Morphological and Structural Diversities of Indigenous Mycorrhiza Communities Associated to Castor Bean from Adamawa Cameroon

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Authors’ contributions

This work was carried out in collaboration among all authors. Author LTT designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors AM and OWY managed the analyses of the study. Author CM managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

This study describes the diversity of arbuscular mycorrhizal fungi (AMF) that enter into association with castor bean growth wild in Subsaharan Africa. Three sites of castor bean stands were selected in each of the three Subdivisions (Ngaoundere I, Ngaoundere II and Nyambaka) of the Vina Division in Adamawa Cameroon. Soil samples and roots were taken from each castor bean rhizosphere. All samples from one site were mixed into a composite sample. Leek was used as trap plant. Mycorrhizal parameters, spores density and specific richness were determined following to the standard methods. After spore extraction, species identification was obtained through the informations provided by the International Vesicular Mycorrhizal fungi collection. Results indicate that Cameroonian castor bean accession was found to be symbiotic with AMF under Sudano-Guinean climate of Adamawa Cameroon. The morphological and structural characterization enabled the description of six AMF species, belonging to three genera: *Glomus fasciculatum*, *Glomus* sp1, *Glomus* sp2, *Scutellospora calospora*, *Scutellospora purpurans*, *Entrophospora infrequens*. These findings open opportunities for domestication and application of AMF for a sustainable castor bean productivity.

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1. INTRODUCTION

*Ricinus communis* L. (Castor bean) occupies a special place among oilseeds, especially because of its tolerance to drought, its robustness, its resistance to some pathogenic attacks, its adaptation to poor soils [1] and it presents no competition with food crops [2]. Castor bean has recently been highly rated as a source of raw material (oil) for biodiesel production due to its high oil content (25 – 55%) [3]. Castor oil is also a raw material for paints, coatings, inks, lubricants and wide variety of other products. It is also used as laxative in human medicine [4]. In Cameroon, castor bean stems are used in the manufacture of rock salt; seed oil is used to treat various diseases and also it is consumed [5]. The major producing countries are Brazil, China, India and the countries of the former Soviet Union. India alone exports 80% of castor oil and therefore largely dominates the market [6]. However castor bean is one of the under-valORIZED and under-exploited species in Sub-Saharan Africa because it grows wild in this part of the world [2].

Several strategies to improve castor bean productivity have been undertaken in recent years. India has made significant progress in the development of high-yielding hybrid castor bean varieties [7,8]. In addition [9] assessed the effect of nitrogen nutrition on castor bean growth and its physiology and reported that this plant is a nitrogen-demanding plant. Our previous studies focused on the physical characterization and geolocation of local castor accessions in the Adamawa-Cameroon Region. 04 local castor bean accessions were listed in this region. These were named Vina, Martap, Nyambaka and Béél accession. Vina accession link more widespread. However, its domestication is still limited [5]. The use of chemical fertilizers in agriculture leads to the destruction of soil structure and pollution of the environment. In this respect, the use of natural fertilizer as Mycorrhizae to improve plants productivity is required.

Mycorrhizal fungi are a major component of the soil microbial community that has successfully established a symbiotic relationship with two-thirds of plant species [10]. These fungi allow plants to obtain an extension of its root system and to optimize its supply in water and mineral elements, to improve its resistance against stresses including cold and drought [11]. The benefit effect of this symbiosis is not limited to both partners, but also relates to ecosystem integrity since it improves soil quality [12,8].

A crucial step in the successful application of arbuscular mycorrhizal fungi (AMF) is the selection of effective and suitable fungal strains. To the best of our knowledge, no work has been carried out on endogenous AMF associated with castor bean in Sub-saharan Africa. In this context, the main objective of current study was to determine castor bean mycorrhizal status in Sudano-Guinean savannahs of Adamawa Cameroon. Specifically it consisted to: (1) Evaluate the castor bean root mycorrhizal colonization; (2) Study the diversity of AMF associated with castor bean rhizosphere in Adamawa Cameroon. The interest of this work was that the endogenous strains of AMF associated with castor bean rhizosphere will constitute a basic data for the formulation of suitable mycorrhizal inoculum for castor bean productivity.

