ARBUSCULAR MYCORRHIZAS IN THE ROOTS OF *AFZELIA AFRICANA*, *ENTADA AFRICANA*, AND *PTEROCARPUS ERINACEUS*

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Abstract. *Afzelia africana*, *Entada africana*, and *Pterocarpus erinaceus* are economically important tropical tree species in Africa. In this study, we performed morphological and molecular analyses to examine the mycorrhizal status of these species in their natural habitat in Benin, West Africa. Fine roots were examined using trypan blue staining. Putative arbuscular mycorrhizal fungi (AMF) were identified by extracting root DNA and sequencing the large subunit region of ribosomal DNA. Operational taxonomic units (OTUs) were positioned on a consensus phylogenetic tree produced using maximum likelihood and neighbor-joining models. Morphological analyses revealed vesicles, arbuscules, and inter- and intracellular hyphae in roots of all three tree species. Molecular analyses revealed 39 AMF OTUs, among which 18 were from roots of *E. africana*, 15 from *P. erinaceus*, and 9 from *A. africana*. These OTUs belonged to *Glomus*, *Nanoglomus*, *Rhizoglomus*, *Sclerocystis*, *Septoglomus*, and *Scutellospora*; two unidentified species belonged to the family Glomeraceae. This is the first study to report AMF in roots of *A. africana*, *E. africana*, and *P. erinaceus* in Benin, and opens new avenues for future studies.
Keywords: *AMF, Benin, LSU, mycorrhiza, plant improvement, tropical tree species, Wari-Maro forest, West Africa*

Introduction

Mycorrhizas are essential symbiotic associations that allow nutrient transfer between fungi and the roots of living plants (Brundrett, 2004). Mycorrhizal symbioses provide multiple benefits for the functioning of natural ecosystems (Smith and Read, 2008), including nutrition enhancement (Clark and Zeto, 2000; Bücking and Kafle, 2015; Bunn et al., 2019), drought stress alleviation (Sharma et al., 2015; Dar et al., 2018), soil pathogen resistance (Talavera et al., 2001; Makoi and Ndakidemi, 2009), and growth of the host plant, as well as soil structure stabilization (Aggarwal et al., 2011). In tropical forests, most Dipterocarpaceous and Myrtaceaous trees form ectomycorrhizal symbioses, and many other angiosperm species form arbuscular mycorrhizas (Wang and Qiu, 2006;

Smith and Read, 2008; Brundrett, 2009). However, information on the mycorrhizal status of valuable tree species is limited for West African tropical forests. Ectomycorrhiza in *Afzelia africana* Sm. have previously been reported (Redhead, 1968; Ba and Thoen, 1990; Yorou et al., 2008); however, the presence of arbuscular mycorrhizal fungi (AMF) in the roots of this species has not been confirmed, and there have been no reports of the mycorrhizal status of *Entada africana* Guill. & Perr. or *Pterocarpus erinaceus* Poir.

Afzelia africana is a vulnerable species (African Regional Workshop, 1998), also known as African mahogany. It is a deciduous tree belonging to the Detarioideae subfamily (Fabaceae), that is valued for the quality of its hard, reddish wood (Bayer and Waters-Bayer, 1999; Sinsin et al., 2004) and for the high nutritional value of its leaves as cattle feed (Sinsin, 1993). Seeds of this species germinate easily; however, the low recruitment rate of seedlings hinders natural regeneration (Padonou et al., 2013), particularly in its fire-prone natural habitats (Bationo et al., 2001). As a consequence of human pressures (Houehanou et al., 2019) and seedling recruitment issues, the population of *A. africana* is decreasing; only adult trees of this species are commonly observed in its natural habitats (Padonou et al., 2013). However, despite the frequent pruning of these adult trees, they continue to persist.

Entada africana, a species of least concern (Botanic Gardens Conservation International, International Union for Conservation of Nature SSC Global Tree Specialist Group, 2019) known as fake locust bean, is a deciduous tree species belonging to the subfamily Caesalpinioideae (Fabaceae); all of its organs are used as traditional medicines (Tibiri et al., 2007, 2010). The roots, stem bark, and leaves of this species contain many phytochemical classes of compounds including polyphenols, terpenes, and alkaloids (Sanogo et al., 1998; Ahua et al., 2007; Tibiri et al., 2007, 2010; Mbatchou et al., 2011), which can be further improved by mycorrhizal associations (Sbrana et al., 2014).

