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Effect of phosphorus fertilization on arbuscular mycorrhizal fungi in the Bambara groundnut rhizosphere

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Tropical soils are highly leached, abundantly clayey and strongly acid, resulting in low mineral availability and especially phosphorus (P). The plants of this region must establish symbiotic relationships enabling them to strengthen their hydromineral nutrition. The aim of this work was to study the effect of P fertilizer application on the diversity of arbuscular mycorhizal fungi (AMF) under the rhizosphere of three Bambara groundnut (*Vigna subterranea*) landraces. To attain it, plants were grown in farm under different simple superphosphate (SSP) levels (0, 50, 100, 150 and 200 kg.ha^{-1} P_2O_5) in 2015 at Mendong and Soa districts. Experimental units were arranged in a randomized complete block design with three replications. Soil and root samples were taken from the rhizosphere of three randomly selected seeds holes per experimental unit at flowering (31 days after sowing) and fruition stages. At harvest, the roots were stored in 50% ethyl alcohol. They were stained with methyl blue and observed under an optical microscope. The soil samples allowed the trapping to be carried out in a greenhouse with sorghum as test plants. Results show that Bambara groundnut landraces were the host of several AMF species and were highly colonized with all showing hyphae, vesicles and even spores. High level of SSP (200 kg.ha^{-1} P_2O_5) significantly ($p<0.001$) reduced the intensity of mycorrhization as well as the diversity indices compared to the control. A density of AMF spores reaching 1930 was obtained in 100 g of trapping substrate. On the basis of morphological characteristics, 16 AMF species belonging to nine genera (*Glomus, Acaulospora, Gigaspora, Racocetra, Rhizophagus, Funneliformis, Septoglomus, Diversispora* and *Claroideoglomous*) were isolated and identified. *Rhizophagus intraradices* and *Septoglomus constrictum* were the most abundant. AMF species identified in this groundnut rhizosphere can be multiplied and used as biological fertilizer to increase its yield.

Key words: Arbuscular mycorhizal fungi (AMF) diversity, Cameroon, morphological characteristics, rhizosphere, root colonization, spore density, symbiosis, phosphate fertilization, Voandzou.

INTRODUCTION

In the world in general and in Africa in particular, about 795 million people are undernourished (one in four in sub-Saharan Africa) and suffer from insufficient energy intake associated with protein, vitamin and mineral deficiencies (WFP, 2016). Moreover, population projections predict an increase in world population. This demographic growth far exceeds that of agricultural production. Indeed, this agricultural production must increase by 70% for a world population of 2.3 billion inhabitants in 2050 (FAO, 2017). The challenge of agricultural research is to contribute to increasing crop yields while safeguarding the environment. Bambara groundnut (*Vigna subterranea* (L.) Verdc.) could play an important role because its seeds are highly caloric, rich in minerals, vitamins, proteins and amino acids (De Kock, 2013; Yao et al., 2015; Tsoata et al., 2017a).

In fact, seed legumes (Bambara groundnut) have an important socio-economic role in tropical Africa, where they are a tradition in the culinary habits of populations (Brink et al., 2006). Bambara groundnut is native to northeastern Nigeria and northern Cameroon (Begemann, 1988). Its world production was estimated at 216575 t in 2013. In Africa, its production has not varied considerably since 1993 and is about 300 000 t/year. In Cameroon, production increased from 5800 t in 1993 to 38075 t in 2014 (FAO, 2017), making it the third largest producer in the world. Bambara groundnut seeds are used as feed for humans, poultry and livestock (Brink et al., 2006).

They contain on average of 63% carbohydrates, 19% protein and 6.5% fat; these values are considered sufficient to make this legume a complete food (Bamishaiye et al., 2011) to consider for food security (De Kock, 2013; Yao et al., 2015). Bambara groundnut has medicinal properties well known to local populations (Brink et al., 2006). One of the main attributes of Bambara groundnut is its tolerance to poor soils (Temegne et al., 2015) and drought (Berchie et al., 2012; Tsoata et al., 2016; 2017b) as well as its ability to produce under conditions where groundnuts fail completely (Jideani and Diederiks, 2014). Despite this panoply of properties, its production remains low. This low production is reflected in rising market prices and scarcity of seeds (IRAD, 2013). This low production is due to diseases and pests but above all poverty of soil (FAO, 2003), in particular phosphorus (P) deficiency.

However, very little information exists on the biological factors that support the Bambara groundnut growth on poor soils. P is an essential mineral for living organisms which is after nitrogen an indispensable element to the good functioning of the plants (Morel et al., 2006). P is mainly extracted from phosphate (P_i) rock (natural P_i). Worldwide reserves of commercial natural P_i are currently estimated at 12 billion tons with an annual consumption of 132 million tons. These reserves are barely enough for a hundred years (Frossard et al., 2004). As a result, P was placed on the red list of raw materials by the European Commission in 2014 (CE, 2014).

Plant production is limited by low P availability due to inability to take P in orthophosphate ions form, either directly through roots or through arbuscular mycorrhizal fungi (AMF). Among the functional groups composing the telluric microflora, AMF play a major role in improving water and mineral nutrition. Thus, many plant species rely heavily on AMF for their survival, especially in the tropics, where the majority of soils are highly leached, clayey and highly acidic, resulting in low mineral availability, especially in P.

