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Arbuscular mycorrhizal fungi in the rhizosphere of *Musa* spp. in western Cuba

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Abstract

Diversity of arbuscular mycorrhizal fungi (AMF) in banana and plantain fields in western Cuba is here reported. Thirty rhizosphere soil samples were collected and used for direct evaluation of the AMF community and establishment of trap cultures. AMF spores were extracted from the soil samples by wet sieving and decanting, and species were identified based on the morphology of the spores. Overall, 56 AMF morphospecies were differentiated within at least 10 genera. From the total number of morphospecies, 25 were identified up to the species level, and 31 were morphologically different from described species. From field samples, 42 morphospecies were verified, with predominance of the genera *Acaulospora* and *Glomus*. However, the most frequent species recovered directly from the field samples were *Claroideoglomus etunicatum* and *Funneliformis geosporum*. Additionally, 14 morphospecies were only obtained in the trap cultures, from which five could be identified up to the species level while the other nine corresponded to apparently undescribed species. It was concluded that banana and plantain fields in western Cuba have highly diverse communities of arbuscular mycorrhizal fungi.

Key words – Agroecosystems – Banana plants – Diversity – Glomeromycota

Introduction

Inorganic fertilizers promote high crop productivity. However, they negatively affect water and soil quality (Morakinyo et al. 2013, Qin et al. 2015). As a result, low–chemical input and organic agriculture have recently received more attention worldwide, aiming preservation of functionality in agroecosystems (Clark & Tilman 2017). Cuban agriculture entered a renovation stage in the middle 1990s, transitioning from a system highly dependent on agrochemicals to a model with lower external inputs, adopting some agroecological principles (Palma et al. 2015).

Several beneficial plant–microorganism interactions with biotechnological potential for agriculture have been described and studied worldwide (Finkel et al. 2017). Among these, the arbuscular mycorrhizal symbiosis is one of the most relevant, with a great diversity of studies at different crops and regions (Baum et al. 2015). Arbuscular mycorrhizal fungi (AMF, phylum Glomeromycota) are probably the most abundant fungal group in agricultural soils, accounting for

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up to 50% of the biomass of soil microbes (Cheng & Baumgartner 2006). These fungi play a key interface between plant hosts and soil mineral nutrients and have gained growing interest, as ecosystem “engineers” and “biofertilizers” (Fitter et al. 2011). On the other hand, most Glomeromycotan fungi have also shown to increase plant tolerance to pathogens (Dar & Reshi 2017, Sennoi et al. 2013).

The arbuscular mycorrhizal symbiosis occurs naturally in banana (*Musa* spp.) (Jefwa et al. 2012, Mahecha–Vásquez et al. 2017, Melo et al. 1997). Previous research has studied, with promising results, the effect of AMF inoculation on the acclimation of banana plantlets and tolerance to pests and diseases that commonly affect this crop (Sampaio et al. 2012, Koffi & Declerck 2015, Ortas et al. 2017). Sampaio et al. (2012), evaluated the response of banana plants inoculated with AMF and fertilized with different concentrations of the Hoagland nutrient solution, or a biofertilizer to *Fusarium oxysporum* f. sp. *cubense* (causing agent of the Panama disease), and found a better response and tolerance of the inoculated plants to the pathogen. In another study, Koffi & Declerck (2015) inoculated micro propagated banana plantlets with *Rizhophagus irregularis* MUCL 41833 and detected increases in their growth and biomass production. More recently, Ortas et al. (2017) inoculated micro propagated banana plantlets with *Glomus caledonium* and *G. macrocarpum,* which resulted in increased growth, root colonization and P uptake by the plants.

Studies on AMF diversity in *Musa* spp. plantations in Cuba are scarce. A better understanding of the occurrence of these fungi and subsequent isolation of naturally occurring AMF species from banana and plantain fields represent the initial steps for future inoculant production and implementation. Considering this, the present study aimed to evaluate the AMF diversity associated with different *Musa* spp. plantations in western Cuba.