Pterocarpus erinaceus, known as African rosewood, is a deciduous tree species belonging to the subfamily Faboideae (Fabaceae), that has high wood quality (Traoré et al., 2010; Segla et al., 2017). This species is selectively logged in natural forests (Adjonou et al., 2010; Rabiou et al., 2015); the processed wood is exported to China, India, and other countries (Segla et al., 2017). The species is also valued as animal feed (Olafadehan, 2013). It is now an endangered species (Barstow, 2018) due to its low growth rate (Dumenu and Bandoh, 2016) and overexploitation (Dumenu, 2019).

Our long-term research goals are to test the potential of mycorrhizas for improving seedling recruitment in *A. africana*, the growth rate of *P. erinaceus*, and the quality and quantity of phytochemical production in *E. africana*. As a first step, in this study, we investigated the mycorrhizal status of these three tree species through anatomical observation of their roots and molecular detection of putative mycorrhizal fungi.

Materials and Methods

Study site and sampling

Sampling was conducted in the Wari-Maro Forest Reserve in Benin, West Africa, in July 2019. The reserve includes dense forests, woodlands, and savannahs in the Sudano-Guinean phytodistrict defined by Aubréville (1970) and White (1983) in the tropical climate zone (8.80–9.20°N, 1.95–2.45°E) overlapping the Tchaourou and Bassila districts (*Fig. 1*) (Salako et al., 2014; DIVA-GIS, 2018; Djotan et al., 2018). Samples were collected and transported to Japan under official regulations.
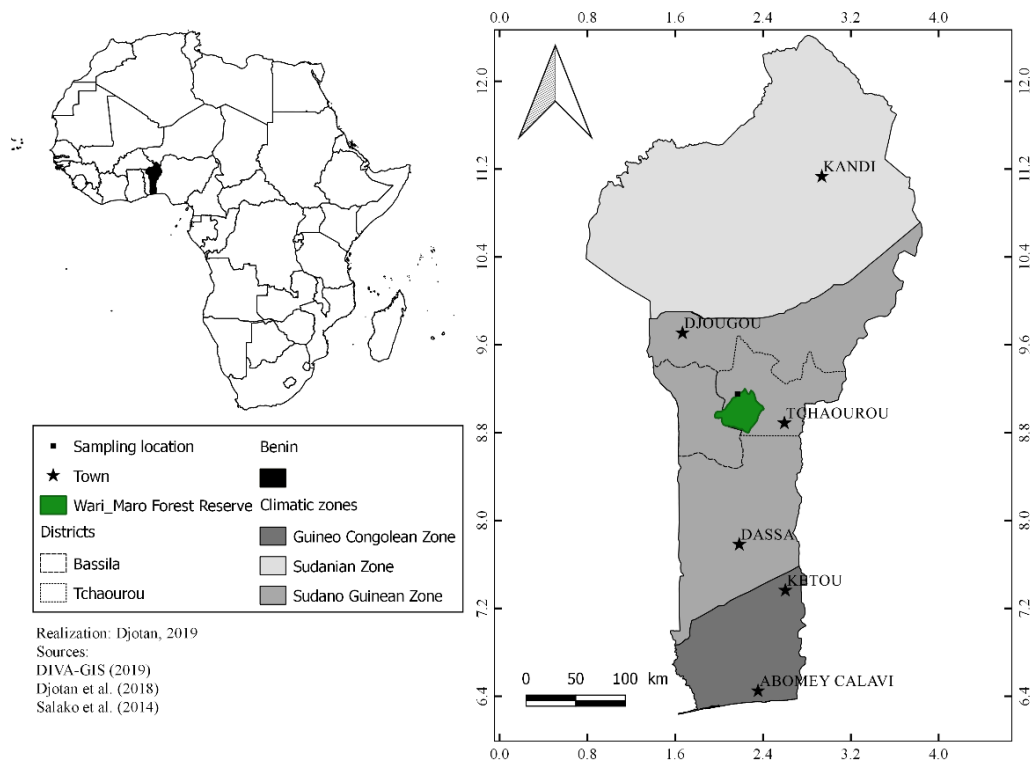
Figure 1. *Sampling locations. Green polygon indicates the Wari-Maro Forest Reserve, which overlaps two districts of central Benin, West Africa. Samples were collected around the black point within the polygon*

Five individual trees of each species were randomly sampled in the forest reserve. We collected 50 g of fine roots, including first- and second-order lateral roots, from each tree. We ensured that the collected roots belonged to the targeted tree by tracing the excavated roots to the base of the tree. Each root system sample was kept fresh within its rhizosphere and transported to the laboratory in a plastic test tube. Roots were washed three times with running tap water to remove soil particles attached to the root surface, and then preserved in 60% ethanol until further use.