Many studies have also shown that AMF improve water and mineral nutrition of plants and in particular the Pi plants supply (Onguene et al., 2011; Taffouo et al., 2014). Some studies showed that phenological stages influence mycorrhizal activity (Mbogne et al., 2015; Johnson et al., 2016) but little is known about the chemical P fertilizer effect on it. Some work done in Cameroon on the mycorrhization of bambara groundnut did not take into account the AMF biodiversity under cultivation (Ngakou et al., 2012; Tsoata et al., 2015). Several researchers (Ngonkeu et al., 2003; Nwaga et al., 2003) recommend the use of indigenous (native) AMF as biological fertilizers because of their adaptation to local conditions. The objective of this work was to evaluate the AMF diversity in the Bambara groundnut rhizosphere under P_i fertilization.

MATERIALS AND METHODS

Plant material

Three Bambara groundnut landraces obtained from the producers were used: V1, the ivory cream (or white) seed coat; V2, the red seed coat and V3, the ivory cream seed coat with grey eyes (hilum) (V3). These landraces were chosen because they are among the most appreciated by the producers.

Study area

The study was conducted in Soa and Mendong localities. The Soa (N 03°58'647", E 011°34'361", altitude: 680 m) and Mendong (N 03°50'12 to 12.5", E 011°27'05.6 to 09", altitude: 717 m) sites are located in the Center region of Cameroon. They are characterized by precipitation from 1,617 to 1,800 mm/year. The average daily air temperature varies from 23 to 24°C (IRAD, 2008). They belong to

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Table 1. Physio-chemical properties of the soil of the experimental sites.

Site	Sand	Clay	Silt	OC	N	C/N	pH	P	CEC	Na^+	K^+	Mg^+	Ca^{2+}
	%					-		µg/g	cmol/kg				
Mendong	46.99	40.99	11.41	2.50	0.21	11.81	6.50	7.59	8.18	0.030	0.45	1.92	8.44
Soa	58.23	31.49	10.28	2.54	0.21	12.36	5.09	4.75	6.59	0.029	0.22	1.30	2.17

OC, Organic Carbon; N, total N; P, phosphorus (Bray 2); CEC, cation exchange capacity.

the agro-ecological zone of bimodal wet forest, characterized by acidic ferralitic soils. The area is characterized by a bimodal rainfall pattern, with four seasons: long rainy season from September to November, long dry season from December to February, short rainy season from March to June and short dry season from July to August. The previous crop of the Mendong study area was rice (*Oryza sativa* L.) with a predominance of species such as *Chromolaena odorata* L. and *Imperata cylindrica* L. The site of Soa was an old shrub fallow.

Soil sampling from the field

In each site, a composite sample of the top soil (0 to 15 cm depth) was collected from the experimental site with an auger before bed preparation following the transect method described by Okalebo et al. (2002). About 200 g of the samples were collected and afterward mixed to obtain a composite sample of the field.

Soil physical and chemical analyses

Physical and chemical properties were analyzed in the International Institute of Tropical Agriculture (IITA) soil laboratory of Nkolbisson (Cameroon). Soil sample was air-dried and ground to pass through a 2 mm sieve. For carbon (C) and nitrogen (N) analysis, soil was further fine ground to pass through a 0.5 mm sieve. Soil pH in water was determined in a 1:2.5 (w/v) soil: water suspension. Organic C was determined by chromic acid digestion and spectrophotometric analysis (Heanes, 1984). Total N was determined from a wet acid digest and analyzed by colorimetric analysis (Anderson and Ingram, 1993). P was extracted using Bray extractant and the resulting extract analyzed using the molybdate blue procedure described by Murphy and Riley (1962). Exchangeable cations - Ca, Mg, K and Na were extracted using the ammonium acetate (NH_4OAC, pH: 7) and determined by flame atomic absorption spectrophotometry. Cation exchange capacity (CEC) was determined using ammonium acetate. Results of the soil physico-chemical properties analysis are presented in Table 1. Mendong soil is weakly acidic (pH: 6.52) and Soa acidic (pH: 4.64). The soils of the two sites are poor in N and P in which Mendong soil is richer in P than in Soa with both sites have sandy-clay soil. The low C/N ratios at both sites reflect rapid decomposition of organic matter. It leads to a malfunctioning of the clay-humic complex.

Experimental design

The experimental set-up was a complete randomized block with three factors: phosphorus levels (0, 50, 100, 150 and 200 $kg.ha^{-1}$ P_2O_5) (Toungos et al., 2010; Nweke and Emeh, 2013), landraces (V1, V2 and V4) and sites (Mendong and Soa).

Experimental layout

In each site, after clearing and then plowing between 20 and 30 cm deep, the blocks and the experimental plots were delimited using a double decameter, a string and stakes. Each experimental unit consisted of one of three landraces of Bambara groundnut combined with one level of phosphorus. The distance between lines was 25 cm and in the line the spacing between the plants was 25 cm. A space of 25 cm was left at the edges of each unit. A total of 49 seed holes/4m m^2, that is, 1,470 seed holes for the 336 m^2 were seeded. Sowing was carried out on September 13, 2015 in Mendong and on September 26, 2015 in Soa. The sorted seeds were sown (3 to 5 cm deep) directly in the field at three to four seeds/seed hole. Thinning was kept as one to two vigorous seedlings per hole. The different levels of phosphorus fertilizer (P_2O_5) were applied two weeks after sowing. Urea (46% N) was supplied as a base fertilizer (25 gm^{-2}) to promote vegetative growth of plants. Weeding was done in order to eliminate weeds and reduce pest attacks and diseases. The first weeding was carried out one week after sowing and subsequently weeding and/or hoeing were carried out according to the frequency of grass growth.

