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Full Length Research Paper

Assessment of the occurrence and abundance of mycorrhizal fungal communities in soils from yam (*Dioscorea* **spp.) cropping fields in Dabakala, North Côte d'Ivoire**

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Abundance and diversity of arbuscular mycorrhizal fungi (AMF) in soils under yam cropping fields in Dabakala, North Côte d'Ivoire were investigated to identify AMF species in order to set endomycorrhizal inoculation technology to improve yam productivity. Samples were collected at the locations of Bonieredougou 1, Bonieredougou 2, Souleymanekaha 1 and Souleymanekaha 2. Soil characteristics, AMF species, spore abundance and species richness were determined. Soils were either sandy loam or loamy sand, acidic and poor in organic matter and P contents. Boniérédougou soils differed from those of Souleymanekaha by their high coarse sand levels. After spore morphotyping, a total of 55 AMF species belonging to 14 genera were isolated with a range of 26 to 34 species recovered in a single location. Spore abundance was not affected by soil parameters while species richness was positively correlated $(R = 0.71; p = 0.007)$ with coarse sand content and **negatively with coarse and fine silt contents (** $R = -0.59$ **;** $p = 0.042$ **and** $R = -0.65$ **;** $p = 0.022$ **). Acaulospora** *paulinae, A. scrobiculata***,** *Ambispora* **sp. 1 and 2,** *Claroideoglomus etunicatum***,** *Funneliformis mosseae***,** *Glomus* **sp. 2 and** *Septoglomus furcatum* **were ubiquitous. These species could be used as potential inoculum to improve yam productivity in the study area.**

Key words: Yam, arbuscular mycorrhizal fungi, soils, Côte d'Ivoire.

INTRODUCTION

Yam (*Dioscorea* spp.) is a main staple food in West Africa. This region considered to be "the yam belt" produced more than 92% of the world yam fresh tubers production as reported in 2011 (FAO, 2013). Increasing yam production in West Africa is mainly due to increased cropping fields. In Côte d'Ivoire for example, these areas that were 505,408 ha in 2000, reached 834,400 ha in 2011, while the mean yields declined from 8.88 to 6.64 t

fresh tuber ha $^{-1}$ at the same period (FAO, 2013). The main constrains challenging yam production are soil fertility decline (Carsky, 2003), nematode damages (Coyne et al., 2006) and pest and disease attacks (Baimey, 2005; Egesi et al., 2006). Moreover, extensive production system is not yet sustainable due to land pressure. Developing technologies based on best control of soil fertility appears to be a solution to yam cultivation

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problems (O'Sullivan and Ernest, 2008). In this view, several researches for improving yam yields through mineral fertilization in different locations had been realized. These studies showed mitigated responses of field conditions (Diby et al., 2004; Ettien et al., 2009), others reported no response to fertilizer spreading (Sotomayor-Ramirez et al., 2003; Baimey, 2005; Diby 2005; Diby et al., 2009). Another way to enhance soil fertility and improve plant growth is making use of natural symbioses between plant and soil microorganisms.

In this agroecological outlook, mycorrhizal symbiosis is a very interesting option. Arbuscular mycorrhizas are known to be the most common underground symbioses that occur between plants and arbuscular mycorrhizal fungi (AMF) (Smith and Read, 2008).

Their importance was recognized in a broad range of basic and practical studies (Walker et al., 2007). Recent studies highlighted potential of AMF for yam productivity improvement.

Arbuscular mycorrhizal colonization was reported to increase yam yields in Nigeria (Oyetunji and Afolayan, 2007) and in Benin (Tchabi et al., 2009; 2010). It was also shown that mycorrhizal symbioses were able to suppress nematode damage and additionally leads to improve yam tuber quality and weight (Tchabi, 2008). AMF diversity was recently estimated in soils from yam cropping fields in Nigeria (Dare et al., 2013).

It was shown that AMF diversity and occurrence in these soils were influenced by different agroecosystems and environmental conditions. In Côte d'Ivoire, the fact that yam is grown on poor soils negatively impact its productivity (Diby et al., 2009). The use of endomycorrhizal inoculation technology could be an interesting option for ecological intensification of yam production systems in Côte d'Ivoire. For this purpose, it was interesting to understand the ecology of arbuscular mycorrhizal fungi in yam cropping fields in Côte d'Ivoire.