Staining and anatomical observation

The root segments were cleaned and stained (Phillips and Hayman, 1970), and stained roots were preserved in lacto-glycerol until observation (Brundrett et al., 1984). For each tree, 10 root segments (length, 1 cm) were randomly cut, mounted with lacto-glycerol, and observed under a microscope (BX50, Olympus, Tokyo, Japan) fitted with a camera (DP71, Olympus) featuring standard bright-field optics. Based on the resulting microscopy images, we calculated the colonization frequencies of arbuscular mycorrhiza for each species as the number of tree samples of the species in which mycorrhizal features were observed (n = 5). Colonization intensity was calculated as the relative length of slide-mounted roots containing mycorrhizal features to the total length of observed roots (Giovannetti and Mosse, 1980; Fujimaki et al., 2001). We confirmed AMF colonization by observation of arbuscules, vesicles, intracellular hyphae, or intercellular hyphae with branches inside cells.

DNA extraction, polymerase chain reaction (PCR) amplification, and sequencing

To identify the root fungal community composition, DNA was extracted from root segments (three randomly selected 1 cm root segments) of each sampled tree using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions.

We performed 10-fold dilution of the extracted root DNA samples for use as a DNA template for PCR. We used the nested PCR method to amplify the large subunit (LSU) region of the fungal ribosomal DNA (rDNA) (Gollotte et al., 2004). We used the eukaryote-specific primer LR1 (5'-GCATATCAATAAGCGGAGGA-3') (Van Tuinen et al., 1998), and the fungus-specific primer FLR2 (5'-GTCGTTTAAAGCCATTACGTC-3') (Trouvelot et al., 1999) in the first round of PCR. The resulting PCR products were diluted 100-fold and used as a template for second-round PCR with the general AMF nested primers FLR3 (5'-TTGAAAGGGAAACGATTGAAGT-3') and FLR4 (5'-TACGTCAACATCCTTAACGAA-3') (Gollotte et al., 2004). Both rounds of PCR were performed in a 6 µL reaction mixture containing 1× KAPA2G Robust HotStart ReadyMix with dye (KAPA Biosystems, Wilmington, MA, USA), 0.5 µM of each primer, and 1 µL DNA template. The PCR protocol for each round consisted of an initial denaturation at 95°C for 2 min, 30 cycles of denaturation at 95°C for 90 s, annealing at 54°C for 10 s, extension at 72°C for 30 s, and a final extension at 72°C for 2 min. The second-round PCR products were cloned into the T-vector pMD20 (Takara Bio, Shiga, Japan) using the TaKaRa DNA Ligation Kit Mighty Mix and *Escherichia coli* HST08 Premium Competent Cells (TaKaRa Bio) according to the manufacturer's instructions. Each recombinant *E. coli* colony was randomly selected and boiled in 30 µL sterile water for 10 min at 98°C. DNA fragments were amplified from the supernatant by PCR using a primer specific to the vector. The PCR products were purified using the Illustra ExoProStar system (GE Healthcare, Chicago, IL, USA) according to the manufacturer's instructions, and sequenced by Macrogen Japan Co. (Tokyo, Japan). Eight colonies were sequenced from each root sample that showed positive results following amplification.

Data analysis and phylogeny

Nucleotide sequences received from Macrogen Japan were cleaned and submitted to the CD-HIT Suite online tool (Huang et al., 2010) for operational taxonomic Unit (OTU) clustering. OTU clusters were formed at 99% similarity. Non-AMF OTUs were removed from the data, and the most representative OTU cluster sequences were blasted using the MEGA-X web service menu (Kumar et al., 2018). Additional sequences of the LSU region of other glomeromycotan species were added to the data, and sequences were aligned using the Muscle alignment tool of MEGA-X. The sequences of OTUs generated in this study are available in the National Center for Biotechnology Information (NCBI) GenBank database (links are provided in the Data Availability section).

Evolutionary history was inferred using a maximum likelihood (ML)-based general time reversible model (Nei and Kumar, 2000), and the neighbor-joining (NJ) method (Saitou and Nei, 1987) with the MEGA-X software (Kumar et al., 2018). For the first method, initial trees for the heuristic search were obtained automatically by applying NJ and BioNJ algorithms to a matrix of pairwise distances estimated using the maximum composite likelihood approach, and then selecting the topology with the highest log likelihood value. A discrete gamma distribution was used to model evolutionary rate

differences among sites (5 categories + G, parameter = 1.0112). In the second method, evolutionary distances were computed using the p distance method (Nei and Kumar, 2000) and expressed as numbers of base differences per site. These analyses involved 83 nucleotide sequences including 39 OTUs obtained in this study; all sequences are available in the GenBank database. We eliminated all positions with less than 95% site coverage using the partial deletion option. In total, 301 positions were included in the final dataset. The ML model tree with the highest log likelihood value (–5161.09) is shown. The tree was outgrouped with *Paraglomus occultum* FJ461883, drawn to scale, with branch lengths measured in terms of the number of substitutions per site. For each model, the percentage of trees in which the associated taxa clustered together in a bootstrap test (Felsenstein, 1985) with 1000 replicates is shown next to each branch (NJ/ML).