Sampling

At flowering and fruition, the roots (three plants per treatment/landrace) were taken from the rhizosphere of the Bambara groundnut pods of each experimental unit at each site. About 5 g of fine roots attached to the main root were thus collected per seed hole and then stored in 50% of ethyl alcohol. After the plant harvest in each experimental unit, soil samples were collected and trapped in the greenhouse at the Institute of Agricultural Research for Development (IRAD).

Root colonization

In the laboratory, roots were thinned and stained (Phillips and Hayman, 1970) before microscopic observation. Approximately 3 to 5 g of each root sample (1 to 2 cm fragment length) of soil were placed in labeled test tubes containing 10 mL KOH (10%, W/V) to empty the contents of the cytoplasm in order to facilitate the observation of mycorrhizal structures. After 24 h, 10 mL of hydrogen peroxide was added to it during 15 min in order to perfect the clarification (Kormanik and McGraw, 1982). The tubes were emptied of the KOH and the roots were thoroughly rinsed three times with tap water. Then, 1% of hydrochloric acid (HCl) was poured into the roots in each tube and left for 15 min to acidify the cell contents. Next, the solution was removed. They were soaked in 10 mL of dye (lactic acid-glycerol-water-methyl blue). After 24 h, the dye (Koske and Gemma, 1989) was removed and three rinses were performed. Discoloration was achieved by introducing the bleach (lactic acid-glycerol-water) into the test tubes. The root fragments (10) were mounted on glass slide. The experiment was repeated three times for each sample. The observation was made with the photonic microscope (OLYMPUS, 100x). For each fragment, the abundance and diversity of the mycorrhizal structures (vesicles, extra and intracellular hyphae, auxiliary cells and spores) were noted. The frequency (F) and the intensity (I) of the mycorrhization were evaluated by the method of Trouvelot et al. (1986).

Table 2. Effect of stage of development on intensity and frequency of mycorhization.

Stage of development	Intensity (%)	Frequency (%)
Flowering	22.12±1.55[a]	41.83±2.55[a]
Fruition	24.00±1.63[a]	43.76±2.35[a]
p	0.181 ns	0.441 ns

Means±Standard error followed by the same letter in a column are not significantly different at the 5% threshold.

AMF trapping in a greenhouse

Approximately 500 g of soil from each sample was sandwiched between two layers of sterile sand in 1 kg plastic bags perforated at the bottom. The latter were seeded with four sorghum seeds (from IRAD) per sachet. These sorghum seeds were previously disinfected with 10% sodium hypochlorite for 15 min and rinsed three times with tap water. The bags were placed in unperforated bins to keep moisture. The plants were allowed to grow under these conditions for three months. Then the sachets were transferred to the shelter in order to provoke a water deficit which can induce sporulation in which after five weeks, the rhizosphere was removed for spore extraction.

AMF spore extraction and identification

For each treatment, a sample of 100 g of substrate taken from each trapping bag was used for the extraction of the spores by wet sieving following the method described by Gerdemann and Nicholson (1963) through a series of sieves ranging from 400 to 38 µm. The spore suspension contained in the sieves was centrifuged on a 50% sucrose gradient (Daniels and Skipper, 1982; Oehl et al., 2003). After rinsing, it was poured onto a filter paper with a grid surface to facilitate the counting of the spores. The spores were counted using a binocular magnifying glass (40× Magnification) according to their size, color, shape, ornamentation and characteristics of their walls and hyphal attachments. The average number of spores was expressed per 100 g of dry substrate. The AMF spores were mounted on glass slides in polyvinyl–lactic acid–glycerine (PVLG) with the Melzer reagent (Josserant, 1983) and identified on the basis of the morphological descriptions published by International Culture Collection of Vesicular Arbuscular Mycorrhizal Fungi (INVAM). AMF types were classified to the genus rank and, if possible, to the species rank and named with the current taxonomy (Schüßler and Walker, 2010; Redecker et al., 2013).

Indices of diversity

The AMF diversity of each site (Castillo et al., 2016) was analyzed using the following indices: species richness (S: number of species in the study area), diversity index (H': Shannon-Wiener (1948) index; Pielou's (1966) evenness index (J'), Simpson's dominance index (*1*) and Simpson's (1949) diversity index (Ds). The indices were calculated by the following formulas:

$$H' = -\sum_{i=1}^{S} Pi \, Log \, Pi; \quad J' = H'/log(S);$$

$$I = \sum[ni(ni-1)]/[N(N-1)]; \quad Ds = 1-I$$

Where, Pi is the proportional abundance or percentage importance of the species (Pi = ni / N); ni is the number of individuals of a species in the sample, N the total number of individuals of all species in the sample and S is the total number of species.

Data analysis

The data were subjected to analysis of variance (ANOVA) using SPSS version 20 software. The Student-Newman-Keuls test was used to compare the means at the 5% threshold. Data that did not comply with the ANOVA assumptions (normality and homogeneity of variance tested through the Shapiro-Wilk's test and Brown and Kolmogrov-smirnov's test, respectively) were subjected to Kruskal-Wallis test. Their means were compared by the Mann-Whitney U test at the 5% threshold.

