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Identification of arbuscular mycorrhizal fungi in plantain producing municipalities in department of Cordoba, Colombia

Identificación de hongos micorrízicos arbusculares en municipios plataneros del departamento de Córdoba, Colombia

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Abstract

Currently plantain cultivation makes excessive use of agrochemicals, condition that threatens a sustainable production system and leads to the degradation of the environment, reducing the quality of the products. Based on the foregoing, and in order to expand knowledge of the arbuscular mycorrhizal fungi associated with plantain cultivation, the aim of this study was to isolate and identify fungal morphotypes that form symbiosis with plantain (*Musa* AAB Simmonds cv. Hartón), in farms from seven municipalities of the department of Córdoba, Colombia. In each farm, soil samples from the first 20 cm of soil depth in the rhizosphere area were taken to the soil laboratory at Universidad de Córdoba, Colombia, where spores were extracted using the OHM and the Jemkins modified methods, grounded on wet sieving in sucrose gradient. Morphological identification of the species was carried out taking into account spores characteristics, and evaluating the following parameters: number of spores of arbuscular mycorrhizal fungi (AMF), relative abundance, and diversity and dominance indexes. In seven localities, the genera *Glomus*, *Acaulospora* and *Scutellospora* were found. The most abundant genus was *Glomus*, recording for *G.* morphospecies 02 the highest number of spores, followed by *G. deserticola*. The municipalities with highest number of spores were Lorica and Moñitos, while the ones with the lowest amount were Tierralta and Valencia.

Key words: Endomycorrhizae, *Musa* AAB, diversity, dominance, *Glomus,* morphotype.

Resumen

Actualmente, el cultivo de plátano hace un uso excesivo de agroquímicos, condición que amenaza sistemas de producción sostenibles y conduce a la degradación del medio ambiente, reduciendo la calidad de los productos. Con base en lo anterior y para ampliar el conocimiento de los hongos micorrízicos arbusculares asociados con el cultivo de plátano, el objetivo de este estudio fue aislar e identificar los morfotipos fúngicos que forman esta simbiosis en el plátano (*Musa* AAB Simmonds) variedad Hartón en fincas de siete municipios del departamento de Córdoba, Colombia. En cada finca se tomaron muestras de suelo en los primeros 20 cm de profundidad en la zona rizosférica llevándose al laboratorio de suelos de la Universidad de Córdoba donde se extrajeron las esporas mediante los métodos OHM y Jemkins modificado, fundamentadas en tamizado en húmedo en gradiente de sacarosa. La identificación morfológica de las especies, se realizó teniendo en cuenta los caracteres de las esporas, evaluando los siguientes parámetros: número de esporas de hongos formadores de micorrizas arbusculares, abundancia relativa e índices de diversidad y dominancia. En las siete localidades se encontraron los géneros *Glomus, Acaulospora* y *Scutellospora*, siendo el más abundante el primero, registrando *G.* morfoespecie 02 el mayor número de esporas, seguida de *G. deserticola.* Los municipios con mayor número de esporas fue Lorica y Moñitos, y los de menor cantidad fueron Tierralta y Valencia.

Palabras clave: endomicorriza, *Musa* AAB, diversidad, dominancia, *Glomus,* morfotipo.

Introduction

Banana and plantain or dessert and cooking bananas are basic family food basket products for most of the Colombian population, with a wide geographical distribution, i.e. Orinoquia, Amazonia, Andean, and the Caribbean regions, as well as the Pacific coast. It generates 0.51 jobs.ha^{-1} annually, which is equivalent to 177853 permanent jobs. This crop has been used in Colombia, not only as monoculture, but as shade for coffee plantations in (Hernández, Riaño & Aristizábal, 2014; Martínez, Peña & Espinal, 2006).

Plantain alone is a socio-economically important crop in the tropical region, with an estimated planted area in 2015 of 600000 ha, with a production of 6 million tons per year. Moreover, its cultivation is considered within the traditional sector of the farmer economy, as a subsistence crop for small-scale producers, of high geographical dispersion and for food security; therefore, plantain production is aimed at meeting mostly domestic demand and only 1% is left for international commercialization, mainly for the United States and European markets.

Specifically in the department of Córdoba, Colombia there are an estimated 23000 hectares of land dedicated to plantain cultivation, located in the municipalities of Moñitos, Los Córdobas, Puerto Escondido, San Bernardo del Viento, Lorica, Tierralta and Valencia that generates ca. 10000 direct jobs.

However, currently in agricultural banana and plantain exploitation systems there are in some cases, an excessive use of agrochemicals (fertilizers, fungicides and herbicides), a condition that threatens the sustainability of the production system, since it leads to the degradation of the environment; this causes moreover, reduction in product quality due loss in soil fertility and an increase in phytosanitary problems. This has been studied by Nicholls & Altieri (2008), who stated that when there is high use of chemicals or synthetic fertilizers such as urea, and especially when applications are excessive, this increases nutritional imbalances in the plants, which in turn makes them more vulnerable to pests and diseases.