Statistical analysis

Differences in colonization intensity between species were tested using one-way analysis of variance (ANOVA), and means were compared using Tukey's honestly significant difference (HSD) test with the R v. 3.5.2 software (R Core Team, 2016).

Results

Mycorrhizas were present in the roots of all of the sampled trees, but at different intensities (*Fig. 2, Table 1*); intensity was higher in *E. africana* (84 ± 19%) than in *P. erinaceus* (48 ± 24%) and *A. africana* (48 ± 30%). There were no significant differences in the intensity of mycorrhizal colonization between *P. erinaceus* and *A. africana*.
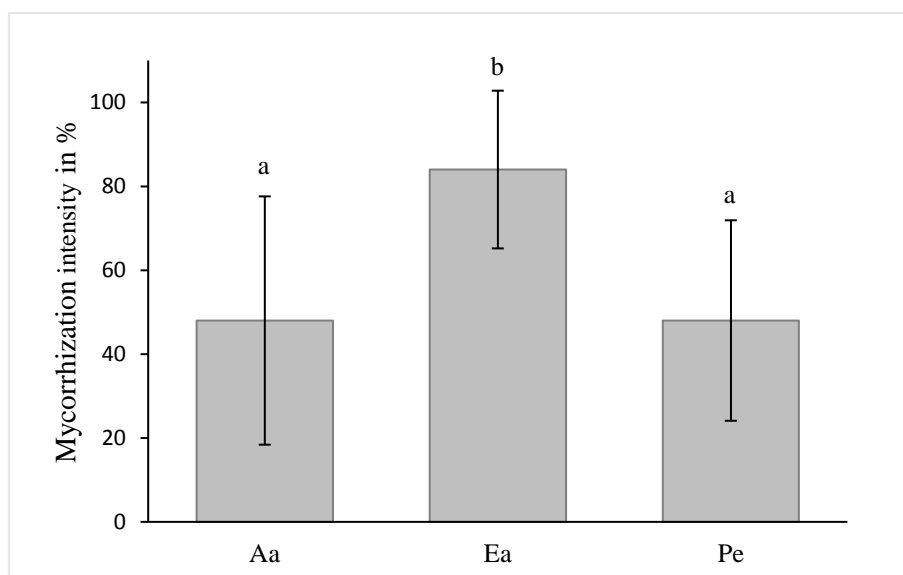

Figure 2. *Mycorrhizal colonization intensity (mean ± standard error) in roots of three tree species. Identical letters indicate no significant difference among the tested species (Tukey's honest significant difference [HSD] test, p < 0.05; n = 5). Aa,* Afzelia africana*; Ea,* Entada africana*; Pe,* Pterocarpus erinaceus

Table 1. *One-way analysis of variance table for the intensity of mycorrhizal colonization (AMF%). The factor "Species" has 3 levels (*Afzelia africana, Entada africana, *and* Pterocarpus erinaceus*). AMF% is significantly different among species*

Factor	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Species	2	4320	2160	5.5385	0.01977*
Residuals	12	4680	390		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