This study aimed at characterizing both the chemophysical parameters of yam cropping field soils and the ecology of AMF communities associated with yam in Dabakala a region of yam production in north Côte d'Ivoire. This study should lead to the selection of potential candidates to initiate AMF inoculation technology for yam productivity in Côte d'Ivoire.

MATERIAL AND METHODS

Study sites

This study was undertaken at Dabakala in North Côte d'Ivoire, a dry Sudan savanna zone where yam is mainly produced for marketing (Doumbia et al*.*, 2006). This zone is characterized by a wet season from June to October with 900 to 1200 mm of rain. The study was conducted at four study fields located into the villages of Boniérédougou and Souleymanekaha (Figure 1). Fields where distant each other from at least 10 km. These study sites were mainly cultivated with *Dioscorea alata* and *Dioscorea cayenensisrotundata*.

Soil sampling

Samples were collected in December, during the harvesting period from four fields: Boniérédougou 1, Boniérédougou 2, Souleymanekaha 1 and 2 (Table 1 for geographical positions). At each field, three samples were collected. According to the regimen proposed by Huang and Cares (2004), samples were obtained by mixing 12 soil cores taken in mounds at 20 cm-depth. A total of 12 samples were collected for the study. The soils were brought up to the laboratory. Each replicate was air-dried, filtered and divided into three sub-sets: To determinate soil parameters, to isolate and identify AMF spores and AMF propagation and spore production in trap cultures.

Soil chemo-physical parameter measurement

The soil samples characteristics were determined by routine analysis methods. Soil texture, pH water, organic C were determined according to Walkley and Black (1934), total N by the Kjeldahl method (Bremner, 1960), total and available P (Olsen, 1952), exchangeable K, Ca and Mg and cation exchange capacity (CEC) were measured. Organic matter (*OM*) was calculated as: *OM* $(%) =$ organic $C \times 1.724$ (Schaffer, 1975).

Trapping culture and mycorrhizal root analyses

For each sampling point, three culture pots (small plastic bucket of 2 L) were established. The inocula consisted of 100 g of soils sampled at each point mixed with 1000 g of autoclaved compost. Two to five day old cowpea (*Vigna unguiculata*) seedlings were added per pot. A total of 15 pots were set up including three nonmycorrhizal control pots of 1000 g of compost substrate previously sterilized in an oven at 120°C for 2 h 30 min. 75 days after, soils core were removed from each pot and root colonization assessed (Trouvelot et al., 1986).

AMF spore isolation and identification

Spores from both field soils and trap cultures were extracted by wet sieving (Gerdemann and Nicolson, 1963). For this purpose, 50 g of air-dried soils were suspended in 1 L of water using a 2 L beaker. The supernatant was decanted through sieves of 500-, 125-, 90 and 45-µm arranged in that order to discriminate particles. The content of the 500-µm sieve, generally composed of debris, was discarded. The contents of the 125-, 90- and 45-µm sieves were collected and centrifuged (2000 rpm for 5 min). The sediment was resuspended in 50% sucrose solution and centrifuged (2000 rpm for one min) again. Spores in suspension were filtered and counted using a compound microscope according to INVAM enumeration method [\(http://invam.caf.wvu.edu\)](http://invam.caf.wvu.edu/). For species identification, healthy spores were mounted on glass microscope slides and stained with polyvinyl alcohol-lacto-glycerol (1vol/1vol) (PVLG) mixed with Melzer's reagent (Brundrett et al*.*, 1994). Spores were cracked open to allow spore substructure characteristics under a stereomicroscope at a magnification of x400. AMF spore morphotyping was based on Oehl et al. (2006, 2011), INVAM and Blaszkowski [\(http://www.zor.zut.edu.pl\)](http://www.zor.zut.edu.pl/) current species description manuals and their classification has been done according to Redecker et al. (2013).

Species relative abundance

AMF relative abundance was recorded after using different scales;

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Figure 1. (A) Location of the study area in Côte d'Ivoire and (B) satellite view of yam cropping fields and sampling points (Google Earth). Sampling points are indicated as Boxy for samples from Boniérédougou x (1 or 2) and Soxy for samples from Souleymanekaha x (1 or 2).

Table 1. Geographical position of the study sites.