2. MATERIALS AND METHODS

2.1 Study Area

Adamawa Cameroon is located between the 6th and 8th degrees of north latitude and between the 10th and 16th degrees of east longitude. Climate of Adamawa Cameroon region is from the Sudano-Guinean type [13]. Vegetation is various and composed of grasslands; grassy, shrubby and tree savannahs [14]. Administratively, Adamawa Cameroon region has 05 Divisions (Vina, Mbéré, Faro & Deo, Djerem, Mayo Banyo). This work took place in three Subdivisions of Vina (Ngaoundere I, Ngaoundere III and Nyambaka). Three sites were chosen at random in each Subdivision as summarized in Table 1.

2.2 Soil Sampling

Three sites of castor bean stands (one stand being defined as a homogeneous grouping of castor bean while site is defined here as being the place where castor bean has been observed over an area of more than one km²) were selected in each of the three Subdivisions (Ngaoundere I, Ngaoundere II and Nyambaka) (Table 1). For each site, ten castor bean plants were chosen at random. Soil samples of 300 g and castor bean roots with diameter less than 1
mm were taken from each plant rhizosphere using an auger. Soil samples were taken at 10 cm depth on average within 20 cm radius in the castor bean rhizosphere [15]. All samples from one site were mixed into a composite sample and labeled [16]. Composite soil samples were collected in plastic bags before storing in a dry, shaded place.

2.3 Trapping of Arbuscular Mycorrhizal Fungi Spores from Collected Soil Samples

"Gros Long d’Eté" variety of *Allium porrum* (Leek) produced by Technisem and distributed in Cameroon market by Semagri are used as trap plant. The use of “Gros Long d’Eté” variety of leek as trap plant is justified by the fact that its roots system is fibrous and it presents short life cycle (90 to 120 days) compared to other leek varieties. Trapping of spore was carried out according to the method described by Gerdeman and Nicolson [17] modified as follows: 04 seeds were sown directly per hole. Leek was sown in 20 different pots, each containing 1 kg of composite soil. The pots placed out (Fig. 1), were left at natural watering rainfall capacity for three months (July to September 2018). The rhizospheric soils were sampled for laboratory analysis.

![Fig. 1. Trapping of arbuscular mycorrhizal fungi spores using leek as trapped plant](image)

2.4 Assessment of Mycorrhizal Parameters

Fine harvested castor bean roots were thinned according to [18] method to highlight endomycorrhizal infestation structures. Castor bean roots were: (1) carefully washed, the youngest taken and cut to 1-2 cm in length; (2) put into a test tube with 10% potassium hydroxide, and heated in a water bath at 90°C for 30 minutes to clear the roots; (3) the potash was discarded, filtered through a sieve, before neutralization by rinsing with acidified water; (4) neutralized roots were dept into cotton blue in a water bath for 15 minutes, filtered again through a sieve, and rinsed with distilled water; (5) some of these roots were mounted in water for direct observations, while other were mounted in glycerine for later observations. The mycorrhizal parameters such as mycorrhizal frequency, mycorrhizal intensity, roots arbuscular content were determinated according to Trouvelot et al. [19]. These mycorrhizal parameters were calculated automatically using "Mycocalc" software.

2.5 Extraction of Arbuscular Mycorrhizal Fungi Spores from the Rhizospheric Castor Bean Soils

Arbuscular mycorrhizal fungi spores were extracted according to the wet extraction method described by Gerdeman and Nicolson [17] modified by the Tobolbai et al. [20]: (a) suspension of soil sample (500 g) in water; (b) mechanical stirring of soil for 15 min (repeated thrice); (c) passing the soil through a series of sieves of size corresponding to the range of spores sizes of between [25 - 400 µm]; (d) creating a density gradient by centrifugation; (e) filtering through a 25 µm sieve for spores collection.