We found vesicles, arbuscules, and intra- and intercellular hyphae in stained roots of *A. africana*, *E. africana*, and *P. erinaceus* (*Fig. 3*). We detected 39 AMF OTUs, including 9 from *A. africana*, 18 from *E. africana*, and 15 from *P. erinaceus*. Three OTUs (1, 8, and 15) were present in two tree species, whereas the others were species-specific (*Table 2*).
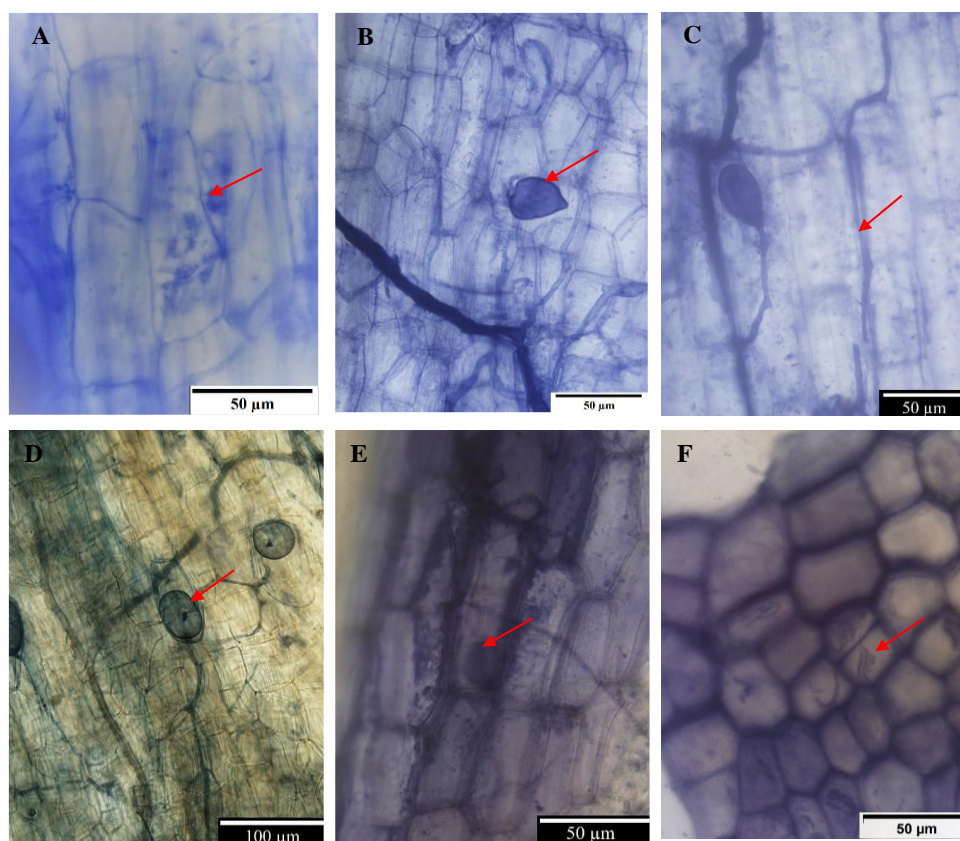
Figure 3. *Micrographs of trypan blue-stained roots taken with an Olympus DP71 camera fitted to an Olympus BX50 microscope. Arrows indicate finely branched arbuscules (A) and terminal vesicles (B) in roots of* A. africana*, intercellular hyphae (C) and terminal vesicles (D) in roots of* E. africana*, and non-terminal vesicles (E) and intercellular hyphae (F) in roots of* P. erinaceus

Using a threshold of percentage of identity equal to or greater than 95%, we identified OTUs 2, 14, 15, and 31 as *Scutellospora erythropa* (in *E. africana*), *Rhizoglomus dalpeae* (in *E. africana*), *Septoglomus deserticola* (in *A. africana*), and *Glomus trimurales* (in *E. africana*), respectively (*Table 2*).

Table 2. *Glomeromycotan operational taxonomic units (OTUs) detected in this study, ordered by their presence and abundance in all three tree host species*