RESULTS

Effect of stage of development on mycorhization

The stage of development (flowering and fruition) did not influence the frequency and intensity of mycorrhization (Table 2).

Effect of P fertilization on mycorrhization and diversity index

Levels of P_2O_5 did not influence the frequency of mycorrhization (Table 3). The P_2O_5 levels greater than 150 $kg.ha^{-1}$ significantly ($p<0,001$) reduced mycorrhization intensity by 60%. The Shannon-Wiener (H'), Simpson (Ds) and Pielou (J') diversity indices were significantly ($p<0.001$) greater in control than the fertilized plots (Table 3). The dominance of Simpson (1) and the total number of spores were significantly ($p<0.001$) lower in the control treatment (Table 3).

Effect of Bambara groundnut landraces on mycorrhization and diversity index

The Bambara groundnut landraces bear arbuscular and vesicular mycorrhizae. The mycorrhizal structures observed in the roots were in the form of arbuscules, vesicles, spores, intra- or intercellular hyphae and auxiliary cells. Numerous external hyphae were also

Table 3. Effect of P levels on mycorrhization and AMF diversity index under Bambara groundnut rhizosphere at Mendong and Soa areas.

P_2O_5 (kg.ha^{-1})	Site	Intensity (%)	Frequency (%)	H'	J'	*I*	D_S	S
0	M	21.8±3.4^{ab}	48.1±4.5^{ab}	1.82±0.04^{bcd}	0.7±0.01^{bc}	0.24±0.01^{bc}	0.76±0.01^{bc}	14±0.3^{a}
	Soa	30.1±4.3^{a}	33.6±3.4^{bc}	2.17±0.01^{a}	0.82±0.01^{a}	0.14±0.01^{d}	0.86±0.01^{a}	15±0.2^{a}
Mean		25.9±2.9^{A}	40.8±3^{A}	1.99±0.05^{E}	0.76±0.02^{E}	0.19±0.01^{A}	0.81±0.01^{D}	14±0.2^{A}
50	M	19.5±3^{ab}	52.8±4.1^{a}	1.84±0.03^{bcd}	0.69±0.01^{bc}	0.23±0.01^{bc}	0.77±0.01^{bc}	15±0.3^{a}
	Soa	29.2±4^{a}	31.1±6.1^{c}	2.07±0.03^{a}	0.79±0.01^{ab}	0.17±0.01^{cd}	0.83±0.01^{ab}	14±0.2^{a}
Mean		24.4±2.5^{A}	41.9±4.1^{A}	1.96±0.04^{D}	0.74±0.02^{D}	0.2±0.01^{B}	0.8±0.01^{C}	15±0.2^{A}
100	M	21.3±3.6^{ab}	57.2±5.7^{a}	1.68±0.04^{d}	0.64±0.01^{c}	0.28±0.02^{ab}	0.72±0.01^{cd}	15±0.5^{a}
	Soa	25.3±2.9^{a}	29.4±4.9^{c}	1.7±0.11^{d}	0.65±0.04^{c}	0.32±0.04^{a}	0.68±0.04^{d}	14±0.4^{a}
Mean		23.3±2.3^{A}	43.3±4.4^{A}	1.69±0.06^{A}	0.64±0.02^{A}	0.3±0.02^{D}	0.7±0.02^{A}	15±0.3^{A}
150	M	25±3.1^{a}	60.8±3.7^{a}	1.74±0.13^{cd}	0.67±0.05^{bc}	0.27±0.05^{ab}	0.73±0.04^{cd}	14±0.2^{a}
	Soa	26.5±4.3^{a}	33.1±5.8^{bc}	1.96±0.06^{abc}	0.74±0.02^{abc}	0.21±0.02^{bcd}	0.79±0.02^{abc}	15±0.3^{a}
Mean		25.7±2.5^{A}	46.9±4.1^{A}	1.85±0.08^{B}	0.7±0.03^{B}	0.24±0.02^{C}	0.76±0.03^{B}	14±0.2^{A}
200	M	21.5±4.1^{ab}	46.8±5.5^{ab}	2.01±0.1^{ab}	0.76±0.01^{abc}	0.2±0.03^{bcd}	0.8±0.03^{abc}	14±0.2^{a}
	Soa	10.6±0.9^{b}	35±4.7^{bc}	1.81±0.08^{bcd}	0.69±0.03^{bc}	0.27±0.03^{ab}	0.73±0.03^{cd}	14±0.3^{a}
Mean		16.1±2.2^{B}	40.9±3.7^{A}	1.91±0.07^{C}	0.73±0.03^{C}	0.24±0.02^{C}	0.76±0.01^{B}	14±0.2^{A}
p (P_2O_5)		< 0.001***	0.513 ns	< 0.001***	< 0.001***	< 0.001***	< 0.001***	0.609 ns
p (Site)		< 0.001***	< 0.001***	< 0.001***	< 0.001***	< 0.001***	< 0.001***	0.552 ns
p (P_2O_5*Site)		< 0.001***	0.142 ns	< 0.001***	< 0.001***	< 0.001***	< 0.001***	0.108 ns

M, Mendong; H, Shannon-Wiener diversity index; J', Pielou's evenness index; I, dominance of Simpson; Ds, Simpson diversity index, S: species richness. Means±Standard errors followed by the same letter in a column are not significantly different at the 5% threshold.

