like mycorrhization frequency ($r = 0.958$), Hill index ($r = 0.974$) and Simpson index ($r = 0.999$) (Table 5). This means that increasing of Nitrogen level in soil will induce enhancement of mycorrhization frequency and also AMF diversity. Additionally, it appeared there was a positive significant correlation between all of the diversity indexes (Table 5).

For AEZ 2, there was a significant negative correlation ($r = -0.970$) between Nitrogen (N) and mycorrhization frequency (F%) as shown in Table 5. So, increasing of N

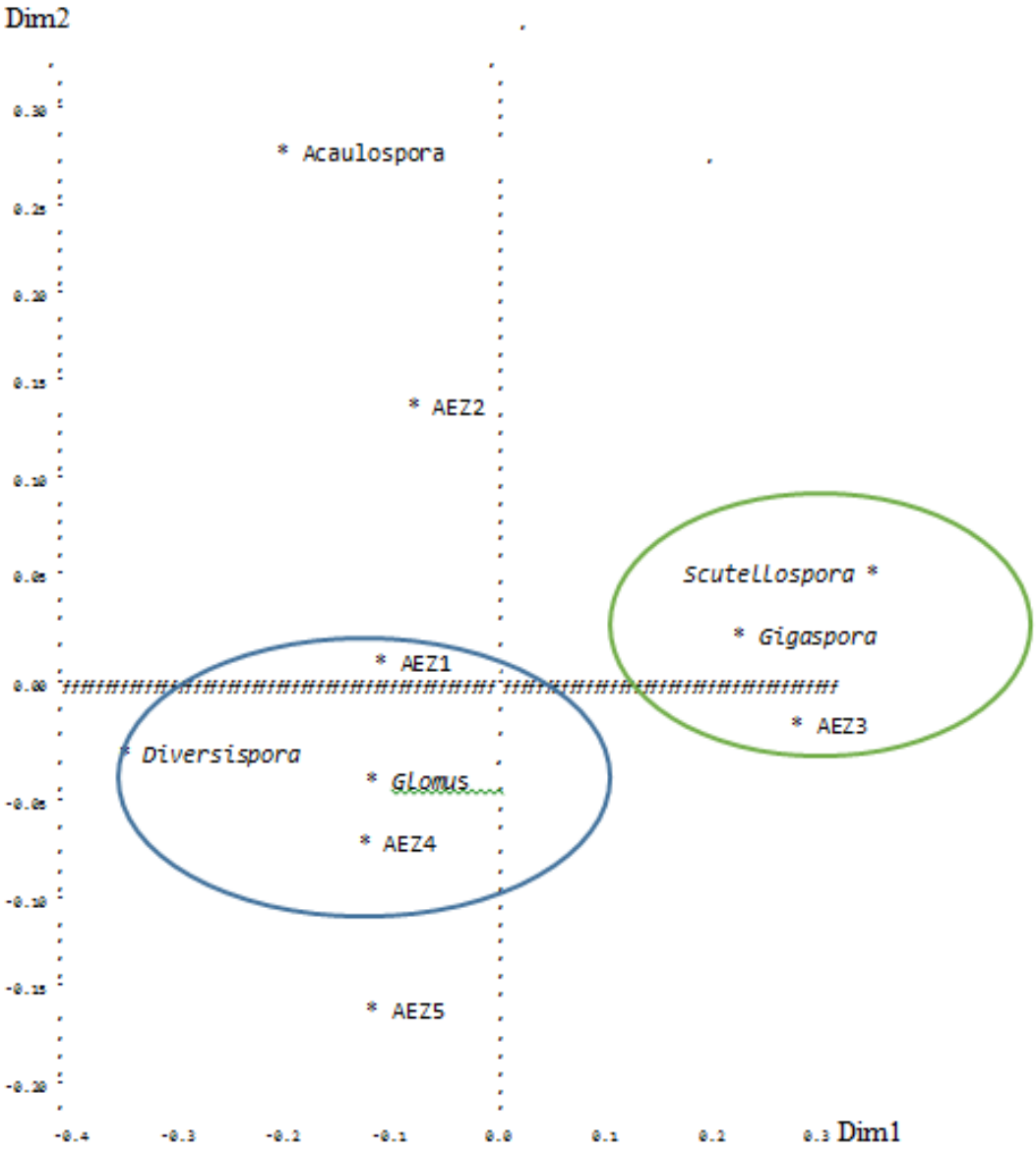

Figure 3. Typology of AEZ according to AMF species.