Materials & Methods

The present study comprised a total of 30 banana and plantain fields distributed within the Artemisia, Havana, Mayabeque and Pinar del Río provinces, in western Cuba. Soils within the sampled fields were classified as Alluvial, Greyish Brown, Greyish Brown without carbonates, Red Ferralitic, Sialithic Brown with carbonates, Sialithic Dark Brown with carbonates and Yellowish Gray. Soil pH values ranged from 5.4 to 8.0, and the percentage of organic matter between 1.5 and 4.1%. The management of these plantations is based on the guidelines established by the Cuban urban agriculture, which includes 28 subprograms. It is characterized by an integral approach and a strong interrelation “crops–animals–environment–man” and is based on the use of organic matter (compost) and/or earthworm humus (Rodríguez Nodals 2000). Pest and disease control are almost entirely based on biological products, such as strains of *Bacillus thuringiensis*, *Beauveria bassiana*, *Metharrhizus anisopliae*, *Trichoderma harcianum* and *Trichoderma virides,* among others.

Rhizospheric soil samples were composed of five subsamples taken at randomly chosen points. Once in the laboratory, each sample was divided into two parts. The first part was used for direct evaluation of the AMF communities present in the field samples. The second part was used to establish trap cultures with *Sorghum bicolor* L. Moench as the host plant. The intention was to promote the sporulation of AMF species that might have not been sporulating when the soil samples were collected. Trap cultures were grown under greenhouse conditions for five months, after which soil was processed to evaluate the presence of AMF spores and species occurrence.

Spores were extracted by wet sieving and decanting of chemically (0.4 N sodium pyrophosphate) and physically (domestic blender) disaggregated soil samples (Herrera et al. 2004). The content form the 140 and 40 µm sieves was deposited on filter papers (over a Buchner funnel, using a vacuum). Posteriorly subsamples were centrifuged at 2500 revolutions per minute for five minutes, using a gradient of water and 2M sucrose (Sieverding 1991).

Species were identified based on the morphology of the spores (morphospecies). Damaged, decomposed, or old spores were discarded from the analysis. The morphological properties of the spores were observed after mounting them on microscopy slides, using polyvinyl alcohol/lactic acid/glycerol (PVLG), and a mixture of PVLG and reagent of Melzer (1:1, v/v) as mounting solutions. The original descriptions of the species and other specialized bibliography were consulted (Błaszkowski 2012, Oehl et al. 2011, Schenck & Pérez 1990, INVAM 2020). Vouchers from species deposited at the Cuban Collection of Arbuscular Mycorrhizal Fungi (http://www.ecosis.cu/hongos-micorrizogenos-arbusculares/) were also examined. Species with morphological characteristics different from those of already described taxa were considered as undescribed species and given a number after the term "morphotype".

The frequency of occurrence (F_i) was calculated according to Zhang et al. (2004), using the equation $F_i = J_i/k$, where J_i is the number of samples at which the morphospecies i occurred and k the total number of samples. Morphospecies were then classified as dominant ($F_i > 50\%$), very common ($30 < F_i \leq 50\%$), common ($10 < F_i \leq 30\%$), and rare ($F_i \leq 10\%$).

Results

A total of 42 morphospecies, distributed in six families and at least ten genera, were obtained directly from field samples (Table 1). From these, 20 were identified up to the species level; while 19 were considered as undescribed species (Morphotypes 1–19); and other three, could not be associated morphologically to any described genera within the Glomeromycota (Morphotypes 20–22). The number of morphospecies per plantation varied between 2 and 16, showing no relationship with province, soil attributes nor type of plantation (banana versus plantain) or cultivar. The best represented families were Glomeraceae and Acaulosporaceae with 19 and 11 morphospecies, respectively. At the genera level, the highest number of identified morphospecies was verified within *Acaulospora* (11) and *Glomus* (9), accounting for 26.19% and 21.43% of the total. Around 52% of the AMF morphospecies were classified as rare (22). Dominant morphospecies, present in more than 16 of the plantations, were represented by *Claroideoglomus etunicatum*, *Funneliformis geosporum*, and Morphotypes 1 and 22. The "common" dominance level was represented by 13 morphospecies. Five morphospecies were represented in at least 50% of the plantations, showing a generalist status. Another 19 morphospecies were present in 2 to 14 plantations (intermediate), and 18 were represented only in one plantation (exclusive).

