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Full Length Research Paper

Potentialities of native arbuscular mycorrhizal fungi strains to improve the quality of macropropagated seedlings of plantain cv Orishele and FHIA 21

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Abstract

In order to assess the effect of native arbuscular mycorrhizal fungi strains on plantain seedlings obtained by tissue multiplication technique, two cultivars of plantain (Orishele and FHIA 21) were inoculated with *Glomus clavisporum, Acaulospora colombiana, Rhizophagus proliferum* and *Acaulospora* sp isolated from Côte d'Ivoire plantain fields. This study was conducted for twelve weeks in a greenhouse. Root colonization, growth, physiological and biomass parameters were assessed. It was shown that mycorrhization enhanced photosynthetic activity and growth of the macropropagated seedlings. Seedlings inoculated with *Rhizophagus proliferum* had the highest rate of mycorrhization (Orishele: 83%; FHIA 21: 92.33%). A better biomass production resulting from a relative mycorrhizal dependency of 79.81% for the Orishele cultivar and 42.55% for FHIA 21 cultivar was observed. The hierarchical cluster analysis showed that *Acaulospora colombiana* and *Rhizophagus proliferum* strains induced higher photosynthetic activity and a better growth of the seedlings. These strains have therefore important potential for mycorrhizal plantain seedlings production.

Keywords: Arbuscular mycorrhizal fungi, Côte d'Ivoire, Native strains, Plantain seedlings, Tissue multiplication technique.

Introduction

The production of good quality planting materials is one of the main concerns for many plantain smallholders (Perrin, 2015). To solve this problem, an innovative and affordable technique for plantain seedlings production based on macropropagation was then developed by CARBAP (African Center for Research on Bananas and Plantain). This technique called "seedlings resulting from stem fragments" (Kwa, 2003; Sadom *et al.*, 2010) which allows the quick production of large quantities of planting materials, in soil-less culture conditions, is a good response to the issues faced by Côte d'Ivoire plantain sector (Traoré *et al.*, 2009). However, macropropagated plantlets obtained by this technique are exposed to declining soil fertility and attacks from pests and diseases; which can cause significant morbidity and mortality rate. To face this situation, chemical fertilizers and pesticides could be used to increase the production and the quality of plantain seedlings. But such practices can endanger nurseryman and the environment. It is therefore necessary and urgent to develop biofertilization and bioprotection strategies to improve the quantity and the quality of the plantain seedlings production. The use of AMF (arbuscular mycorrhizal fungi) could be an alternative for good growth and better protection of macropropagated plantlets. *In vivo* mycorrhization improves growth, plant nutrition and plant protection against ground pathogens (Jefwa *et al*., 2010; Willis *et al.*, 2013). The inoculation with AMF during the plantain seedlings acclimatization is therefore a key step to obtain vigorous seedlings resistant to soil pathogens attacks.

This work was conducted in order to study the impact of four AMF native strains (*Glomus clavisporum, Acaulospora colombiana*, *Rhizophagus proliferum, Acaulospora* sp) on the quality of macropropagated seedlings of plantain cultivars Orishele and FHIA 21.

Materials and methods

Source of plant materials

Plantain seedlings cv. Orishele (*Musa* AAB) and FHIA 21 (*Musa* AAAB) were obtained from National Center for Agricultural Research in Côte d'Ivoire (CNRA). Seedlings were produced by macropropagation using the "seedlings from stem fragments" technique (Kwa, 2003).

Isolating native mycorrhizal strains

Soil samples were randomly collected from plantain fields of three agro-ecological areas of Côte d'Ivoire (Fotso *et al.*, 2016). The soil samples collected were bulked and brought to the laboratory in well labeled polyethylene bags. Pot-cultures were established in greenhouse using original soil samples mixed with autoclaved sand 1:3 (v/v) as the substrate for growth of cowpea (*Vigna unguiculata*) to propagate AMF strains. Plants were watered and fertilized with Long Ashton's no-P fertilizer. The most abundant AMF spore morphotypes were recovered and used to start monospecific culture on 10 days old cowpea seedlings grown in a sterile mix of sand-perlite 2:1 (v/v). Watering and fertilization were done similarly as for the AMF trapping. The best isolates were selected according to their abundance, their ubiquity and their ability to colonize efficiently plantain roots. After six months of trapping culture and purification, four strains of arbuscular mycorrhizal fungi: *Glomus clavisporum, Acaulospora colombiana, Rhizophagus proliferum, Acaulospora* sp (Figure 1) were isolated. The inoculum was a homogeneous mixture of spores, root soil and host plant roots.