2.6 Morphological and Structural Characterization of Arbuscular Mycorrhizal Fungi Spores

For the identification of Arbuscular Mycorrhizal Fungi (AMF), the extracted spores were grouped by morphotype under criteria such as size, shape and color. Two groups of spores from each morphotype were mounted between slide and coverslip, thus one in PVGL (Polyvinyl-Lactic Acid-Glycerol), and the other in the PVGL-Melzer Reagent mixture (1:1/v:v) [21]. The morphotypes determination of the genus was made based on the classifications described by Morton and Benny [22]. The original descriptions of species, as well as the descriptions provided on the website of the International Vesicular Mycorrhizal fungi collection (INVAM): http://invam.caf.wv.edu/fungi taxonomy/species ID.htm were used as the reference during the identification process. Morphological characters of spores were compared with those of standard specimens and the reference strains. Several parameters were used to characterize AMF
Table 1. Description of soil sampling sites

<table>
<thead>
<tr>
<th>Subdivisions</th>
<th>Sampling sites</th>
<th>Latitude (°)</th>
<th>Longitude (°)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ngaoundere I</td>
<td>Regional hospital</td>
<td>N 07°19'012</td>
<td>E 013°35 210'</td>
</tr>
<tr>
<td></td>
<td>Bantai</td>
<td>N 07°19'131</td>
<td>E 013°35 433'</td>
</tr>
<tr>
<td></td>
<td>Douze poteaux</td>
<td>N 07°19'356</td>
<td>E 013°35 336'</td>
</tr>
<tr>
<td>Ngaoundere III</td>
<td>Bini (Shalom City)</td>
<td>N 07°25'168</td>
<td>E 013°33 184'</td>
</tr>
<tr>
<td></td>
<td>Bini (Pharen City)</td>
<td>N 07°24'672</td>
<td>E 013°33 067'</td>
</tr>
<tr>
<td></td>
<td>Dang (BOCOM)</td>
<td>N 07°26'311</td>
<td>E 013°33 318'</td>
</tr>
<tr>
<td>Nyambaka</td>
<td>Dibi I</td>
<td>N 07°25'077</td>
<td>E 013°32 789'</td>
</tr>
<tr>
<td></td>
<td>Dibi II</td>
<td>N 07°08'604</td>
<td>E 013 43 688'</td>
</tr>
<tr>
<td></td>
<td>Belel-Dibi</td>
<td>N 013°46'397</td>
<td>E 013°35 210'</td>
</tr>
</tbody>
</table>

spores and were evaluated based on the formula proposed by Sghir [23]. The species richness (R) refers to the total number of different morphotypes recorded in a 100 g soil sample, and was expressed by: R (%)=N/100 g, where N is the number of different specimens. The specific density (D) indicates the number of spores recovered in 100 g soil sample, and was express as: D (%)=N/100 g, where N is the number of spores. The diversity of arbuscular mycorrhizal fungi species in all the sites was calculated using Shannon-Weaver diversity index (H) [24]. The Shannon index is given by the formula below: \( -H = -\sum p_i \ln p_i \), where \( p_i = S/N \), S is the total number of individuals of one species, N is the total number of all individuals in the sample and \( \ln \) = logarithm to base e. The proportion of species relative to total number of species (\( p_i \)) was calculated, and multiplied by natural logarithm of this proportion (\( \ln p_i \)). The results were summed across the species, and multiplied by -1.

2.7 Data Analysis

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

3. RESULTS

3.1 Arbuscular Mycorrhizal Colonization of Castor Bean Roots

Castor bean roots from different soils sampled were colonized by Arbuscular Mycorrhizal Fungi. The analysis of variances (ANOVA) showed that there is no significant difference between localities relative to root mycorrhizal frequency (77±6% for Ngaoundere I, 84±5% for Ngaoundere III and 89±11% for Nyambaka). In contrast, there is a significant difference (p<0.05) between localities relative to mycorrhizal intensity as well as root arbuscular content. Mycorrhizal intensity was higher in Ngaoundere III (70±3%) and Nyambaka (72±8%) localities than in Ngaoundere I locality (61±4%). Arbuscular content of castor bean roots from Nyambaka (55±10%) was higher than those from Ngaoundere I (39±6%) (Fig. 2).