OTUs	OTUs Freq (%)				Access no.	Closest BLAST match				
	Aa	Ea	Pe	All species		Score	e value	Access no.	Identity (%)	Species
1	42	0	8	14	MN920713	652	0	GQ149222	98.13	uncultured Glomeromycotina
2	0	13	0	5	MN920719	551	1.00E-152	AM086173	98.71	*Scutellospora erythropa*
3	21	0	0	5	MT408134	654	0.00E+00	HG515449	98.91	uncultured Glomeromycotina
4	0	0	17	5	MT408150	616	3.38E-172	HE858414	96.28	uncultured *Glomus*
5	0	10	0	4	MN920715	580	1.00E-161	MH504073	94.71	uncultured *Sclerocystis*
6	0	10	0	4	MN920717	610	2.00E-170	MH504060	96.04	uncultured *Sclerocystis*
7	0	10	0	4	MT408139	628	4.32E-176	MH504023	97.07	uncultured *Sclerocystis*
8	0	3	8	4	MT408144	462	4.67E-126	JF798534	90.42	uncultured Glomeromycotina
9	0	0	13	4	MT408154	660	0.00E+00	HG515449	99.18	uncultured Glomeromycotina
10	0	0	8	3	MN920710	632	4.00E-177	HE858414	97.07	uncultured Glomerales
11	0	6	0	3	MN920712	612	5.00E-171	MH504023	96.28	uncultured *Sclerocystis*
12	0	6	0	3	MN920714	606	2.00E-169	KM879707	95.99	uncultured Glomeromycotina
13	0	6	0	3	MN920716	588	9.00E-164	HQ857172	95.17	uncultured fungus
14	0	6	0	3	MN920718	682	0	MN130954	99.73	*Rhizoglomus dalpeae*
15	5	0	4	3	MT408133	592	5.65E-165	JQ048925	95.21	*Septoglomus deserticola*
16	0	0	8	3	MT408149	588	7.27E-164	GQ149222	95.19	uncultured Glomeromycotina
17	0	0	4	1	MN920708	645	0	HG515449	98.90	uncultured Glomeromycota
18	0	0	4	1	MN920709	466	4.00E-127	JF798534	90.50	*Glomus* sp.
19	0	3	0	1	MN920711	588	9.00E-164	JQ048925	94.96	*Septoglomus deserticola*
20	5	0	0	1	MN920720	523	3.00E-144	GQ149222	92.25	uncultured Glomeromycotina
21	5	0	0	1	MT408132	641	5.57E-180	GQ149222	97.59	uncultured Glomeromycotina
22	5	0	0	1	MT408135	652	0.00E+00	HG515449	98.91	uncultured Glomeromycotina
23	5	0	0	1	MT408136	518	9.56E-143	GQ149222	91.98	uncultured Glomeromycotina
24	5	0	0	1	MT408137	551	9.42E-153	GQ149222	93.58	uncultured Glomeromycotina
25	5	0	0	1	MT408138	641	5.57E-180	GQ149222	97.59	uncultured Glomeromycotina

OTUs	OTUs Freq (%)				Access no.	Closest BLAST match				
	Aa	Ea	Pe	All species		Score	e value	Access no.	Identity (%)	Species
26	0	3	0	1	MT408140	617	9.35E-173	MH504023	96.53	uncultured *Sclerocystis*
27	0	3	0	1	MT408141	580	1.23E-161	MH504073	94.71	uncultured *Sclerocystis*
28	0	3	0	1	MT408142	586	2.64E-163	MH504073	94.97	uncultured *Sclerocystis*
29	0	3	0	1	MT408143	575	5.71E-160	MH504073	94.44	uncultured *Sclerocystis*
30	0	3	0	1	MT408145	595	4.38E-166	MH504023	95.47	uncultured *Sclerocystis*
31	0	3	0	1	MT408146	636	2.56E-178	JN937265	97.58	*Glomus trimurales*
32	0	3	0	1	MT408147	566	3.45E-157	JQ048925	93.90	*Septoglomus deserticola*
33	0	3	0	1	MT408148	531	1.00E-146	HG515434	92.88	uncultured Glomeromycotina
34	0	0	4	1	MT408151	652	0.00E+00	HG515449	98.91	uncultured Glomeromycotina
35	0	0	4	1	MT408152	649	0.00E+00	HG515449	98.63	uncultured Glomeromycotina
36	0	0	4	1	MT408153	652	0.00E+00	HG515449	98.91	uncultured Glomeromycotina
37	0	0	4	1	MT408155	652	0.00E+00	HG515449	98.91	uncultured Glomeromycotina
38	0	0	4	1	MT408156	660	0.00E+00	HG515449	99.18	uncultured Glomeromycotina
39	0	0	4	1	MT408157	649	0	HG515449	98.63	uncultured Glomeromycotina
Total	100	100	100	100						