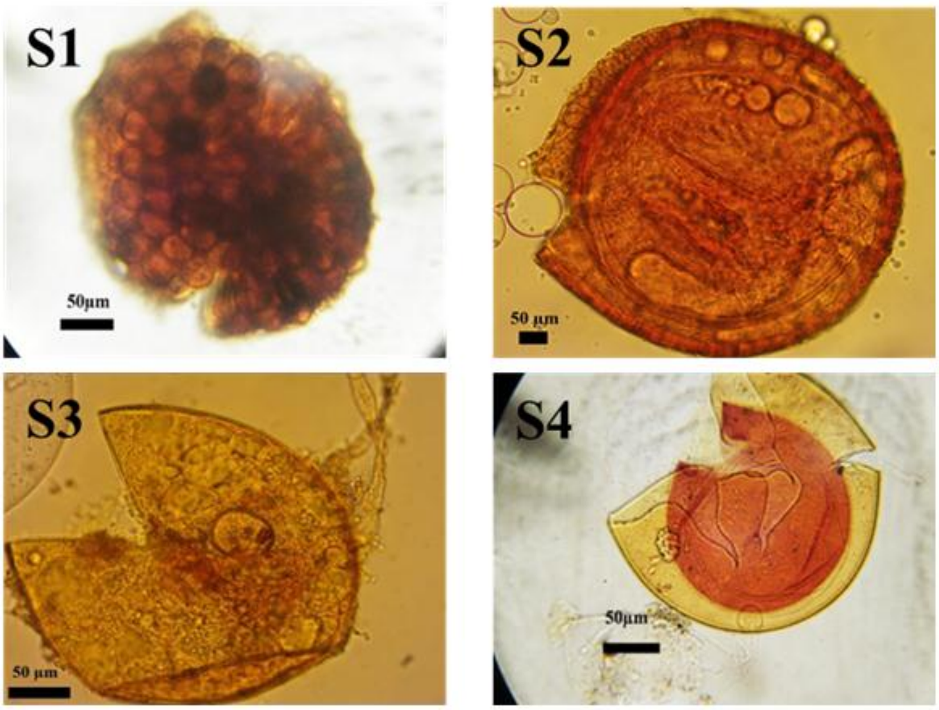

Figure 1: Spores of arbuscular mycorrhizal fungi strains used in the study (stained with Melzer's reagent). S1: *Glomus clavisporum,* S2: *Acaulospora colombiana,* S3: *Rhizophagus proliferum* and S4: *Acaulospora* sp

Soil characteristics

The characteristics of the substrate are described in Table 1. Soil nutrient composition (nitrogen, phosphorous, potassium, carbon, magnesium, calcium, sodium), CEC (Cation Exchange Capacity) and pH were determined according to standard procedures (Anderson & Ingram 1993; Okalebo *et al.* 2002).

Table 1: Physico-chemical parameters of substrates

	pH $_{H2O}$	C (%)	N (%)	P (ppm)	CEC (cmol.kg^{-1})	Ca^{2+} (cmol.kg^{-1})	Mg^{2+} (cmol.kg^{-1})	K^{+} (cmol.kg^{-1})	Na^{+} (cmol.kg^{-1})
Sand	6.7	0.1	0.01	2	0.48	0.217	0.139	0.106	0.13
Soil	7.1	1.63	0.15	55	11.4	4.215	0.973	0.197	0.132

Inoculation process

After deflasking, plantain seedlings were acclimatized for 4 weeks on a coconut fiber substrate. They were then transplanted into 5-liter-container with sterilized soil at 120 ° C under 1 bar for 1 hour. Inoculation of the plantlets occurred during the transplantation. Inoculation was done on 3-leaves stage plantlets with a height of 5 ± 1 cm. Three replicates were considered per treatment. Control experimental units (without inoculation) were also included with three replicates. The experimental units were arranged in a completely randomized design under greenhouse conditions.

Assessment of growth parameters

Every two weeks, shoot height (from base), area of the second leaf from the sword shoot and the girth (at two cm from the base) of the stem were recorded. Eight weeks after inoculation, chlorophyll a was extracted from the second leaf with ethanol (80%) (Sadasivan & Manickam, 1996) and assessed using a spectrophotometer (JENWAY 7315). Then twenty pieces (1 cm) of fine roots were collected for each plantlet and stained with trypan blue (Phillips & Hayman, 1970), in order to determine the root colonization (Trouvelot *et al.,* 1986). At the end of the experiment, shoots and roots were harvested and dried at 80°C for 72 hours. Dry biomass weight was then assessed. The relative mycorrhizal dependency (RMD) were calculated for each treatment according to the following formula (Gerdemann, 1975):

$$\text{RMD} = \frac{\text{Dry weight of inoculated seedlings} - \text{Dry weight of control seedlings}}{\text{Dry weight of control seedlings}} \times 100$$

Data analysis

In order to assess the effects of mycorrhizal strains, data collected were subjected to analyses of variance (Generalized Linear Models). Means found to be significantly different at $p \leq 0.05$ were separated using Fisher's LSD (Least Significant Difference) test. Hierarchical cluster analysis based on all measured parameters was performed to classify treatments; the Ward clustering method with Euclidean distance was used.