Table 4. Effects of landraces on mycorrhization and AMF diversity index.

Land-race	Intensity (%)	Frequency (%)	H'	J'	I	DS	S
V1	26.77±2.01c	45.16±3.4a	1.87±0.05a	0.71±0.02a	0.24±0.02c	0.76±0.02a	15±0.16a
V2	22.5±1.77b	41.58±2.77a	1.90±0.05b	0.72±0.02b	0.22±0.02a	0.78±0.02c	14±0.14a
V3	19.91±1.98a	41.64 ±2.81a	1.86±0.05a	0.71±0.02a	0.23±0.02b	0.77±0.02b	14±0.19a
p	< 0.001***	0.408 ns	< 0.01**	< 0.05*	< 0.001***	< 0.001***	0.220

H, Shannon-Wiener diversity index; J', Pielou's evenness index; I, dominance of Simpson; Ds, Simpson diversity index; S, species richness. Means±Standard errors followed by the same letter in a column are not significantly different at the 5% threshold.

observed. The intensity of mycorrhization was higher at V1 and lower at V3; but the landrace did not affect mycorrhizal frequency (Table 4). V2 had the Shannon-Wiener (H'), Simpson (Ds) and Pielou (J') diversity indices higher than those of V1 and V3 (Table 4). The total number of spores of V1 significantly exceeded those of V2 and V3 (p<0.001) by 21 and 43%, respectively.

Effect of site on mycorrhization and diversity index

The frequency of mycorrhization was 1.6 times higher in Mendong than in Soa (Table 5). However, the intensity of mycorhization was higher at Soa than Mendong by 11.6%. The Shannon-Wiener (H'), Pielou (J'), Simpson (Ds) indices and the total number of spores were significantly (p<0.001) higher at Soa than Mendong (Table 5).

Identification of arbuscular mycorrhizal fungi

Bambara groundnut roots showed Mycorrhizal structures in the form of arbuscules, vesicles, hyphae, spores and auxiliary cells (Figure 1). Figure 2 shows some AMF spores isolated from the rhizosphere soil of Bambara

Table 5. Effect of study site on mycorrhization and AMF diversity index.

Sites	Intensity (%)	Frequency (%)	H'	J'	*I*	D_S	S
Soa	24.32±1.65[a]	32.44±2.23[b]	1.94±0.04[b]	0.74±0.01[b]	0.22± 0.02[b]	0.78±0.02[b]	14±0.12[a]
Mendong	21.8±1.53[b]	53.15±2.16[a]	1.82±0.04[a]	0.69±0.01[a]	0.24±0.01[a]	0.76±0.01[a]	14±0.15[a]
p	< 0.001***	< 0.001***	<0.001***	<0.001***	< 0.001***	<0.001***	0.557

H, Shannon-Wiener diversity index; J', Pielou's evenness index; I, dominance of Simpson; Ds, Simpson diversity index; S, species richness. Means±Standard errors followed by the same letter in a column are not significantly different at the 5% threshold.

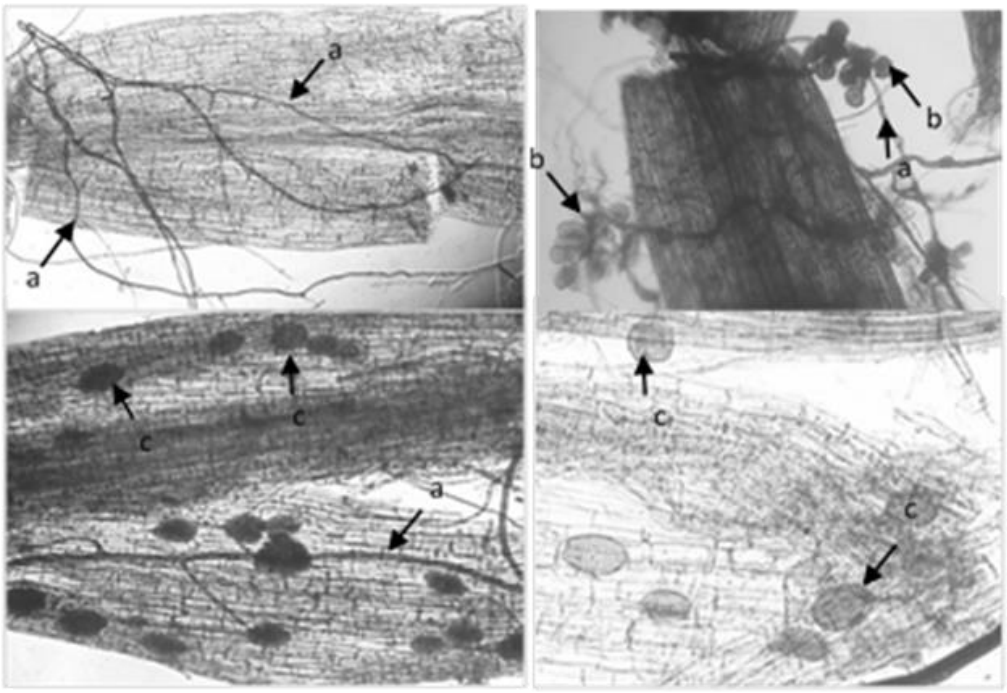

Figure 1. Mycorrhizal structures in Bambara groundnut roots. **a**, hyphae; b, auxiliary cells and c: vesicles.

groundnut. The 16 AMF species (Table 6) identified in the two sites could belong to six different families (Acaulosporaceae, Diversisporaceae, Entrophosporaceae, Gigasporaceae, Glomeraceae and Racocetraceae) and nine genera of *Glomeromycota* branch. The number of AMF spores of various species are presented in Table 7. The AMF diversity was identical in both sites.