Table 1 Morphospecies of arbuscular mycorrhizal fungi identified in 30 banana and plantain fields in western Cuba.

AMF Family/Species and Morphotypes	F_i (%)	Class
Acaulosporaceae		
Acaulospora elegans Trappe & Gerd.	20.00	C
Acaulospora mellea Spain & N.C. Schenck	3.33	R
Acaulospora rehmii Sieverd. & S. Toro	3.33	R
Acaulospora scrobiculata Trappe	36,67	VC
Acaulospora sp. 1 "yellow thick component U" (Morphotype 9)	13.33	C
Acaulospora sp. 2 "yellow Guatao" (Morphotype 10)	16.67	C
Acaulospora sp. 3 "sL4 San Antonio" (Morphotype 11)	13.33	C
Acaulospora sp. 4 "coraliform" (Morphotype 12)	3.33	R
Acaulospora sp. 5 "dwarf orange" (Morphotype 13)	3.33	R
Acaulospora sp. 6 "greenish yellow small" (Morphotype 14)	3.33	R
Acaulospora sp. 7 "vermiform warts" (Morphotype 15)	3.33	R
Archaeosporaceae		
Archaeospora trappei (R.N. Ames & Linderman) J.B. Morton & D. Redecker	16.67	C
Entrophosporaceae		
Claroideoglomus claroideum (N.C. Schenck & G.S. Sm.) C. Walker & A. Schüssler	3.33	R
Claroideoglomus etunicatum (W.N. Becker & Gerd.) C. Walker & A. Schüssler.	86.67	D
Entrophospora infrequens (I.R. Hall) R.N. Ames & R.W. Schneid.	3.33	R
Gigasporaceae		
Gigaspora margarita W.N. Becker & I.R. Hall	3.33	R
Gigaspora sp.1 "orange" (Morphotype 16)	3.33	R

Table 1 Continued.

AMF Family/Species and Morphotypes	F_i (%)	Class
Glomeraceae		
Funneliformis geosporum (T.H. Nicolson & Gerd.) C. Walker & A. Schüssler	73.33	D
Funneliformis mosseae (T.H. Nicolson & Gerd.) C. Walker & A. Schüssler	26.67	C
Glomus brohultii Sieverd. & R.A. Herrera	6.67	R
Glomus sp. 1 "brown dichotomic" (Morphotype 1)	60.00	D
Glomus sp. 2 "white biconcave septum" (Morphotype 2)	13.33	C
Glomus sp. 3 "flexible tan" (Morphotype 3)	13.33	C
Glomus sp. 4 "peridium brown coupled" (Morphotype 4)	13.33	C
Glomus sp. 5 "brown very thick wall" (Morphotype 5)	6.67	R
Glomus sp. 6 "dwarf white" (Morphotype 6)	6.67	R
Glomus sp. 7 "brown cucumber" (Morphotype 7)	3.33	R
Glomus sp. 8 "layer 2nd very thick" (Morphotype 8)	3.33	R
Rhizoglomus aggregatum (N.C. Schenck & G.S. Sm.) Sieverd., G.A. Silva & Oehl	16.67	C
Rhizoglomus clarum (T.H. Nicolson & N.C. Schenck) Sieverd., G.A. Silva & Oehl	3.33	R
Rhizoglomus fasciculatum (Thaxt.) Sieverd., G.A. Silva & Oehl	33.33	VC
Rhizoglomus intraradices (N.C. Schenck & G.S. Sm.) Sieverd., G.A. Silva & Oehl	3.33	R
Rhizoglomus microaggregatum (Koske, Gemma & P.D. Olexia) Sieverd., G.A. Silva & Oehl	13.33	C
Sclerocystis clavispora (Trappe) R.T. Almeida & N.C. Schenck	3.33	R
Sclerocystis coremioides (Berk. & Broome)	6.67	R
Sclerocystis sinuosa (Gerd. & B.K. Bakshi)	50.00	VC
Scutellosporaceae		
Scutellospora sp. 1 "greenish yellow" (Morphotype 17)	13.33	C
Scutellospora sp. 2 "brown gemmated" (Morphotype 18)	3.33	R
Scutellospora sp. 3 "Caimito" (Morphotype 19)	3.33	R
Other morphotypes		
"Barina–spore banana" (Morphotype 20)	3.33	R
"Pseudo–*Acaulospora* xerophytic" (Morphotype 21)	16.67	C
"Vesicle–spore hyaline Guatao" (Morphotype 22)	56.67	D