Results

Root colonization

The root colonization analysis showed that all treated seedlings were colonized by mycorrhizal structures. The strain *Rhizophagus proliferum* had the highest root colonization frequencies regardless the cultivars (Orishele: 83%; FHIA 21: 92.33%) (Table 2). The control seedlings showed no mycorrhizal structure.

Table 2: Plantain root colonization frequencies eight weeks after inoculation with native AMF strains

Treatments	**Root colonization frequency (%)**	
	Orishele	**FHIA 21**
Control	-	-
Glomus clavisporum	63.33 ± 20.28 a	66.67 ± 17.64 a,b
Acaulospora colombiana	83.33 ± 8.82 a	36.67 ± 21.86 a
Rhizophagus proliferum.	83.33 ± 16.67 a	92.33 ± 2.62 a
***Acaulospora* sp**	40 ± 25.17 b	63.33 ± 23.33 a,b
Cultivars	**p** = 0.9498 **F**=0.0041	
Treatments	**p** = 0.0004 **F**=8.2256	
Cultivars×Treatments	**p** = 0.2777 **F**=1.3760	

(Values within the same column with different letters are significantly different)

Effects of AMF native strains on plantain seedlings growth

Eight weeks after inoculation, plants of the Orishele cultivar had significantly higher average height (18.66 cm) than the FHIA 21 cultivar. However, the girth of the FHIA 21 seedlings was significantly higher (2.08 cm) than the girth of Orishele cultivar (Table 3). Overall, inoculated seedlings had significantly better growth performance compared to control. Seedlings treated with mycorrhizal strains had higher height ($p = 0.0027$), girth ($p < 0.0001$) and leaf area ($p < 0.0001$) than non-treated plants (Table 3). *Acaulospora colombiana* had the most significant effect on seedlings height (Orishele: 20.57 cm, FHIA 21: 18.83 cm) and leaf area regardless the cultivar. Seedlings inoculated the strains *Glomus clavisporum* and *Acaulospora* sp showed the highest girth respectively for Orishele (2.05 cm) and FHIA 21 (2.35 cm).

Table 3: Growth parameters of plantain seedlings eight weeks after inoculation with native AMF strains

Treatements	Height (cm)		Girth (cm)		Leaf area (cm²)	
	Orishele	FHIA 21	Orishele	FHIA 21	Orishele	FHIA 21
Control	16.83 ± 0.33 c	16.6 ± 0.46 b	1.5 ± 0.09 b	1.78 ± 0.06 c	135.89 ± 15.65 b	163.29 ± 16.33 c
Glomus clavisporum	18.833 ± 0.60 a,b,c	17.13 ± 0.52 a,b	2.05 ± 0.08 a	1.98 ± 0.08 b,c	260.16 ± 15.04 a	247.93 ± 29.16 b
Acaulospora colombiana	20.57 ± 0.81 a	18.83 ± 1.09 a	1.92 ± 0.07 a	2.17 ± 0.09 a,b	307.28 ± 22.76 a	313.92 ± 27.27 a
Rhizophagus proliferum	17.83 ± 0.83 b,c	16.67 ± 0.44 a,b	1.97 ± 0.09 a	2.1 ± 0.06 a,b	258.61 ± 19.18 a	261.11 ± 11.58 a,b
***Acaulospora* sp**	19.23 ± 0.64 a,b	18 ± 0.76 a,b	2.03 ± 0.02 a	2.35 ± 0.13 a	308.96 ± 2.07 a	293.85 ± 12.18 a,b
Cultivars	**p** = 0,0109 **F**=7.866		**p** = 0,002 **F**=12.604		**p** = 0,8781 **F**=0.024	
Treatments	**p** = 0,0027 **F**=5.841		**p** <0,0001 **F**=12.535		**p** <0.0001 **F**=23.366	
Cultivars×Treatments	**p** = 0,8113 **F**=0.393		**p** = 0,1640 **F**=1.823		**p** = 0,7963 **F**=0.414	