level by one unit led to 97% decrease of F%. Additionally, the Shannon and Hill indexes are positively correlated and both of them increased or decreased in the same way.

In AEZ 3, a significant positive correlation (p = 0.0285) between soil pH and spore density was recorded (Table 5). So increasing or decreasing soil pH could affect spore density in soil. Additionally, a significant negative correlation (r = -0.952) appeared between P and I%. So, a P increase leads to I% decrease in 95.2%. Positive correlation was found among diversity indexes.

For Zone AEZ 4, mycorrhization frequency is negatively correlated with Phosphorus (r = -0.99) and all of diversity indexes (Table 5). Increase of phosphorus level in soil leads to a decrease in root colonization frequency of mycorrhizae. Moreover, negative correlation between diversity index and mycorrhization frequency can suggest that fungal species which really mycorrhize Bambara groundnut roots were few present in this zone. We have earlier seen that this zone has acceptable diversity; but species which are most capable of forming mycorrhizal infections are rarely observed. If diversity declined, efficient fungi became abundant and could induce high mycorrhization frequency levels. Additionally, it appeared that Phosphorus positively affected the Shannon index and also all of diversity indexes are positively correlated (Table 5).

For AEZ 5, correlation results were in general not

Table 3. Different diversity index values in each of the zones.

Studied factor	Modality	H'	1-D	1-Hill
AEZ	AEZ 1	1.358 ± 0.02^{ab}	0.639 ± 0.007^{ab}	0.597 ± 0.013^{a}
	AEZ 2	1.402 ± 0.03^{ab}	0.677 ± 0.009^{a}	0.636 ± 0.016^{a}
	AEZ 3	1.149 ± 0.04^{c}	0.604 ± 0.013^{bc}	0.471 ± 0.033^{b}
	AEZ 4	1.469 ± 0.08^{a}	0.654 ± 0.033^{ab}	0.639 ± 0.043^{a}
	AEZ 5	1.240 ± 0.02^{cb}	0.553 ± 0.007^{c}	0.476 ± 0.176^{b}

AEZ: Agro-ecological zone. Means which have the same letter are not significantly different (*P*>0.05) based on the Student Newman-Keuls test.