Frequency of occurrence (Fi %) categories: D = Dominant, VC = very common, C = common, R = rare

In addition to the morphospecies found through direct analysis of the soil samples, other 14 morphospecies were recovered from trap cultures and grouped within three families and three genera (Table 2). Five of these species were identified up the species level, eight could only be identified up to the genus level (Morphotypes 23–30), and one did not associate with any described genera within the Glomeromycota (Morphotype 31). The genus *Diversispora*, which was not represented in the field samples, appeared to have four morphospecies within the studied areas.

Considering samples analyzed directly from the field and those from trap cultures, the total AMF species richness obtained associated with banana and plantain fields in western Cuba, reached a value of 56 morphospecies. From these, 25 were identified up to the species level and 27 were identified up to the genus level and were referred to as undescribed species or morphotypes. Other four morphotypes had morphological characters that did not correspond with those of any described AMF genera (Morphotypes 20–22, 31).

Table 2 Additional morphospecies of arbuscular mycorrhizal fungi verified in the trap cultures. MA refers to the maximal abundance (categories 1 to 6) at which species were able to reproduce from minimal to maximal spore populations.

AMF Family/Species and Morphotypes	MA
Glomeraceae	
Glomus sp. 9 brown cork–covered (Morphotype 23)	4
Glomus sp. 10 brown geosporum–like (Morphotype 24)	4

Table 2 Continued.

AMF Family/Species and Morphotypes	MA
Glomus sp. 11 brown narrow sporophore (Morphotype 25)	4
Glomus sp. 12 brown thick walled sporophore (Morphotype 26)	4
Glomus sp. 13 brown white sporophore (Morphotype 27)	4
Glomus sp. 14 yellow right septum (Morphotype 28)	2
Acaulosporaceae	
Acaulospora kentinensis (C.G. WU & Y.S. Liu) Kaonongbua, J.B. Morton & Bever	4
Acaulospora sp. 7 brown–sandwich (Morphotype 29)	6
Acaulospora sp. 8 light yellow (Morphotype 30)	4
Diversisporaceae	
Diversispora eburnea (L.J. Kenn., J.C. Stutz & J.B. Morton) C. Walker & A. Schüssler	3
Diversispora spurca (C.M. Pfeiff., C. Walker & Bloss) C. Walker & A. Schüssler	3
Diversispora trimurales (Koske & Halvorson) C. Walker & A. Schüssler	3
Diversispora versiformis (P. Karst.) Oehl, G.A. Silva & Sieverd	3
Other morphotypes	
Dornamented (Morphotype 31)	2