DISCUSSION

High level of phosphate fertilizer (200 $kg.ha^{-1}$ P_2O_5) significantly reduced the intensity of mycorrhization. The application of this level of fertilizer might have increased the acidity of the soil. Indeed, the extreme acidity of soils could be the cause of the low density of spores observed in certain species of arbuscular mycorrhizal fungi (AMF) (Bivoko et al., 2013; Mbogne et al., 2015). Bhadalung et al. (2005) pointed out that chemical P fertilization reduces the total number of AMF spores in the long-term. They noted with maize that, only *Acaulospora* sp. maintains its number of spores unchanged under fertilization on the nine species listed, it is therefore insensitive to chemical fertilization.

On the other hand, they noted that the absolute number of spores of *Glomus* sp. and *G. geosporum* decreased in response to fertilization but not relative abundance. *Glomus* sp. and *G. geosporum* are therefore slightly sensitive to fertilization (Bhadalung et al., 2005). However, soils with low availability of phosphorus favor greater mycorrhizal colonization and possibly greater formation of spores (Smith and Read, 2008). The results of this study show that Glomeraceae and Entrophosporaceae are slightly sensitive, Acaulosporaceae are insensitive, Gigasporaceae and

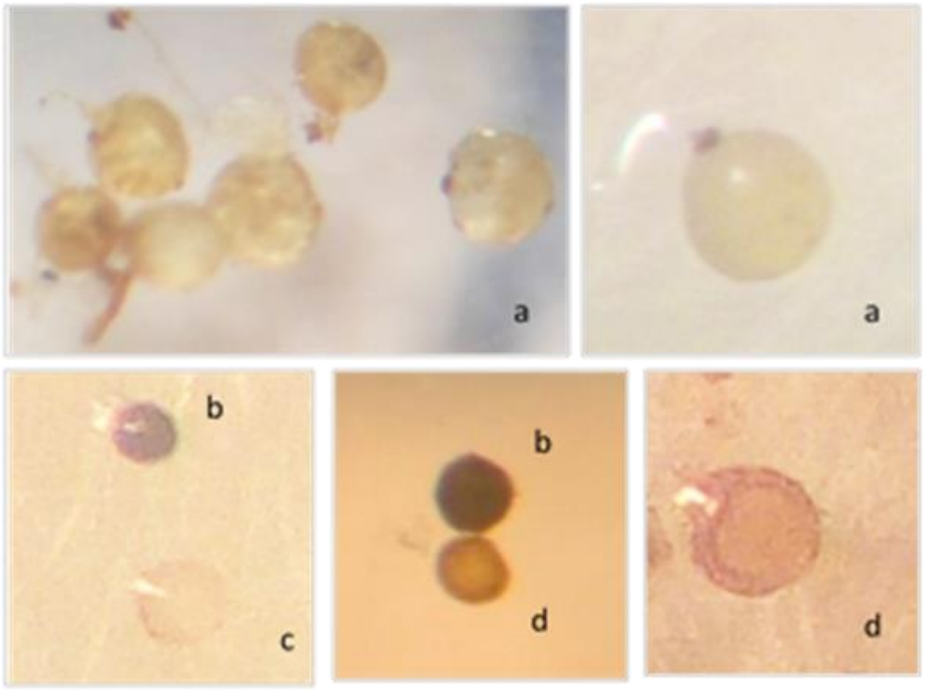

Figure 2. Arbuscular mycorrhizal fungi spores isolated from rhizosphere soil of Bambara groundnut. a, *Gigaspora margarita*; b, *Septoglomus constrictum*; c, *Acaulospora delicate*; d, *Claroideoglomus etunicatum*,

Table 6. AMF species identified in the study sites on the basis of morphological traits.

Family	Genera	Species
Acaulosporaceae	*Acaulospora* Gerd. and Trappe emend. Berch	*A. delicata* *A. laevis* *A. foveata* *A. tuberculta*
Diversisporaceae	*Diversispora* Walker C. and Schüßler A.	*D. eburnea*
Entrophosporaceae	*Claroideoglomus* Walker C. and Schüßler A.	*C. etunicatum*
Gigasporaceae	*Gigaspora* Gerd. and Trappe	*G. margarita*
	Gigaspora Gerd. and Trappe	*G. decipiens*
Racocetraceae Oehl, sieverd and Souza	*Racocetra* Oehl, Souza F.A. and Sieverd.	*R. coralloidea*
Glomeraceae	*Glomus* Tul. and Tul. C.	*G. ambisporum*
	Funneliformis Walker C. and Schüßler A.	*F. geosporum*
	Funneliformis Walker C. and Schüßler A.	*F. mossae*
	Rhizophagus Walker C. and Schüßler A.	*R. clarus*
	Rhizophagus Walker C. and Schüßler A.	*R. intraradices*
	Septoglomus Sieverd., Sliva G.A. and Oehl	*S. constrictum*
	Septoglomus Sieverd., Sliva G.A. and Oehl	*S. viscosum*

Diversisporaceae are highly susceptible to phosphate fertilization. Abbott and Robson (1991), Bagyaraj (2014) emphasize that Acaulospora species are commonly found in tropical soils and are well suited to soils with a

Table 7. Average of total number of AMF spores in 100 g of soil.