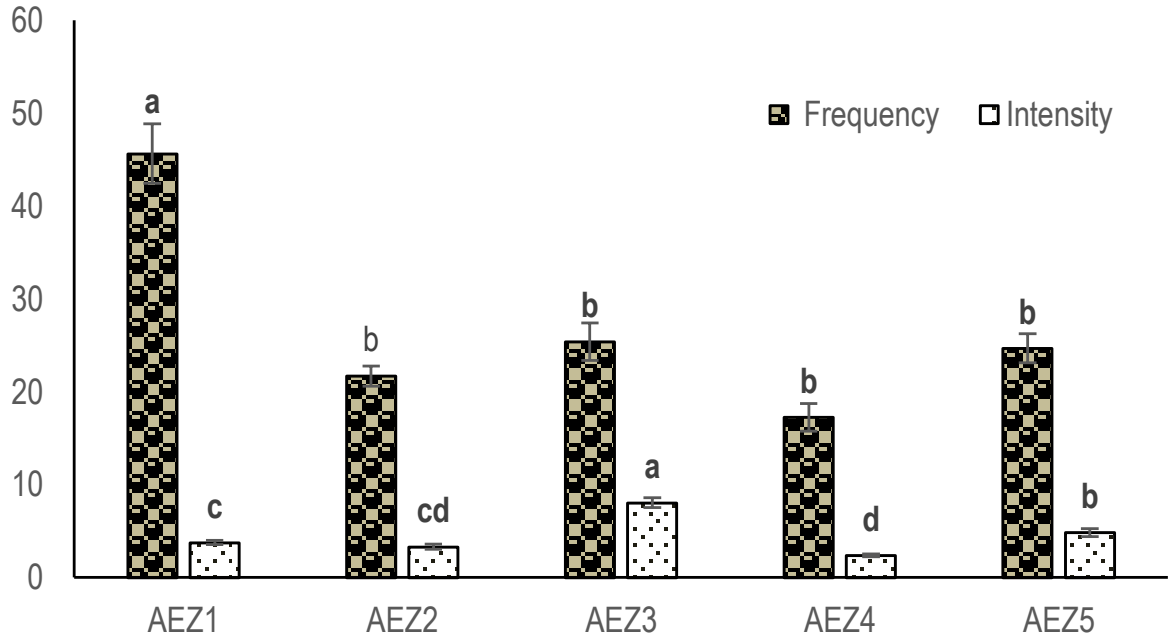

Figure 4. Mycorrhization percent frequency and intensity according to AEZ.

Table 4. Soils characteristics of surveyed zones.

Studied factor	Modality	pH	Phosphorus (ppm)	Nitrogen (%)	Carbon (%)
AEZ	AEZ 1	5.48±0.03^{d}	26.64±0.43^{b}	0.04±0.0009^{c}	0.35±0.006^{d}
	AEZ 2	5.82±0.03^{c}	29.50±0.39^{a}	0.063±0.0015^{a}	0.63±0.012^{a}
	AEZ 3	6.15±0.04^{b}	26.61±0.78^{b}	0.07±0.0014^{a}	0.54±0.008^{b}
	AEZ 4	6.25±0.09^{b}	21.98±0.38^{c}	0.068±0.0013^{b}	0.52±0.019^{b}
	AEZ 5	6.58±0.18^{a}	28.84±0.46^{a}	0.037±0.0016^{c}	0.45±0.015^{c}

AEZ: Agro-ecological zone. Means which have the same letters are not significantly different (*P*>0.05) on the basis of a Student Newman-Keuls test. Different letters represent the SNK groups ranking.

significant except mycorrhization frequency and Shannon index which have shown significant positive correlation (r = 0.986; p = 0.0135) (Table 5). It can be notice that only correlations statistically significant have been taken into account here.

DISCUSSION

Density of spores associated to Bambara groundnut

Most of the surveyed zones have a high level of spore

Table 5. Summary of different significant correlations between studied parameters in all surveyed zones.

Correlation of Pearson	AEZ 1	AEZ 2	AEZ 3	AEZ 4	AEZ 5
Nitrogen * Frequency	0.958*	-0.970*	ns	ns	ns
Nitrogen * Simpson	0.999***	ns	ns	ns	ns
Nitrogen * Hill	0.974*	ns	ns	ns	ns
Frequency * Phosphorus	ns	ns	ns	-0.990*	ns
Intensity * Phosphorus	ns	ns	-0.952*	ns	ns
Shannon * Phosphorus	ns	ns	ns	0.969*	ns
Density * pH	ns	ns	0.972*	ns	ns
Frequency * Simpson	0.960*	ns	ns	-0.956*	ns
Frequency * Shannon	ns	ns	ns	-0.987*	0.986*
Frequency * Hill	ns	ns	ns	-0.970*	ns
Shannon * Hill	0.991**	0.986*	0.995**	0.996*	ns
Simpson * Hill	0.967*	ns	0.996**	0.985*	ns
Shannon * Simpson	ns	Ns	0.983*	0.984*	ns