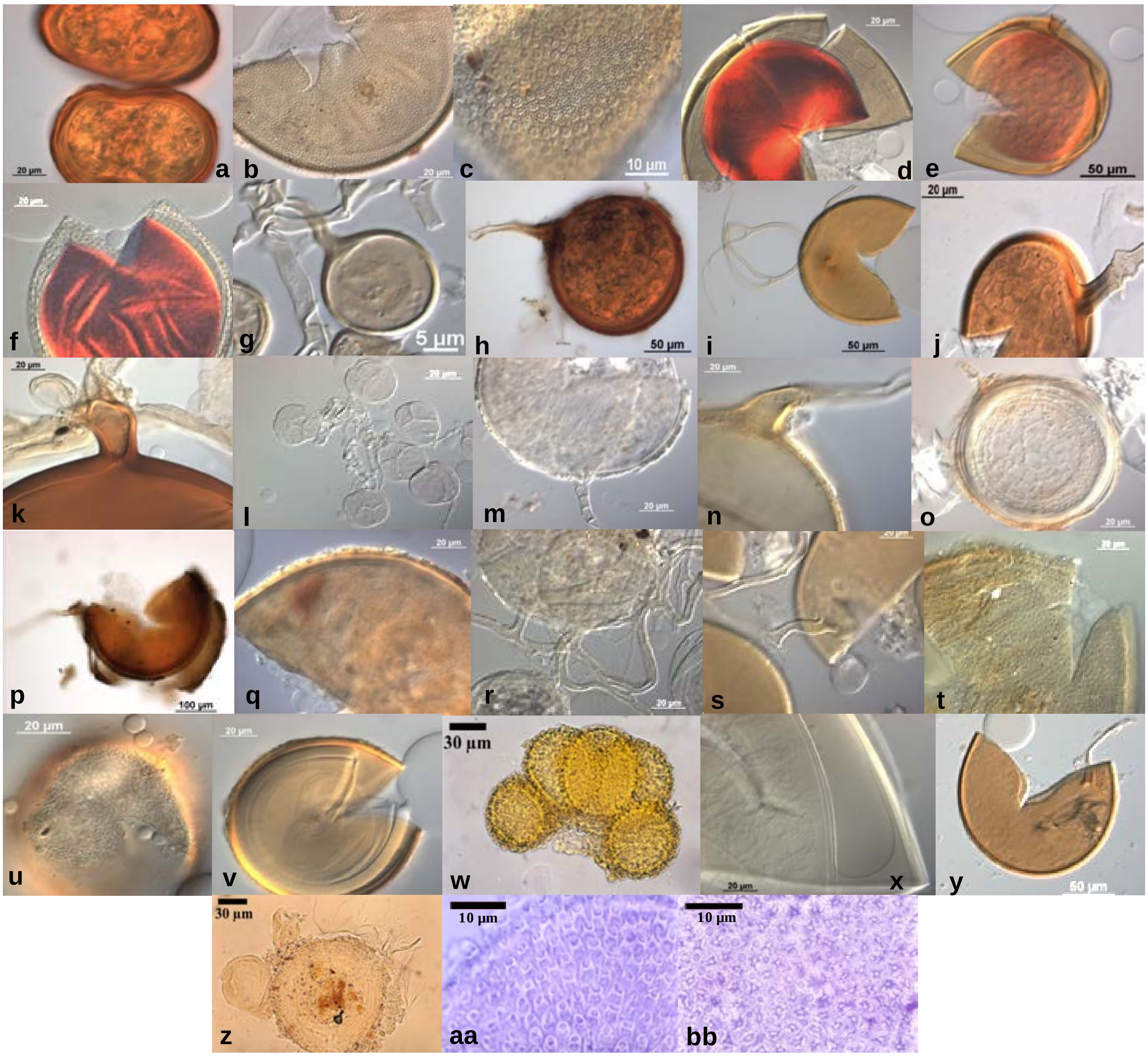

Fig. 1 – Spores of some morphospecies of arbuscular mycorrhizal fungi verified at banana and plantain fields in western Cuba. a *Acaulospora* sp. 7. b *Acaulospora* sp. 3. c *Acaulospora elegans*. d *Acaulospora* sp. 8. e *Acaulospora* sp. 2. f *Acaulospora scrobiculata*. g *Glomus* sp. 1.

h *Funneliformes geosporum*. i *Glomus* sp. 11. j *Glomus* sp. 12. k *Glomus* sp. 13. l *Glomus* sp. 6. m *Diversispora eburnea*. n *Funneliformes mosseae*. o *Diversispora spurca*. p *Glomus* sp. 9. q *Diversispora versiformis*. r *Glomus* sp. 2. s *Glomus* sp. 14. t *Acaulospora kentinensis*. u Morphotype 31. v Morphotype 21. w Morphotype 20. x *Scutellospora* sp. 1. y *Claroideoglomus etunicatum*. z Morphotype 22. aa, bb Morphotype 31.

Within the undescribed species, Morphotype 20 "Barina–spore banana" formed glomoid spores grouped in dense sporocarps, covered by a mantle of sinuous hyphae (Fig. 1w). Although the mantle of this morphotype resembles that of *Glomus tortuosum* and *Glomus sinuosum*, it differs morphologically from these two. Morphological differences between Morphotype 20 and *G. tortuosum* include spore size (60–80 µm vs. 120–220 µm), length of the subtending hypha (≤6 µm vs. ≤ 15 µm) and a less sinuous hyphal mantle in Morphotype 20. Also, the peridium in Morphotype 20 is formed by thinner hyphae (6–7 µm) compared to *G. sinuosum* (≤19 µm). Finally, spores of Morphotype 20 are lighter in color than those of *G. tortuosum* and *G. siniuosum*.