3.2 Number of Spores Depending on Size and Sampling Sites

Size is a classification index of spores observed to distinguish and separate different isolated strains. There was a variability of spore’s size relative to sampling sites, in directly soils sampled or those obtained after trapping (Table 2). In Ngaoundere I and Ngaoundere III, the number of spores after trapping was significantly (p<0.05) higher than that from direct counting while there is no significant difference between number of spores from direct counting and counting after trapping in Nyambaka. Globally these spores are higher (> 400 spores) in Nyambaka and Ngaoundere III than Ngaoundere I (< 400 spores). The size of isolated spores varied from 500 to 50 μm, divided into three main classes, namely [500; 250], [250; 100] and [100; 50]. The number of spores from [250; 100] class was very high (54.22%), that from [100; 50] class was moderately high (37.38%) and very low value (8.40%) of this parameter was from [500; 250] class.

3.3 Diversity of Arbuscular Mycorrhizal Fungi

The morphological analysis (color, shape, size and attachment hypha) revealed the presence of 6 different morphotypes (Fig. 3).
Fig. 2. Root mycorrhizal parameters depending on sampling sites
Values of uniform bands affected by the same letter are no significantly different (p<0.05)
Fig. 3. Morphological and structural diversity of isolated spores

a: Entrophospora infrequens; b: Scutellospora purpurascens; c: Glomus fasciculatum; d: Scutellospora calospora; e: Glomus sp1; f: Glomus sp2; C1: outer layer; C2: inner layer; C3: outer wall layer; BI: suspended hypha; Hy: hyphal branching; Pg: germination wall

Table 2. Number of spores in 100 g soil depending on size, soil sampling and extraction method

<table>
<thead>
<tr>
<th>Spores size (µm)</th>
<th>Ngaoundere I</th>
<th>Ngaoundere III</th>
<th>Nyambaka</th>
</tr>
</thead>
<tbody>
<tr>
<td>[100-50]</td>
<td>59±10</td>
<td>129±26</td>
<td>175±34</td>
</tr>
<tr>
<td>[250-100]</td>
<td>95±5</td>
<td>193±4</td>
<td>216±40</td>
</tr>
<tr>
<td>[500-250]</td>
<td>9±2</td>
<td>45±4</td>
<td>18±7</td>
</tr>
<tr>
<td>Total</td>
<td>163±17a</td>
<td>367±34a</td>
<td>408a±81b</td>
</tr>
<tr>
<td>p-Value</td>
<td>0.0000</td>
<td>0.0067</td>
<td>0.0938</td>
</tr>
</tbody>
</table>

DE: Direct extraction; ET: Extraction trapping; values of line affected by the same letter are not significantly (p < 0.05) different for each of the two extraction methods.
4. DISCUSSION

The observation of mycorrhizal structures of castor bean from Vina Division of Adamawa Cameroon corroborate partially [25] findings who revealed the presence of arbuscules, vesicles and mycelial hyphae (characteristics of mycorrhizal symbiosis establishment) on castor bean roots in Senegal after 04 months greenhouse growth. Globally mycorrhizal colonization of castor bean roots is higher in Ngaoundere III and Nyambaka compared to Ngaoundere I, thus suggesting that Ngaoundere III and Nyambaka localities are more favorable for castor bean productivity, but this remain to be studied.

The variability of number of spores on sampling soils would be justified by that these soils would present different physicochemical properties.