***: Very highly significant (p<1‰), **: Highly significant (p<1%) *: Significant (p<5%) ns: Not significant at 5%.

densities. These spore densities under Bambara groundnut range from 2826 to 5713 spores per 100g of soil. These recorded densities were less than those recorded by Bossou et al. (2019) under maize (6260 spores per 100 g dry weight soil) but higher than those by Johnson et al. (2013) under cowpea (202 ± 42 per 100 g dry weight soil), by Balogoun et al. (2015) in cashew orchards and by Houngnandan et al. (2009) in *Isoberlinia doka* habitats (237 to 258 spores per 100g of soil). These high spore levels could be due to different factors; and Brundrett (2009) indicated that the presence and natural distribution of glomales were controlled not only by floristic composition but also by environmental conditions. Additionally, legumes had an ability to promote development of fungal propagules by releasing some exudates in their rhizosphere, which favoured development and increase of microorganisms including mycorrhizal fungi (Scheublin et al., 2004). Indeed, a crop species can directly influence mycorrhizal fungal spore's abundance and composition (Eom et al., 2004; Lovelock et al., 2003). The results in the current study have shown that the largest spore amounts were recorded in AEZ 2, which is a cotton cropping area. In this part of Benin, the land-use system is mostly an agroforestry system in which many forest species such as *Vitellaria paradoxa*, *Parkia biglobosa* and *Tamarindus indica* (Gnangle et al., 2012) were kept in fields during land occupation. Indeed, species such as *Parkia biglobosa* and *Tamarindus indica* are also legumes harbouring in their soil and root habitat significant fungal flora that increase in population over many years; and these floras are beneficial for subsequent crops (Guissou et al., 1998). In addition, the cropping fields constitute a continual rotating environment where involved crops promote, with times, a high spore's abundance (Houngnandan et al., 2009). Furthermore, in the current study, zones where high spore densities were found are mostly characterized by sandy or sandy loamy soils.

This could also explain high densities recorded in these zones because sandy soils are said to favour high glomales populations (Dalpé, 1989). Ferruginous soils on sandstone and the presence of many rocks and concretions (in AEZ 4) as well as heavily degraded soils (in AEZ 5) have limited fertility and may be the cause of low spore densities in these soils compared to others zones. It is also important to note that the samples were taken during the flowering period. Knowing that the crops establishment is done during the raining period, the unimodal climate (a long dry season followed by a long rainy season), and the period from sowing to flowering associated with favourable environmental conditions (moisture and presence of the roots of the plant) could have allowed an activation and multiplication of spores that could explain the high densities recorded. Indeed, Bohrer et al. (2003) reported that spores number is higher in the soil exposed to relatively long water stress conditions (dry season) thanks to the production of spores.

Furthermore, small-sized spores are most abundant compared to those with large size in all surveyed zones. These results are supported by research findings of Bossou *et al.* (2019) and Johnson et al. (2013) who indicated that AMF spore densities were proportionally inverse with respect to their sizes. These study results are consistent with findings from Bossou et *al.* (2019) who showed a largest abundance of black spores followed by white spores in all surveyed zones. However, these results are in opposite with findings from Johnson

et al. (2013) who reported that black spores became first in terms of density but followed by brown spores. These different results could be explained due to differences in plant species, which were not the same in these different studies areas.