The second unidentified morphospecies, Morphotype 21 "Pseudo-*Acaulospora* xerophytic", is morphologically similar to unidentified species already reported in dry ecosystems within San Juan de Lagunillas, Venezuela and California, USA. The spores of this morphotype are characterized by forming a thick internal wall (Fig. 1v), which might represent a functional adaptation to dry environments. However, thicken walls are an uncommon characteristic within the genus *Acaulospora*. Morphotype 22 "Vesicle-spore hyaline-Guatao", forms hyaline spores with an external mucilaginous layer which retains organic matter and soil particles (Fig. 1z). The formation of vesicle-like structures within this layer was also observed. Finally, Morphotype 31 "D ornamented", forms gigasporoid spores with ornamentations (Fig. 1aa, 1bb).

Discussion

The occurrence of AMF species associated with several banana and plantain fields in Cuba is here reported for the first time. The diversity of AMF species in banana and plantain fields has been poorly studied worldwide (Gaidashova et al. 2010, Nidheesh et al. 2017). Taking previous studies into account, the species richness here presented can be considered high when compared to other *Musa* spp. fields. Melo et al. (1997) studied the AMF community associated with banana cropping systems in four plantations in the Pernambuco and Bahia States in Northeast Brazil. The authors collected a total of 80 composed soil samples and identified 15 AMF species. Jefwa et al. (2010) summarized results obtained from previous research comprising the AMF symbiosis in banana and plantain fields in Africa, concluding that in Rwanda, Uganda and Kenya up to 20 AMF species have been identified associated to *Musa* spp. In subsequent research, conducted in 50 banana and plantain farms and a total of 350 plants in central Kenya, 22 AMF species were detected, from which only 27% of the fungi were classified up to the species level (Jefwa et al. 2012).

The species richness obtained in the present study resembles that verified by Mahecha–Vásquez et al. (2017), who evaluated the AMF diversity in three banana production states in Colombia under monoculture and polyculture systems. The latter study comprehended four plantations per state (12 plantations) and six plants per plantation (72 samples), reporting minimum and maximum values of 11 and 18 AMF species per plantation, and a total richness of 58 AMF species. However, only 21 species were represented by more than five individuals (according to their abundance), and from these 14 were classified up to the genus level.

The extensive use of agrochemicals in conventional agriculture systems must also be considered when evaluating AMF diversity in banana and plantain fields (Schreiner & Bethlenfalvay 1997, Usuga & Franco 2002, Nicholls & Altieri 2008). Plantations of *Musa* spp. tend to be highly dependent on pesticides to control the several pests and diseases that affect this crop, causing significant yield decreases (Adriano-Anaya et al. 2006, Gowen et al. 2005). Furthermore, cultivation generally requires plenty of fertilization, partly due to the high nutritional requirements of the plants, mainly of potassium (K) and nitrogen (N) (Borges & Oliveira 2000). In high phosphorus level soils planted with banana, pH is the main factor affecting the richness of AMF species (Mahecha–Vásquez et al. 2017). The negative effect of agrochemicals on AMF diversity and levels of root colonization has already been reported (Karpouzas et al. 2014, Rivera–Becerril et al. 2017). High nutrient levels in the soil due to mineral fertilization might also cause a decrease in the AMF dependency of the plants (Nouri et al. 2014, Williams et al. 2017). The management system adopted in the plantations of *Musa* spp. sampled in the present study, based on the use of organic matter application and biological control of pests and diseases, might explain the higher AMF species richness here reported when compared to previous studies conducted in other regions of the world (Usuga et al. 2008, Cabrales Herrera et al. 2018).