(Values within the same column with different letters are significantly different)

Effects of AMF native strains on plantain seedlings physiology and biomass dry weight

Control seedlings had significantly (p = 0.0015) lower chlorophyll a content compared to seedlings treated with mycorrhizal strains. *Rhizophagus proliferum* increased significantly the chlorophyll a content of Orishele seedlings (51.74 µg/cm²). While *Glomus clavisporum* increased significantly the chlorophyll a content of FHIA 21 seedlings (52.55 µg/cm²). Biomass analysis showed that inoculation with mycorrhiza increased significantly plants dry weight (p = 0.0138). Orishele plants inoculated with *Acaulospora colombiana* and *Glomus clavisporum* strains had the highest plants dry weight, increasing respectively biomass by 90.18% and 90.02% compared to control. However, *Rhizophagus proliferum* and *Acaulospora* sp improved more significantly FHIA 21, respectively by 62.67%, 69.60% compared to control. (Table 4). The cultivar Orishele showed more dependency to mycorrhiza inoculation than to the FHIA 21 cultivar. The relative mycorrhizal dependency of Orishele cultivar was 79.81% and 42.55% for FHIA 21 cultivar.

Table 4: Physiological and biomass parameters of plantain seedlings eight weeks after inoculation with native AMF strains

Treatments	Chlorphyll a (µg/cm²)		Biomass dry weight (g)		RMD (%)	
	Orishele	FHIA 21	Orishele	FHIA 21	Orishele	FHIA 21
Control	31.65 ± 1.71 b,c	33.32 ± 2.59 b	8.35 ± 3.06 b	12.45 ± 4.25 b	-	-
Glomus clavisporum	45.79 ± 6.77 a,b	52.55 ± 0.86 a	15.86 ± 1.91 a	14.03 ± 3.85 b	90.02	12.72
Acaulospora colombiana	40.97 ± 3.78 a,b,c	43.78 ±1.19 a,b	15.87 ± 2.87 a	15.58 ± 5.63 a,b	90.18	25.20
Rhizophagus proliferum.	51.74 ± 2.40 a	37.98 ± 2.62 a,b	14.69 ± 0.28 a,b	20.25 ± 4.05 a	76.04	62.67
***Acaulospora* sp**	27.13 ± 5.78 c	31.02 ± 1.21 b	13.61 ± 1.41 a,b	21.11 ± 5.93 a	63.02	69.60
Cultivars	**p** = 0.9267 **F**=0.0087		**p** = 0.0285 **F**=5.5758		**p**<0,0001 **F**=26.2564	
Treatments	**p** = 0.0015 **F**=6.6051		**p** = 0.0138 **F**=4.0968		**p** =0,0033 **F**=5.6488	
Cultivars×Treatments	**p** = 0.2427 **F**=1.4904		**p** = 0.1470 **F**=1.9162		**p** =0,0878 **F**=2.3618	

(Values within the same column with different letters are significantly different)

Hierarchical clustering of AMF native strains

The hierarchical clustering of AMF strains based on growth, physiological and biomass parameters revealed three clear clusters of performance. Cluster I, *Rhizophagus proliferum* and *Acaulospora colombiana* strains; cluster II, *Glomus clavisporum* strain; and cluster III, *Acaulospora* sp and control (Figure 2). Seedlings inoculated with *Rhizophagus proliferum* and *Acaulospora colombiana* strains had the same behavior. Only seedlings inoculated with Acaulospora sp strain had the same characteristics as control seedlings.