AMF diversity

The fourteen different morphotypes collected and identified based on the morphological characters belong to the genera of *Glomus, Gigaspora, Acaulospora, Scutellospora* and *Diversispora*. From these genera, *Glomus* and *Gigaspora* were more frequently recorded from all surveyed AEZs. The richness of these genera was higher than those obtained in Benin under different crop habitats including corn (Bossou et al., 2019), cashew (Balogoun et al., 2015) and *Isoberlinia doka* (Craib and Stapf) (Houngnandan et al., 2009). On the other hand, this genera richness was low as compared to that obtained by Tchabi et al. (2008) in the sub-Saharan savannahs (08 genera), by Johnson et al. (2013) under cowpea in all AEZs in Benin and by Diop et al. (1994) in Senegal (15 species). Results from the present study showed that the spore diversity of fungi varies relatively little from one AEZ to another, with *Glomus* the most abundant genus in all zones. Indeed, this predominance of *Glomus* was reported in AMF morphotypes in various tropical soils (Tchabi et al., 2008; Houngnandan et al., 2009) and in agricultural soils from temperate zones (Oehl et al., 2003; Mathimaran et al., 2005). Abundance of this genus in different agro-ecosystems might indicate that it is the most adaptable and available in different environments (Daniell et al., 2001; Brito et al., 2012). Diversity analysis has also shown that AEZ 1 and AEZ 4 were characterized by genera *Glomus* and *Diversispora* and AEZ 3 by *Gigaspora* and *Scutellospora.* This might indicate that some genera are typical to each zone. This is an important aspect to consider for the determination of the type of inoculum appropriate for each zone.

Mycorrhization frequency and intensity

Mycorrhization frequencies and intensities varied amongst zones. The area recording the highest mycorrhization frequency had neither a good infection level nor the highest levels of soil chemical parameters. At the same time, the area with the highest levels of spore density and different soil chemical parameters recorded low mycorrhization frequency and intensity. This could indicate that low levels of certain soil parameters (nitrogen, carbon, phosphorus and pH) favour the colonization capacity of Bambara groundnut roots by the fungal species present. Indeed, these results corroborate a previous report from Houngnandan et al. (2009) who showed that plots with low phosphorus levels were those with high frequencies of mycorrhization. Conversely, Liu et al. (2012) have shown that high levels of Nitrogen and Phosphorus have reduced Glomeromycetes populations in the soil. There is a very weak correlation between density, frequency and intensity of mycorrhization. It is said that living spores of AMF present in the soil may not function as propagules, but they may be quiescent (inactive because soil conditions are unsuitable) or have an innate period of dormancy-mechanisms, which may help them survive during periods of adverse soil conditions (Brundrett, 2009). In addition, soil parameters might diversely influence establishment of the symbiosis between the mycorrhizal fungi and Bambara groundnut roots.

Correlation between different studied parameters

There is much evidence supporting the hypothesis of a large and diversified influence of soil properties on AM fungi (Sano et al., 2002; Johnson et al., 2005; Mechri et al., 2008; Gryndler et al., 2009). It appeared that AM species may survive and function well within a range of soil and environmental conditions. In the current study, almost no significant correlation was found between soil chemical parameters and spore density, with the exception of soil pH in AEZ 3, where positive correlation with density occurred. Tchabi et al. (2008) reported similar results showing spore production increase with soil pH. In contrast, Houngnandan et al. (2009) found correlation amongst most parameters and indicated a negative correlation not only between Phosphorus, Nitrogen and Soil carbon with spore density but also between mycorrhizal frequency and spore density. In an earlier report, Subramanian et al. (2006) have already indicated that application of Phosphorus can influence spore production either positively or negatively.

In the current study, there was no relationship between mycorrhization frequency and intensity. Indeed, the zone with the highest frequencies did not necessarily have strong intensities of mycorrhization and vice versa. However, negative correlation was denoted between mycorrhization frequency or intensity with diversity index. These might stipulate that efficiency of mycorrhization depends on the diversity of species or genera present in the plant rhizosphere. In this study, negative correlations between Nitrogen and Phosphorus with the frequency and intensity of mycorrhization were found. This might show that increase occurrence of some soil parameters may lead to reduction of mycorrhization level. Furthermore, species diversity was positively correlated with some soil parameters (Nitrogen and Phosphorus). This could stipulate that certain species have preferences and tolerance levels in terms of physico-chemical soil parameters; and this could lead to their appearance or disappearance in different soils. These results were opposite to those of Liu et al. (2012) who did not indicate

any relationship between soil parameters and diversity. Also, Johnson et al. (2013) found that AMF diversity indexes are negatively correlated with both available and total Phosphorus. All these results may be natural, because AMF are living organisms and have different preference or tolerance levels to some environmental factors.