Figure 2. Soil physical composition at the yam cropping fields. Data were reported as means and standard errors for three replicate plots per site. For each soil physical content, nonsignificant differences between fields are indicated by identical letters above the bars and were assigned by comparison using U Mann-Whitney test at 5% level.

rate 1: 0, 1-3% (rare), spores from a taxon were rarely found; rate 2: 3.1-20% (frequent), spores from a taxon were found frequently; rate 3: > 20% (abundant), spores from a taxon were dominant (Tchabi, 2008). Rate 0 was the absence of spores for a specific taxon.

Statistical analyses

AMF spore abundance was recorded as spore number.g⁻¹ soil. The species richness was recorded as the species number. Occurrence (%) was defined as the number of samples in which a taxon (species or genus) was observed on the total number of samples (= 12). Root colonization rate (*r*) was calculated as:

r (%) = (95*N5* + 70*N4*+ 30*N3* + 5*N2* + *N1*) × total number of root observed

Where *Ni* is the number of fragments corresponding to cote i (Trouvelot et al., 1986).

All the statistical analyses have been realized using STATISTICA program (version 7.1, 2005). For the evaluation of differences between sites, non-parametric tests, ANOVA of Kruskal-Wallis and *U* Mann-Whitney test (*p* < 0.05) were used. To determine the natural clusters in the collected data, a hierarchical cluster analysis (HCA) based on Euclidian distance measure and Ward aggregation method were performed. Principal Component Analyses (PCA) were also performed to determine relationships between soil chemo-physical characteristics and AMF parameters. Linear regressions were analyzed with Spearman *R* correlation to determine significant relationships between factors.

RESULTS

Texture and chemical characteristics of soils under yam cropping fields

In terms of physical contents, the soils from the four yam cropping fields were significantly (*p* < 0.05) different (Figure 2), but clay content was the same. The texture of these soils was generally sandy loam except at Boniérédougou site where soils were loamy sand (Figure 1). Hierarchical cluster analysis performed with soil physical composition (Figure 3A) showed that Boniérédougou soils were clearly different from those of Souleymanekaha.

In terms of chemical parameters, soils were not significantly different except Ca^{2+} values (Table 2). Organic matter content was essentially inferior to 2%. At Boniérédougou 2, it was superior to 2%. N content ranged from 0.07 to 0.11% for all soils. The C/N ratio generally ranged from 8 to 12. Despite the highest total P values at Souleymanekaha (231-260 ppm against 139- 197 ppm at Boniérédougou), available P values were low (24-26 against 27-32 ppm at Boniérédougou). Soil pH ranged from 5 to 6 and the lowest values were obtained at Boniérédougou 2. CEC values ranged from 7 to 10 cmol/kg at Souleymanekaha and higher than 10 cmol/kg at Boniérédougou. Unlike soil physical composition (Figure 3A), hierarchical cluster analysis performed with soil chemical properties (Figure 3B) showed that yam cropping fields from Boniérédougou were not distinct from those of Souleymanekaha according to soil fertility.

AMF spore abundance and root colonization assessment

AMF spore abundance ranged from 22 to 28 spores.g⁻¹ of soil (Figure 4). Seventy-five days after trap culture, spore number increased in pots containing soil from Boniérédougou 2.

Table 2. Soil chemical parameters recorded as mean values ± standard errors.

Significant differences between fields are indicated by different letters associated to means values and assigned by comparison with U Mann-Whitney test at 5% level.

Figure 4. Spore densities from field soils and trap culture samples. Data were reported as means and standard errors for three replicate plots per field site. No significant difference was found between fields before and after trap culture by assigning comparison using U Mann-Whitney test at 5% level.

Figure 5. AMF species richness for each genus at the four yam cropping fields.

In contrast AMF spore abundance decreased in the others trap culture. However, no significant difference was obtained between sites before and after trap culture. All root fragments were colonized except the non-mycorrhizal control pots. Root colonization rate varied from 42 to 63% for Boniérédougou 1, 60 to 75% for Boniérédougou 2, 63 to 74% for Souleymanekaha 1 and 58 to 79% for Souleymanekaha 2.