Genera	Study site									
	Mendong					Soa				
P_2O_5	0	50	100	150	200	0	50	100	150	200
Acaulospora	69	82	63	45	178	145	189	132	130	165
Claroideoglomus	19	24	15	11	67	43	56	31	33	38
Diversispora	33	45	39	28	54	68	99	81	71	80
Funneliformis	30	38	33	24	49	60	76	65	72	68
Gigaspora	0	1	1	0	1	2	2	3	3	2
Glomus	55	57	78	77	57	54	58	47	58	46
Racocetra	1	2	2	1	2	1	1	0	1	0
Rhizophagus	290	326	422	267	403	198	318	760	432	791
Septoglomus	193	257	247	122	206	134	234	184	227	174

pH below 5. This is similar to results obtained by Mbogne et al. (2015) who worked on the effect of fertilizer types on the endomycorrhizal biodiversity of pumpkins in Cameroon and Benin.

However, phosphate fertilization did not affect the frequency of mycorrhization in Bambara groundnut. Indeed, the simple superphosphate (P_2O_5) is a slow fertilizer whose solubilisation is progressive. This property could explain this result. Unlike other chemical fertilizers (NPK), it behaves like organic fertilizers (natural phosphate (rock), residual household waste, chicken droppings, etc. that do not reduce the frequency of AMF mycorrhization (Leyval et al., 2009, Mbogne et al., 2015).

This study highlights existence of a significant AMF diversity (16 species) in Mendong and Soa soils under Bambara groundnut culture. Nevertheless, the growing conditions of the Bambara groundnut could favor adaptation of species like *Rhizophagus intraradices* and *Septoglomus constrictum*. In fact, the Glomeraceae family is considered to be the most abundant of all AMF in the tropical arid zone (Maksoud et al., 1994) and is generally associated with acid soils (Abbott and Robson, 1991; Mbogne et al., 2015). It adapts to varied range of soils at different levels of nutrient availability (Straker et al., 2010).

According to Singh et al. (2008), the high competitiveness of Glomeraceae and their adaptive capacity, which allows them to establish better than other AMF families, could explain their predominance in the tropics. This adaptation is also due to their low-perturbable developmental cycle, which is unaffected by soil rehearsals compared to other families such as Acaulosporaceae, Gigasporaceae and Diversisporaceae (Oehl et al., 2003). Glomeraceae easily spread by spores, which are forms of AMF resistance under difficult conditions, while Acaulosporaceae, Gigasporaceae and Diversisporaceae propagate more with other propagule types such as extraracinar mycelial fragments and hyphae (Brito et al., 2012).

Several other studies (Johnson et al., 2013; Castillo et al., 2016) have noted the predominance of Glomeraceae in various tropical soils. Nevertheless, Acaulosporaceae were more abundant (four species) than Gigasporaceae (two species). This diversity of Acaulosporaceae is due to their tolerance to acid soils and their insensitivity to chemical fertilization. Species of this type are highly effective in P-uptake and transfer to the host plant, compared to Glomeraceae species (Jakobsen et al., 1992). The Gigaspora genus often predominates in sandy soils such as dunes (Lee and Koske, 1994).

However, majority of agricultural soils in southern Cameroon have a low percentage of sand. This could justify the low density of Gigaspora spores in the study sites. In addition, species of the genus Gigaspora produce large spores (260 to 440 µm for Gigaspora margarita) that require a longer developmental period than small spores (Hepper, 1984). They adapt more to changes in environmental conditions (Stutz and Morton, 1996) and are most often associated with wild plants than open-field crops (Gai et al., 2006). Species of the genus Gigaspora are able to cope with nutrient-rich environments (Liu et al., 2012).

The stage of development did not influence the frequency and intensity of mycorrhization. This result is contrary to those of Johnson et al. (2016) and Mbogne et al. (2015) who found that the frequency and intensity of mycorrhization are more important to fruition than flowering. The Bambara groundnut root infection process by AMF was certainly rapid and early so that the frequency and intensity of mycorrhization was already optimal at flowering.

Mycorrhization frequencies are around 50% and confirm the mycorrhizal status of the Bambara groundnut. The intensities of mycorrhization were low (less than 30%). This result could reflect the weak capacity of infection of the indigenous AMF of Cameroon. The landraces of Bambara groundnut used in this study seem to have root system with little ramification. However, the finer roots are the most likely to be infected. Furthermore, Duponnois et al. (2001) pointed out that mycorrhizal

infection of plants varies greatly from one plant to another but also within the same species. Nevertheless, Ngonkeu et al. (2003) noted that low root colonization does not imply a low symbiotic efficiency.

Trapping was necessary to observe the diversity of spores under Bambara groundnut culture. Indeed, several authors observed no spore after direct extraction from the native soil. However, after trapping, more than a dozen different spores of AMF were isolated in the same soil (Nwaga et al., 2003). The spore density was higher at Soa than at Mendong. Spore density varies greatly from one soil to another and the natural spatial distribution of AMF species is more closely related to environmental conditions, the complex structure of the soil rhizosphere constituents and/or the floristic composition (Begoude et al., 2016; Castillo et al., 2016).