Conclusion

The present study has shown that there is an important spore density of AMF associated with Bambara groundnut that differs significantly from one AEZ to another. In addition, it appeared that soil parameters diversely influence both mycorrhization frequency and intensity, but also AMF diversity. Also, it should be noted that having high spore densities does not imply higher levels of symbiosis. Furthermore, it appeared that AEZ 1 and AEZ 4 were characterized by *Glomus* and *Diversispora* genera; but *Gigaspora* and *Scutellospora* characterized AEZ 3. So it would be appropriate that further studies be carried out in order to confirm these results. Also, achievement of molecular characterization will allow most accuracy in species identification. This will enable an evaluation of effectiveness and efficiency of different collected species in order to develop some ecological technologies in Bambara groundnut fertilization based on AMF.

CONFLICTS OF INTERESTS

The authors have not declared any conflicts of interests.

REFERENCES

Aboubacar K, Ousmane ZM, Amadou HI, Issaka S, Zoubeirou AM (2013). Effet de la co-inoculation du rhizobium et de mycorhizes sur les performances agronomiques du niébé au Niger. Journal Applied Bioscience 72:5846-5854.

Balogoun I, Saïdou A, Kindohoundé NS, Ahoton EL, Amadji GL, Ahohuendo BC, Babatoundé S, Chougourou D, Baba-Moussa L, Ahanchédé A (2015). Soil Fertility and Biodiversity of Arbuscular Mycorrhizal Fungi Associated with Cashew's (*Anacardium occidentale* L.) Cultivars Characteristics in Benin (West Africa). International Journal of Plant and Soil Science 5(1):50-63.

Bamshaiye OM, Adegbola JA, Bamishaiye EI (2011). Bambara groundnut: An Under-Utilized Nut in Africa. Advances in Agricultural Biotechnology (1):60-72.

Bohrer G, Kangan-Zur V, Roth-Bejerano N, Ward D, Beck G, di Bonifacio E (2003). Effect of different Kalahari-desert VA mycorrhizal communities on mineral acquisition and depletion from soil by hast plants. Journal of Arid Environment 55:193-208.

Bossou LDR, Houngnandan HB, Adandonon A, Zoundji C, Houngnandan P (2019). Diversité des champignons mycorhiziens arbusculaires associés à la culture du maïs (Zea mays L.) au Bénin. International Journal of Biological and Chemical Sciences 13 (2):597-609.

Brito I, Goss MJ, De Carvalho M (2012). Effect of tillage and crop on arbuscular mycorrhiza colonization of winter wheat and triticale under Mediterranean conditions. Soil Use and Management 28:202-208.

Brundrett M (2009). Mycorrhizal associations and other means of nutrition of vascular plants: understanding the global diversity of host plants by resolving conflicting information and developing reliable means of diagnosis. Plant Soil 32:37-77.

Dalpé Y (1989). Ericoid mycorrhizal fungi in the Myxotrichaceae and Gymnoascaceae. New Phytologist 113(4):523-527.

Daniell TJ, Husband R, Fitter AH, Young JPW (2001). Molecular diversity of arbuscular mycorrhizal fungi colonising arable crops. FEMS Microbiology Ecology 36:203-209.

Dansi A, Vodouhè R, Azokpota P, Yedomonhan H, Assogba P, Adjatin A, Loko YL, Dossou-Aminon I, Akpagana K (2012). Diversity of the Neglected and Underutilized Crop Species of Importance in Benin. The Scientific World Journal, pp. 932-947.

Diop TA, Gueye M, Dreyfus BL, Plenchette C, Strullu DG (1994). Indigenous arbuscular mycorrhizal fungi associated with *Acacia albida Del*. in different areas of Senega*l.* Applied and Environmental Microbiology 60(9):3433-3436.