Aa, Afzelia africana; Ea, Entada africana; Pe, Pterocarpus erinaceus

Phylogenetic groups based on consensus trees and 80% bootstrap replicates revealed 3 genera (*Nanoglomus, Rhizoglomus,* and *Septoglomus*) in roots of *A. africana*; 4 genera (*Rhizoglomus*, *Scutellospora*, *Nanoglomus*, and *Septoglomus*) in the roots of *E. africana*; and 4 genera (*Glomus*, *Rhizoglomus*, *Sclerocystis*, and *Nanoglomus*) in roots of *P. erinaceus* (*Fig. 4*). Overall, phylogenetic inference classified 39 OTUs among which 2 (OTUs 8, and 18) were unidentified at the genus level, in the Glomeraceae, and only one OTU, which was identified as *Scutellospora erythropa*, in the Gigasporaceae.
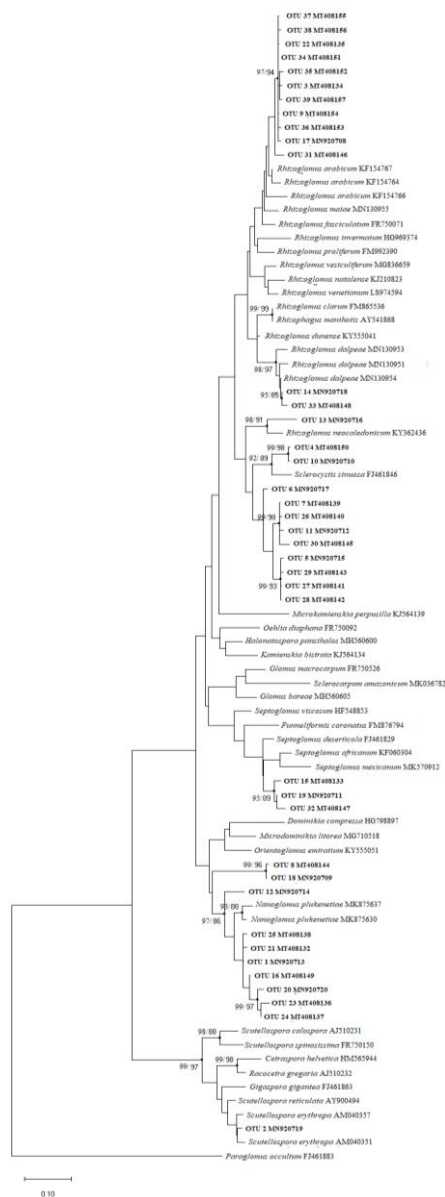
Figure 4. *Maximum likelihood (ML) phylogenetic tree obtained following analyses of large subunit (LSU) sequence data from arbuscular mycorrhizal fungi (AMF) in roots of A. africana, E. africana, and P. erinaceus sampled in Benin. The ML tree was constructed using a general time-reversible model assuming a discrete gamma distribution.* Paraglomus occultum *FJ461883 was used as an outgroup. Neighbor-joining (NJ) and ML bootstrap values ≥ 80% (NJ/ML) are provided at nodes marked with black dots. Operational taxonomic units (OTUs) generated in this study are indicated as OTU#; the scientific names of downloaded OTUs are indicated, followed by their accession numbers*

Discussion

To the best of our knowledge, this study is the first to report microscopic and molecular evidence of arbuscular mycorrhiza in roots of *A. africana*, *E. africana*, and *P. erinaceus* in their natural habitats. Structurally, *E. africana* showed the highest intensity of mycorrhizal colonization. Our basic local alignment search and phylogeny analyses revealed *Nanoglomus* spp., *Rhizoglomus* spp., and *Septoglomus* sp. as putative AMF in the roots of *A. africana*. *Rhizoglomus* spp., *Scutellospora sp.*, *Nanoglomus* spp., *Septoglomus* spp., and one genus-unidentified Glomeraceaous species (OTU 8) were putative AMF in the roots of *E. africana*, whereas those found in the roots of *P. erinaceus* were *Glomus* sp., *Rhizoglomus* spp., *Sclerocystis* spp., *Nanoglomus* sp., and one genus-unidentified Glomeraceaous species (OTU 18).

A critical review (Brundrett, 2009) emphasized past misdiagnosis related to the types of mycorrhizal features used during identification of mycorrhiza. McGonigle et al. (1990) recommended caution in the use of hyphae and vesicles, because unlike arbuscules, these features can appear sporadically in some non-mycorrhizal species. In the present study, we relied not only on the observation of morphological characteristics such as arbuscules, vesicles, and intra- and intercellular hyphae in stained roots, but also on DNA analyses. We also referred to illustrations of morphological features (Yuan et al., 2011; Priyadharsini et al., 2012; Claassens et al., 2018) to confirm features observed in our micrographs.

Some LSU sequences generated in the current study matched uncultured arbuscular mycorrhizal (AM) species according to the Basic Local Alignment Search Tool (BLAST), indicating a lack of molecular and mycological data on the tropical AMF community. This lack of data may also explain the positions of two OTUs on the phylogenetic tree, which corresponded to Glomeraceaous species but were not identified to the genus level.

Most OTUs (38 of 39) belonged to Glomeraceae, whereas only one belonged to Gigasporaceae. This result is consistent with a previous review of AMF species in tropical forests, which described Glomeraceae (78 of 228 species) as the most dominant family and Gigasporaceae (6 of 228 species) as one of the least represented families (Marinho et al., 2018). *Rhizoglomus dalpeae*, recently described by Błaszkowski et al. (2019) in Benin as a new species, was identified from roots of *E. africana*. The host tree in our study was collected from an inselberg within the Wari Maro forest reserve. The habitat within this reserve is a harsh environment, similar to the location where the fungus described by Błaszkowski et al. (2019) was collected.