Sixteen species in nine genera were identified. This specific richness is similar to that obtained in Cameroon and Benin by Mbogne et al. (2015) with pumpkins; as well as in Benin by Johnson et al. (2013) with cowpea. Indeed, the AMF communities' composition may vary from one region to another and from one type of habitat to another. In addition, there are obvious differences between ecosystems under different disturbance regimes (Öpik et al., 2006). For example, low AMF specific richness (only 5) was found in rhizospheric soils associated with pioneer plant species that grow at the mouth of Budi Lake in Chile (Medina et al., 2015). On the other hand, an enormous specific richness was found in biological tomato in a Mediterranean site with 58 species of AMF belonging to 14 genera (Njeru et al., 2015).

The number of genera obtained (nine) is contrary to those obtained by Mbogne et al. (2015) who noted four genera in the pumpkins rhizosphere by the classification of Schüßler et al. (2001), but similar to those of Johnson et al. (2013) who found eight genera under cowpea cultivation with the new classification of Schüßler and Walker (2010) and Redecker et al. (2013). The high number of genera is explained by the great progress made in the AMF identification by molecular tools. This work has led, in particular, to the division of *Glomus* genus in several genera, such as *Glomus*, *Funneliformis*, *Rhizophagus*, *Septoglomus* (Schüßler and Walker, 2010, Oehl et al., 2011, Redecker et al., 2013). The genera *Cetraspora*, *Dentiscutata*, *Racocetra* were also extracted from the genus *Scutellospora* (Morton and Msiska, 2010; Redecker et al., 2013).

At both sites, the average values of the Shannon-Wiener index ranged from 1.82 to 1.94. Effectively, this index can range from 0.45 for the AMF community present in an arable field (Daniell et al., 2001) to 3.0 in a forest (Becerra et al., 2011). According to Brower and Zar (1984), a high diversity community is characterized by low dominance (few species dominate AMF community). The morphotype V2 has a higher Shannon-Wiener index (1.90) than those of V1 (1.87) and V3 (1.86). The AMF diversity under Bambara groundnutt culture could be influenced by the genotype of the plant. Glomeraceae, Gigasporaceae and Acaulosporaceae are seemingly well-suited for the production of inocula in the humid agroecological zone.

The mean values of the Pielou's evenness index ranged from 0.69 to 0.74 in both sites, which means that each species is represented by almost the same number of individuals. The values of the Shannon-Wiener, Simpson and Pielou's evenness index are lower in plots with phosphate fertilizer. Indeed, the work of Mbogne et al. (2015) showed that chemical fertilizer (NPK) reduces diversity index values under pumpkins crop.

The total number of spores was significantly higher in Soa than in Mendong. Before the experiment, the Soa site (pH: 5.09) was an old shrub fallow, and that of Mendong (pH: 6.5) a young fallow. Some studies (Isobe et al., 2007; Mbogne et al., 2015) found a relationship between the AMF spores density and certain chemical properties of the soil like pH, available phosphorus and organic matter. Johnson (1991) and Mohammad et al. (2003) found that sporulation increases with soil pH and organic carbon.

The soils at both sites were acidic. Nevertheless, the optimum pH for AMF spore germination varies depending on the fungal species. In addition, Johnson et al. (2013) believe that the relationship between the density of AMF spores and the chemical properties of soils might not be stable, but could vary depending on the composition of the *Glomeromycota* community. Borriello et al. (2012) also pointed out that intensive plowing in conventional cropping systems negatively affects the AMF community and decreases the number of species thus reduces the sustainability of the system.

In this study, AMF diversity indices are negatively correlated with assimilable P. Johnson et al. (2013) also found that the species richness and AMF diversity associated with cowpea were negatively correlated with the P available in soils. Some species could be more sensitive to the available P and might become less abundant in soils with a high P level. The specific richness is also a reflection of the floristic richness of the previous cultivation of the various sites

Conclusion

The results obtained in this study show that AMF were present in the Bambara groundnut rhizosphere grown in Cameroon. All the landraces used formed the arbuscular mycorrhizae with characteristic structures such as arbuscules, hyphae, vesicles, auxiliary cells and spores. The spores were morphologically different in size, color, shape, presence or absence of the hypha and its mode of attachment to the spore. From these morphological criteria, 16 AMF species belonging to nine genera (*Rhizophagus*, *Septoglomus*, *Racocetra*, *Gigaspora*, *Acaulospora*, *Claroideoglomus*, *Diversispora*, *Funneliformis*

and *Glomus*) were identified. The species *Rhizophagus intraradices* was preponderant. The specific richness was identical in the two sites (Mendong and Soa), and between landraces. High level of chemical phosphate fertilizer (200 $kg.ha^{-1}$ P_2O_5) significantly reduced the intensity of mycorrhization. P fertilization reduced diversity index of AMF species in the Bambara groundnut rhizosphere. But the different P levels did not influence the frequency of mycorrhization and the specific richness. Thus, the choice of landraces, sites and especially P fertilizer level to be applied according to the initial richness of the soil is the factors to take into account to favor the establishment of the mycorrhizal symbiosis for a profitable culture of Bambara groundnut.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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