The high mycorrhizal dependency of *Musa* spp. might also help explain the high number of AMF species associated with this crop. Although this parameter was not evaluated in the present study, previous research has indicated high levels of mycorrhizal dependency and colonization for *Musa* spp., as well as positive effects of inoculation with AMF species (Srivastava et al. 2014). Anaya et al. (2010) studied the dynamics of the AMF colonization in roots of banana plants (Clonal cultivar *Gran Enano*) in three different phenological stages of the crop in Chiapas, Mexico, verifying colonization at all the evaluated stages, with the highest mean values (55%) detected during the flowering season. In another research, Jefwa et al. (2010) reported colonization levels of 58–66% for dessert, cooking and beer banana in Kenya, and of 63–90% in Uganda. Another field study conducted within banana and plantain cultivations systems in Kenya reported AMF colonization between 26 and 52.6% (Jefwa et al. 2012). Ortas et al. (2017) evaluated the effects of inoculation with *Glomus caledonium* and *G. macrocarpum* on micro propagated banana plantlets, detecting better acclimatization and survival of the inoculated plantlets, with colonization by *G. macrocarpum* being 68.8–71.2% and by *G. caledonium* 50.8–55.3%.

The predominance of the *Acaulospora* and *Glomus* genera within AMF communities has been widely documented for natural and agricultural ecosystems worldwide (Pereira et al. 2014, Trejo et al. 2016, Xiang et al. 2014). This pattern, which was also detected in the present study, has been reported for other banana and plantain fields (Jefwa et al. 2012, Melo et al. 1997). In our geographic region, Cabrales Herrera et al. (2018) isolated and identified fungal morphospecies associated with plantain (*Musa* AAB Simmonds cv. Hartón) in farms, from seven municipalities of the department of Córdoba, Colombia. In seven of the sampled localities, the genera *Glomus*, *Acaulospora* and *Scutellospora* were found. The most abundant genus was *Glomus*, with *G.* morphospecies 02 accounting for the highest number of spores, followed by *G. deserticola*.

Sumathi & Thangavelu (2016) explored AMF communities associated with different banana cultivars in India. Soil samples belonging to approximately eight soil types were collected from different banana growing regions of India. The results revealed that a maximum of 50.8% of AMF spores was present in the high clay level soil and a minimum of 0.2% in the silty clay and loamy soils. AMF spores belonged to three different genera *Glomus* (seven species), *Acaulospora* (two species) and *Scutellospora* (two species). The authors also noted that 91.1% of the AMF spores isolated belonged to *Glomus* sp., being the dominating AMF, in the studied banana germplasm accessions.

In this study, *Claroideoglomus etunicatum* was present in 26 of the 30 sampled fields. This is one of the most frequently recorded species from nature and is often used in laboratory studies (Pawlowska et al. 1999). *C. etunicatum* has also been reported in other *Musa* spp. plantations (Mahecha–Vásquez et al. 2017, Melo et al. 1997). However, previous research did not indicate this species as being dominant in banana or plantain fields when spore abundance is considered. Melo et al. (1997) reported *Acaulospora scrobiculata* and *Glomus mosseae* as the most abundant species for the rhizosphere of banana in some plantations in Northeastern Brazil. In the extensive study of AMF diversity within banana crops in Colombia, Mahecha–Vásquez et al. (2017) reported *A. scrobiculata*, *A. excavata* and *Glomus brohultii* as the most abundant species. Jefwa et al. (2012) also documented *A. scrobiculata* as the most abundant AMF species in the rhizosphere of several banana and plantain cropping systems in Kenya.

A. scrobiculata, which was also identified in the present study on banana and plantain fields in western Cuba, represents a common species in *Musa* spp. plantations in several places of the world. This species should have further studies to evaluate the effect of its inoculation on banana and plantain plantlets. Native AMF strains isolated from diverse plantain fields over the world have shown high mycorrhization capacity, as well as a significant effect on the growth and physiological parameters of banana and plantain seedlings (Amoa et al. 2017, Kavoo–Mwangi et al. 2014).

The present work is one of the first studies in the diversity of AMF associated with *Musa* spp. in Cuba. Banana and plantain fields in the country present high AMF diversity, which could represent an indicator of possible strong mycorrhizal dependency of this crop. Future studies should consider the incorporation of molecular approaches to identify the high number of undescribed AMF morphospecies occurring in these agroecosystems.

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