Thoen and Ba (1989) reported that *A. africana* is ectomycorrhizal but is not associated with any endomycorrhiza. The same authors evaluated *A. africana* seed germination and seedling infection, and found that its seedlings exhibit early ectomycorrhizal infection. Yorou et al. (2008) also described the formation of ectomycorrhiza between *A. africana* and *Tomentella* species; however, they did not discuss its arbuscular mycorrhizal status. In the present study, we applied morphological and molecular methods to confirm that *A. africana* roots contained AMF, and therefore hypothesize that this species may be dual-mycorrhizal. In our study, all sampled *A. africana* individuals were adult trees, implying that arbuscular mycorrhiza are formed in adult trees of this species. In addition, Thoen and Ba (1989) conducted their research in Senegal, whereas our study was conducted in Benin. Dual mycorrhizae have been reported in West Africa for *Afzelia pachyloba* Harms, *Gilbertiodendron dewevrei* (De Wild.) J. Leonard, *Uapaca guineensis* Müll. Arg., and *Uapaca staudtii* Pax (Newbery et al., 1988; Thoen and Ba, 1989).

Therefore, we agree with the claim of Thoen and Ba (1989) that roots of adult individual trees should also be examined to avoid erroneous conclusions, because dual mycorrhizal colonization may be more common than previously thought. The high resistance to harsh environmental conditions shown by *A. africana* may be explained by such dual mycorrhizal colonization. We could not identify the *Nanoglomus* fungus found in roots of *A. africana* to the species level; however, its arbuscules and terminal vesicles were comparable to those reported by Corazon-Guivin et al. (2019) describing *Nanoglomus plukenetiae* as new fungal species.

AMF colonization intensity and the number of OTUs were higher in *E. africana* roots than in those of *A. africana* and *P. erinaceus*. The high mycorrhizal dependency of this species may be explained by its ecology. All sampled *E. africana* individuals were growing on shallow and rocky soils of an inselberg, where the absorption of soil resources may be difficult without fungal associations. *R. dalpeae* was previously found to originate from an inselberg with severe environmental conditions in Benin (Błaszkowski et al., 2019), an environment like the one of our research site. The association between *E. africana* and *R. dalpeae* as a mycorrhizal fungal partner could explain the adaptation strategies of the plant to such a harsh environment. A previous study (Tamilarasi et al., 2008) suggested that rhizosphere microorganisms influence the antibacterial properties of medicinal plants; the effects of AMF colonization on medicinal properties should be examined in *E. africana*, which is a medicinal tree species that contains many types of phytochemical compounds (Sanogo et al., 1998; Ahua et al., 2007; Tibiri et al., 2007, 2010; Mbatchou et al., 2011).

Because *P. erinaceus* is a slow-growing species (Dumenu and Bandoh, 2016), it is not used in plantations, and its overexploitation in natural forests is causing a gradual decline in its population (Barstow, 2018). In 2016, to prevent its extinction from natural habitats, as has occurred in other African countries, the Government of Benin banned the logging and export of *P. erinaceus* to China, India, and other countries. Previous studies have reported mycorrhizae in *P. angolensis* DC. (Hengari et al., 2004), *P. soyauxii* Taub. (Onguene et al., 2011), *P. marsupium* Roxb. (Verma et al., 2016), and *P. officinalis* (Saint-Etienne et al., 2006; Fougnies et al., 2007) but not in *P. erinaceus*. The current study reports the first evidence that *P. erinaceus* can form arbuscular mycorrhizae, which may be exploited to improve *P. erinaceus* growth in West Africa.

Conclusion

This study was the first to examine AMF in the roots of three valuable tropical forest tree species in their natural habitats. We confirmed that *A. africana*, *E. africana*, and *P. erinaceus* are all AMF host trees species. *E. africana* showed a relatively high dependence on AMF. At a genus level, 3 genera were identified in *A. africana*, and 4 in *E. africana* and *P. erinaceus*. At a species level, *Glomus trimurales*, *Scutellospora erythropa*, and *Rhizoglomus dalpeae* were identified from the roots of *E. africana*, and *Septoglomus deserticola* from the roots of *A. africana*. Our findings represent a theoretical basis for future applications of mycorrhizal technologies to improve the recruitment and growth of these three tree species following germination of seeds in a controlled environment. Future researches should examine the contribution of AMF to the resistance of *A. africana* in harsh environmental conditions, the effects of AMF colonization on medicinal properties in *E. africana*, and the potential growth-promoting activities of AMF in *P. erinaceus*.

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