Do Rego FA, Diop I, Sadio O, Da Sylva MC, Agbangba CE, Touré O, Kane A, Neyra M, Ndoye I, Krasova-Wade T (2015). Response of cowpea to symbiotic microorganism's inoculation (Arbuscular mycorrhizal fungi and Rhizobium) in cultivated soils in Senegal. Universal Journal of Plant Science 3(2):32-42.

Eom AH, Hartnett DC, Wilson GWT (2004). Host plant species effects on arbuscular mycorrhizal fungal communities in tallgrass prairie. Ecology 122:435-444.

Gbaguidi AA, Dossou-Aminon I, Agre AP, Dansi A, Rudebjer P, Hall R, Vodouhe R (2016). Promotion de la chaine des valeurs des espèces négligées et sous-utilisées au Bénin : cas du voandzou (*Vigna subterranea* L. Verdcourt.). International Journal of Neglected and Underutilized Species 2:19-32.

Gbaguidi AA, Sanoussi F, Orobiyi A, Dansi M, Akouegninou BA, Dansi A (2015). Connaissances endogènes et perceptions paysannes de l'impact des changements climatiques sur la production et la diversité du niébé (*Vigna unguiculata* (L.) Walp.) et du voandzou (*Vigna subterranea* (L) Verdcourt.) au Bénin. International Journal of Biological and Chemical Sciences 9(5):2520-2541.

Gnangle PC, Afouda YJ, Yegbemey RN, Glèlè Kakaï RL, Sokpon N (2012). Rentabilité économique des systèmes de production des parcs à karité dans le contexte de l'adaptation au changement climatique du Nord-Bénin. African Crop Science Journal 20(2):589-602.

Gryndler M, Hrselová H, Cajthaml T, Havránková M, Rezáčová V, Gryndlerová H, Larsen J (2009). Influence of soil organic matter decomposition on arbuscular mycorrhizal fungi in terms of asymbiotic hyphal growth and root colonization. Mycorrhiza 19:255-266.

Guissou T, Bâ AM, Ouadba J-M, Guinko S, Duponnois R (1998). Responses of Parkia biglobosa (Jacq.) Benth, Tamarindus indica L. and Zizyphus mauritiana Lam. to arbuscular mycorrhizal fungi in a phosphorus-deficient sandy soil. Biology and Fertility of Soils 26:194-198.

Haougui A, Souniabe PS, Doumma A, Adam T (2013). Evolution of mycorrhizal fungi population on weeds of four garden in the Maradi region of Niger. International Journal of Biological and Chemical Sciences 7(2):554-565.

Haro H, Sanon KB, Krasova-Wade T, Kane A, N'doye I, Traore AS (2015). Réponse à la double inoculation mycorhizienne et rhizobienne du niébé (variété, KVX396-4- 5-2D) cultivé au Burkina Faso. International Journal of Biological and Chemical Sciences 9(3):1485-1493.

Houngnandan P, Yemadje RGH, Kane A, Boeckx P, Van Cleemput O (2009). Les glomales indigènes de la forêt claire à *Isoberlinia doka* (Craibet Stapf) à Wari-Maro au centre du Bénin. Tropicultura 27(2):83-87.

Igue AM, Saidou A, Adjanohoun A, Ezui G, Attiogbe P, Kpagbin G, Gotoechan Hodonou H, Youl S, Pare T, Balogoun I, Ouedraogo J, Dossa E, Mando A, Sogbedji JM (2013). Evaluation de la fertilité des sols au sud et centre du Bénin. Bulletin de la Recherche Agronomique du Bénin (BRAB) 12(23):1840-7099.

Johnson JM, Houngnandan P, Kane A, Sanon KB, Neyra M (2013). Diversity patterns of indigenous arbuscular mycorrhizal fungi associated with rhizosphere of cowpea (*Vigna unguiculata* (L.) Walp.) in Benin, West Africa. Pedobiologia 56:121-128.