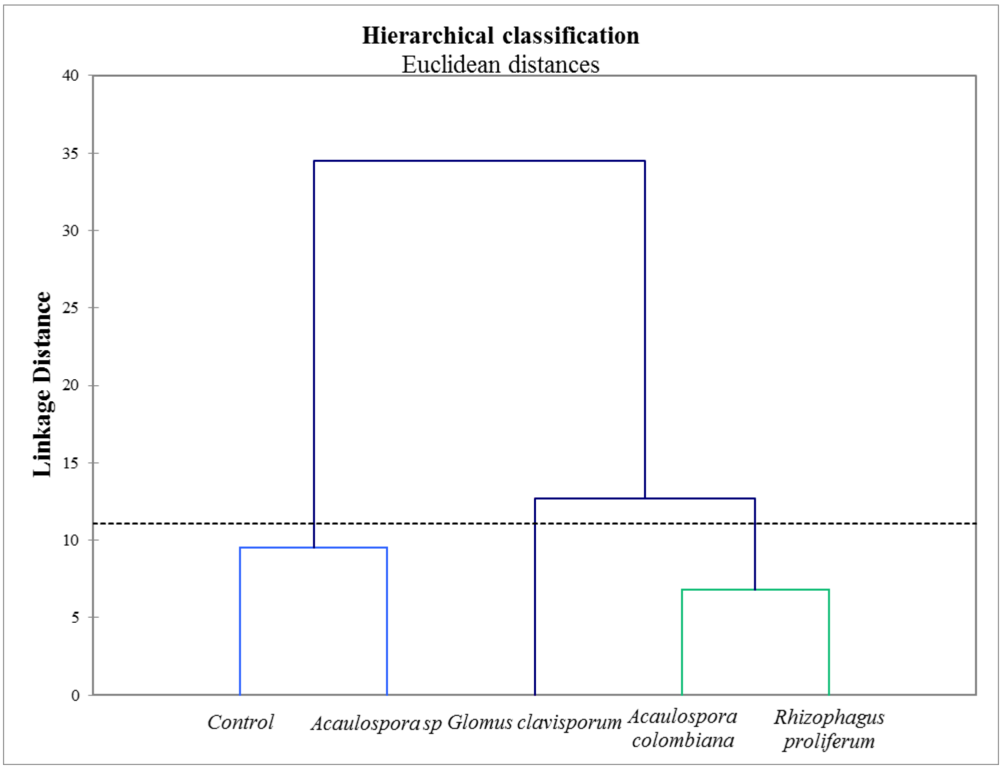

Figure 2: Hierarchical clustering of AMF inoculants based on growth, physiological and biomass parameters of plantain seedlings

Discussion

Knowledge on the status of AMF in plantain cropping systems is limited but increasing due to recent rising interest. Mainly conducted in East and Central Africa, research on bananas and plantain mycorrhization showed that AMF offer significant promise to help address soil fertility and pests and disease constraints. While most studies in Africa on the effect of mycorrhiza on growth of plantain and bananas seedlings were focused on micropropagated plantlets (Jefwa *et al.*, 2010), the present study investigates the effect of those microorganisms on macropropagated plantain seedlings.

The high level of colonization of roots by AMF strains (higher than 40%) is due to the efficiency of native AMF strains isolated from plantain fields. In addition, plantain plants appear to be highly mycorrhizal (Msiska, 2001; Jaizme-Vega *et al.*, 2002). The highest colonization frequencies were observed on plants inoculated with *Rhizophagus proliferum* belonging to the Glomeraceae family. The study conducted by Declerck *et al.* (1995) confirms these observations, showing that the species of the Glomeraceae family had the best root colonization efficiency. The root colonization by arbuscular mycorrhizal fungi could be determinant in plants vigor improvement as observed by Harrier & Watson (2004) and Li *et al.* (2006).

The high levels of chlorophyll a content of inoculated plants could be due to a good phosphorus uptake by inoculated plants (Tavasolee *et al.*, 2011; Birhane *et al.*, 2012). This would result in better photosynthetic activity in inoculated plants (Adolfsson *et al*., 2015). The improvement of vegetative growth (Osorio *et al.*, 2008) observed in mycorrhizal plants would be the combined result of better nutrient uptake on the one hand and good photosynthetic activity on the other (Valentine *et al.*, 2001). The improvement of vegetative growth and biomass by mycorrhizal fungi was also reported on micropropagated plantain seedlings by Tsané *et al.* (2005) in Cameroon and by Kavoo-mwangi *et al.* (2014) in Kenya.

The local cultivar Orishele (*Musa* AAB) had a higher relative mycorrhizal dependency. The cultivar FHIA 21 (*Musa* AAAB), established a symbiosis with the mycorrhizal fungi, but showed less dependency to mycorrhiza. This could be caused by the difference between the two genotypes, the local strain showing more sensibility to inoculation with native strains. *Acaulospora colombiana* and *Rhizophagus proliferum* appeared to be the best strains for plantain seedlings production in Côte d'Ivoire. They were more effective in terms of root colonization, physiology and growth parameters of the Orishele and FHIA 21 cultivars seedlings. The efficiency of *Acaulospora colombiana* was also reported on cassava (Sery *et al.*, 2016).

Conclusion

Our results showed that native AMF strains isolated from Côte d'Ivoire plantain fields have good mycorrhizal ability as well as significant effect on the growth, physiological and biomass parameters on macropropagated plantain seedlings. The *Rhizophagus proliferum* and *Acaulospora colombiana* strains induced a better photosynthetic activity and a better vegetative growth of macropropagated plantain seedlings.

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