AMF diversity and species occurrence

At the four yam cropping fields investigated at Dabakala,

55 AMF species belonging to 13 genera and 8 families were identified. The genera *Glomus*, *Acaulospora*, *Scutellospora* and *Ambispora* were the most diversified species in all the sites (Figure 5). Besides these genera, *Claroideoglomus*, *Funneliformis* and *Septoglomus* were also important because of their presence in all the yam cropping fields. Species occurrence, relative abundance as well as total number of AMF species identified at each site are shown in Table 3. *Acaulospora paulinae, A scrobiculata*, *Ambispora* sp 1 and 2, *Claroideoglomus etunicatum*, *Funneliformis mosseae*, *Glomus* sp 2 and *Septoglomus furcatum*, were frequent both in terms of occurrence $($ > 50%) and relative abundance $($ > 3%).

Table 3. AMF species relative abundance and occurrence per site.

Table 3. Contd.

The relative abundance (r) of each AMF species was recorded following the scale: - (absent): no specimens found; + (rare): 0 < r < 3%; ++ (frequent): 3% < r < 20%; +++ (abundant): r > 20%. Boniéré = Boniérédougou and Souley = Souleymanekaha. According to Oehl et al. (2011), * classified in the genus *Entrophospora*. ** classified in the genus *Glomus*. *** classified in the genus *Orbispora*.

Figure 6. Species richness recorded as means and standard errors for three replicate plots per field site. Non significant differences between sites were indicated by identical letters above the bars and were assigned by comparison with U Mann-Whitney test at 5% level.

Other species which were also frequent (*relative abundance* > 3%) were only found at Boniérédougou sites (Table 3). Finally the total species number per site was higher at Boniérédougou (ranged from 31 to 34) compared to those obtained at Souleymanekaha sites (ranged from 26 to 27). U Mann-Whitney test performed on species richness showed significant difference between sites (Figure 6).

Relationship between soil chemo-physical parameters and AMF spore abundance and species diversity

Principal component analysis performed on soil parameters, AMF spore abundance and species number showed that Axes 1 and 2 accounted respectively for 35.88 and 26.76% of variability between cropping field

Figure 7. (A) Projection of both soil and AMF parameters on the factorial plan and (B) factorial map of sampling points. On the factorial map, Boxy indicates samples from Boniérédougou x (1 or 2) and Soxy indicates samples from Souleymanekaha

(Figure 7A). Coarse sand, fine and coarse silt, total and available P proportion (available P/Total P), and Ca^{2+} as well as species number were the principal parameters linked to Axis 1. Species number was positively correlated with coarse sand and available P proportion and negatively with fine and coarse silt, total P and Ca^{2+} . The linear Spearman R correlation (Figure 8) showed that species number was significantly correlated with coarse sand, fine and coarse silt and total P (*p* < 0.05).

Spore abundance, which was near to the axes origin, was not a significant factor of discrimination between samples. The principal parameters linked to Axis 2 were

Figure 8. Relationships between species number and some soil parameters performed with Spearman *R* linear correlation test.

fine sand, clay, C, N, Mg^{2+} and CEC. Among these parameters, fine sand was negatively correlated to the others factors.

Sample projection on factorial map (Figure 7B) showed an AMF species richness increasing gradient from Souleymanekaha sites to Boniérédougou sites. Boniérédougou 2 differed from the other sites by its high CEC and coarse sand values.

Relationship between soil chemo-physical parameters and AMF genus relative abundance

Principal component analysis performed with AMF genus relative abundance as active variables and secondarily with soil chemo-physical parameter projections to explain genus projection on the factorial plan showed that Axes 1 and 2 accounted respectively for 31.32 and 22.28% of variability between sites (Figure 9). Among the genera, *Ambispora*, *Claroideoglomus*, *Gigaspora*, *Septoglomus* and *Scutellospora* where negatively correlated with Axis 1 on which *Glomus* was positively correlated. At the same time, silt, pH and total P where negatively correlated with the Axis 1 while coarse sand where positively correlated. Linear regressions between genus relative abundance and soil parameters linked to the Axis 1 (Table 4) showed that the relative abundance *Ambispora* spp. was significantly decreased with coarse sand while it was significantly increased with fine soil particles (silt and fine sand) and pH. The relative abundance of *Claroideoglomus* spp. was significantly decreased with coarse sand and significantly increased with fine soil particles total P. The relative abundance of *Septoglomus* spp. significantly increased with CEC level and decreased with pH. Relative abundance of *Scutellospora* spp. was significantly increased with coarse silt.