Liu Y, Shi G, Mao L, Cheng G, Jiang S, Ma X, An L, Du G, Johnson NC,

Feng H (2012). Direct and indirect influences of 8yr of nitrogen and phosphorus fertilization on Glomeromycota in an alpine meadow ecosystem. New Phytologist 194:523-535.
Lovelock CE, Andersen K, Morton JB (2003). Arbuscular mycorrhizal communities in tropical forests are affected by host tree species and environment. Ecologia 135:268-279.
Massawe FJ, Mwale SS, Azam-Ali SN, Roberts JA (2005). Breeding in Bambara groundnut [*Vigna subterranea* (L.) Verdcourt]: Strategic considerations. African Journal of Biotechnology 4(6):463-471.
Mathimaran N, Ruh R, Vullioud P, Frossard E, Jansa J (2005). *Glomus intraradices* dominates arbuscular mycorrhizal communities in a heavy textured agricultural soil. Mycorrhiza 16:61-66.
Mazahib AM, Nuha MO, Salawa IS, Babiker EE (2013). Some nutritional attributes of bambara groundnut as influenced by domestic processing. International Food Research Journal 20(3):1165-1171.
Mechri B, Mariem FB, Baham M, Elhadj SB, Hammami M (2008). Change in soil properties and the soil microbial community following land spreading of olive mill wastewater affects olive trees key physiological parameters and the abundance of arbuscular mycorrhizal fungi. Soil Biology Biochemical 40:152-161.
Ndiang Z, Bell JM, Missoup AD, Fokam PE, Amougou A (2012). Etude de la variabilité morphologique de quelques variétés de voandzou [*Vigna subterranea* (L.) Verdc] au Cameroun. Journal of Applied Biosciences 60:4410-4420.
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.
Phillips JM, Hayman DS (1970). Improved procedures for cleaning roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Transactions of the British Mycological Society 55:158-161.
Saïdou A, Etèka AC, Amadji GL, Hougni D-GJM, Kossou D (2012). Dynamique des champignons endomycorhiziens dans les jachères manioc sur sols ferrugineux tropicaux du Centre Bénin. Annales des Sciences Agronomiques 16(2):215-228.
Sano SM, Abbott LK, Solaiman MZ, Robson AD (2002). Influence of liming, inoculum level and inoculum placement on root colonization of subterranean clover. Mycorrhiza 12:285-290.
Scheublin TR, Ridgway KP, Young JPW, van der Heijden MGA (2004). Nonlegumes, Legumes, and Root Nodules Harbor Different Arbuscular Mycorrhizal Fungal Communities. Applied Environmental Microbiology 70(10):6240-6246.
Smith SE, Read DJ (2008). Mycorrhizal Symbiosis. Academic 787 P.
Subramanian KS, Santhanakrishnan P, Balasubramanian P (2006). Responses of field grown tomato plants to arbuscular mycorrhizal fungal colonization under varying intensities of drought stress. Scientia Horticulturae 107:245-253.
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 18:181-195.
Touré Y, Koné M, Silué YS, Kouadio J (2013). Prospection collecte et caractérisation agromorphologique des morphotypes de voandzou [*Vigna Subterranea* (L.) Verdcourt (Fabaceae)] de la zone savanicole en Côte d'Ivoire. European Scientific Journal 9(24):1857-7881.
Trouvelot A, Kouch J, Gianinazzi-Pearson V (1986). Les mycorhizes, physiologie et génétique. INRA, Dijon, pp. 217-221.
Usharani G, Sujitha D, Sivasakthi S, Saranraj P (2014). Effect of arbuscular Mycorrhizal (AM) fungi (*Glomus fasciculatum* L.) for the improvement of growth and yield of maize (*Zea mays* L.). Central European Journal of Experimental Biology 3(2):30-35.
Vertès F, Jeuffroy MH, Louarn G, Voisin AS, Justes E (2015). Légumineuses et prairies temporaires : des fournitures d'azote pour les rotations. Fourrages 223:221-232.