The six species belong to 03 families and 03 genera. The genus Glomus of the Glomeraceae family had the highest number of species (03) (Glomus sp1, Glomus sp2 and Glomus fasciculatum); the genus Scutellospora of the Gigasporaceae family had 02 species such as Scutellospora calospora and S. purpurans; for the Entrophosporaceae family, the only species identified was Entrophospora infrequens. Concerning their abundances, the number of spores in 100 g of soil is represented in Table 2. E. infrequens was the most abundant in all sampling soils with 170, 131 and 90 spores/100 g of soil, respectively for Nyambaka, Ngaoundere III and Ngaoundere I. G. fasciculatum had 16 and 162 spores/100 g of soil respectively for Nyambaka and Ngaoundere III while S. calospora presented 111 spores / 100 g of soil only in Nyambaka. Furthermore, S. purpurans was completely absent at Ngaoundere III and was the least abundant with only 10 and 12 spores/100 g of soil respectively for Ngaoundere I and Nyambaka. Specific richness in Ngaoundere I, Ngaoundere III and Nyambaka localities were 6, 5 and 6 species respectively. The Shanon index revealed a low diversity in the number of species in castor bean rhizosphere (Table 3).

### Table 3. Specific richness, Shannon index and Pielou’s equitability index

<table>
<thead>
<tr>
<th>Sites</th>
<th>Specific richness</th>
<th>Shannon index</th>
<th>Pielou’s equitability index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ngaoundere I</td>
<td>6</td>
<td>0.7</td>
<td>0.3</td>
</tr>
<tr>
<td>Ngaoundere III</td>
<td>5</td>
<td>0.6</td>
<td>0.2</td>
</tr>
<tr>
<td>Nyambaka</td>
<td>6</td>
<td>0.7</td>
<td>0.2</td>
</tr>
</tbody>
</table>

Indeed, several authors [26,27] reported that the distribution, abundance and viability of endogenous AMF species results from soil physicochemical properties. Globally isolated spores was mostly small because 91% of its were less than 250 μm. This result corroborates that of Zézé et al. [28] who worked on the distribution and abundance of AMF in different types of forests in Ivory coast and revealed that 99% of spores were less than 250 μm. The number of spores is higher in trapping soils than direct extraction soils. This could be explained by the fact that the conditions which plants are grown in pot could improve root colonization and fungal strains sporulation [22]. In addition, the substrate was sterile in pot condition, the competition between AMF and other microorganisms would be reduced, thus allowing these microorganisms to flourish in their environment. These results corroborate those of Tobolbai et al. [20] and Zézé et al. [28] who reported that trapping system stimulates mycorrhizal fungi development.

Globally in this study the genus Glomus from Glomeraceae family had the largest species number (G. sp1, G. sp2 and G. fasciculatum). Thus, the genus Glomus appears to be tolerant to a wide range of soil types. This result corroborates that of Morton and Benny [22] who revealed that Glomus is the most abundant in the tropics. In addition, the dispersion of spore’s types throughout the study area is not homogeneous.

Pielou Equitability tends towards 0, thus suggest that almost all of the number was concentrated in one genus (Glomus). Our results corroborate those of Diallo et al. [25] who reported that the genus Glomus better stimulates plant castor bean growth and development in Senegal under greenhouse cultivation.

The diversity of Arbuscular Mycorrhizal Fungi in Vina Division varied depending on sampling sites but Shannon diversity index remains low with 0.7. This could be explained by the fact that the edaphic-climatic conditions are not suitable for some AMF species development. Tobolbai et al. [20] obtained similar results, indeed their
result revealed very low species diversity (0.48%) in the Northern Cameroon. This result suggests that there are few successful endomycorrhizal species in the Northern Cameroon soils.

5. CONCLUSION

Cameroonian castor bean accession was found to be colonized with arbuscular mycorrhizal fungi in the Sudano-Guinean savannas of Cameroon. Six Arbuscular Mycorrhizal Fungi (AMF) species were involved in this symbiosis in the Vina Division of the Adamawa Cameroon which are Glomus fasciculatum, Glomus sp1, Glomus sp2, Scutellospora Calospora, Scutellospora purpurata, Entrophospora infrequens. The identification of these endogenous endomycorrhizal spores structures in soils is a potential opportunity for production of endomycorrhizal inoculants to improve castor bean productivity in this part of the world.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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