DISCUSSION

This study was an investigation on the AMF ecology associated with yam cropping fields in Dabakala, North Côte d'Ivoire. In this yam production region, soil texture was generally sandy loam. However, hierarchical cluster analyses based on soil physical parameters indicated clearly that Boniérédougou soils differed from those of

Figure 9. Principal component analysis performed with AMF genus relative abundance as active variables and soil chemo-physical parameters as supplementary variables.

Table 4. Linear regressions (Spearman *R* and *p*) between AMF genera and soil chemo-physical parameters.

Genus x soil parameter	Spearman R	p	Genus x soil parameter	Spearman R	p
Ambispora x Coarse sand	-0.59	0.041	Claroideoglomus x Coarse sand	-0.70	0.012
Ambispora x Fine sand	0.69	0.013	Claroideoglomus x Fine sand	0.62	0.030
Ambispora x Coarse silt	0.69	0.012	Claroideoglomus x Coarse silt	0.69	0.013
Ambispora x Fine silt	0.58	0.048	Claroideoglomus x Fine silt	0.65	0.022
Ambispora x pH	0.70	0.012	Claroideoglomus x pH	0.33	0.298
Septoglomus x pH	0.72	0.008	Claroideoglomus x Total P	0.67	0.016
Septoglomus x CEC	-0.60	0.040	Scutellospora x Coarse silt	0.59	0.042

Souleymanekaha through the proportion of coarse sand that was higher at Boniérédougou and the important presence of fine particles in Souleymanekaha soils. These soils had a C/N ratio that ranged from 9 to11. These values could be considered as average as compared to normal standard (Gagnard et al*.*, 1988). This clearly shows evidence that there was an effective organic matter mineralization and a good microorganism activity in these yam cropping fields. However in Boniérédougou soils, total P was lower while available P was higher. This could probably be explained by the fact that these soils were such acidic that they may negatively influence P availability as demonstrated earlier (Betencourt, 2012). AMF spores were abundantly found in all yam cropping fields without significant difference

Mohammad et al., 2003; Tchabi et al., 2008), organic C (Tchabi, 2008; Hu et al., 2013) and available P suggesting that neither soil chemo-physical characteristics nor soil fertility parameters did affect spore abundance in these yam fields. Moreover no correlation was found between spore abundance and any of the soil parameters as indicated earlier (Oehl et al., 2010). This could be explained by the no significant difference found between field soils in terms of chemical parameters. However, it was indicated in other studies that soil parameters such as pH (Johnson et al., 1991; (Neumann and George, 2004; Subramanian et al., 2006) could affect AMF spore abundance.

Overall, 55 AMF species belonging to 14 genera were identified in this agroecological zone considered as a dry

savanna area. A comparable AMF species diversity was also found in both yam cropping fields and natural savanna in Benin (Tchabi et al., 2009). However, this diversity was higher compared to the one observed in yam cropping fields in Benin and Nigeria (Tchabi et al., 2008, 2009; Dare et al., 2013) where 31 to 40 AMF species were found. Our results indicated that species distribution was not uniform and was significantly different between the four yam cropping fields. It was shown that AMF species richness was positively affected by coarse soil particles and negatively by both fine particles and total P. This could explain why AMF species richness was higher at Boniérédougou were soils were characterized by high coarse sand level and low silt. AMF communities were dominated by *Glomus* spp. and *Acaulospora* spp. in each field as was indicated in Benin and Nigeria (Tchabi et al., 2008, 2009; Dare et al., 2013). Several studies had reported *Glomus* dominance in several ecosystems and climatic conditions (Gai et al*.*, 2006, Pande and Tarafdar, 2004). Moreover, different *Glomus* species were shown to be effective on plant growth (Cornet et al*.,* 1982; Bulakali et al*.,* 2000; Bourou et al*.*, 2011; Tchabi, 2009). Other genus such as *Acaulospora* also occurred in all the yam cropping fields that were studied.

Conclusion

In conclusion, this study highlighted an important diversity of AMF associated with yam rhizosphere in Dabakala region, but many of the species identified were not frequent at any location. However, some of them such as *Acaulospora paulinae, A. scrobiculata*, *Ambispora* sp. 1 and 2, *Claroideoglomus etunicatum*, *Funneliformis mosseae*, *Glomus* sp. 2 and *Septoglomus furcatum* were ubiquitous. These species are recommended for research about their effectiveness to improve yam productivity in the study area and then in Côte d'Ivoire.

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