Under this perspective, soil has increasingly been given more importance as a living element of the system, and is now considered an active component, *i.e.* it is composed by physical, chemical and biological factors. The latter has usually been of little relevance, which has led to soil deterioration as a living entity in agroecosystems; this concept is however, currently becoming increasingly important as it contributes to the sustainability of ecosystems and production systems (Van, Moraetis, Lair, Bloem, Nikolaidis, Hemerik & Ruiter, 2015).

Given the increasing environmental contamination caused by indiscriminate use of fertilizers and harmful substances, with the further production of contaminated fruits, agricultural land in these regions has a low biological population; this in turn implies, for farmers, an increase in the use of synthetic substances to maintain the sustainability of the productive systems; moreover, an increase in fertilizer costs and the difficulty in obtaining these, has led instead to enrich the soils biologically, in order to make the production system sustainable again with low investments.

In this sense, Fernández, Castellanos & Fuente (2015), carried out studies on soil biodiversity to evaluate soil quality from an agricultural point of view; furthermore, Bautista, Bolaños, Massae & Villegas (2015), worked with mycorrhizas in plantain fields and obtained excellent results against nematodes.

As for mycorrhization, there are many vascular plant species that can live in symbiosis with fungi belonging to *Phylum Glomeromycota*, which are commonly called mycorrhiza (Shüssler, Schwarzott & Walker, 2001). Yet, the arbuscular ones are the most interesting as they can be used for short-cycle crops and are moreover, dominant in natural conditions. It has been estimated that they are dominant in a 95 % of the cases without specificity, but the responses obtained in each species of mycorrhized plants are different. Therefore, it is of utmost convenience and importance to specify that fungi that forms arbuscular mycorrhizae (AMF) are natural soil inhabitants with a particular characteristic. These are biotrophs that depend on the presence of hosts to supply their carbon needs (Allen, Swenson, Querejeta, Egerton-Warburton & Treseder, 2003; Mukhongo, Kavoo-Mwangi, Kahangi, Ateka, Were, Okalebo, ... & Jefwa, 2015).

According to review works carried out by Srivastava, Singh & Singh (2014), plantain is a mycotrophic species capable of benefiting from the presence of AMF, and where the beneficial effects of symbiosis are widely known. AMF are important in tropical soils where plantain grows, mostly when soils have low fertility, and especially when they are poor in phosphorus, with salinity and erosion problems, among others. In this sense, the success of the use or inclusion of soil biota in any crop management system lies in its diversity, as suggested by Zheng, Guo, Zhang, Song, Fang, Zhang & Sun (2014), which can be measured, among others, by enzymatic activity found in soil.

Based on the above mentioned and in order to expand the knowledge on arbuscular mycorrhizal fungi associated with plantain (*Musa* AAB Simmonds cv. Hartón), the aim of this study was to isolate and identify fungal morphotypes that form symbiosis with this cultivar, carried out in seven municipalities of the department of Córdoba, Colombia.

Materials and methods

Study area and sampling sites

The study was carried out in seven municipalities of the department of Córdoba where plantain production is one of the main agricultural activities of economic importance: Moñitos, San Bernardo del Viento, Puerto Escondido, Lórica, Los Córdoba, Tierralta and Valencia. The first five are located in the Caribbean coastal region and the last two in the upper basin of the Sinú River. These municipalities are located to the south and northwestern region of the department of Córdoba, Colombia with variability in agroecological conditions (Table 1); in each municipality, 10 sampling points were chosen in plots with an average crop age between two and three years, all with conventional management.

Table 1. Characteristics of the municipalities in the department of Córdoba where samplings were carried out.

Municipality	Coordinates	Climatic conditions
Upper basin of the Sinú river		
Tierralta	08° 10' 34" latitude N 76° 03' 46" longitude W	Mean temperature: 27°C; Mean annual rainfall: 2200–2300 mm; RH of 85%
Valencia	08° 16' latitude N 76° 09' longitude W	Mean temperature: 28°C; Mean annual rainfall: 1800-2000 mm; RH of 85%
Caribbean coastal region		
San Bernardo del Viento	09° 21' 22" latitude N 75° 57' 21" longitude W	Mean temperature: 28°C; Mean annual rainfall: 1300–1500 mm; RH of 80–85%
Lorica	09° 13' 54" latitude N 75° 49' 11" longitude W	Mean temperature: 28°C; Mean annual rainfall: 1300–1500 mm; RH of 80–85%
Puerto Escondido	09° 01' 19" latitude N 76° 15' 54" longitude W	Mean temperature: 28°C; Mean annual rainfall: 1200–1300 mm; RH of 80–85%
Moñitos	09° 15' 00" latitude N 76° 07' 50" longitude W	Mean temperature: 28°C; Mean annual rainfall: 1200–1300 mm; RH of 80–85%
Las Córdobas	08° 54' 00" latitude N 76° 21' 35" longitude W	Mean temperature: 28°C; Mean annual rainfall: 1200–1300 mm; RH of 80–85%

Variables measured

In each of sampling points, composite samples of rhizospheric soil were taken, at soil depth ranged from 0-20 cm; these were labeled and taken to the Soil Laboratory of Universidad de Córdoba, Colombia where population analysis and identification of AMF was carried out. AMF populations were established through spore counts in three rhizospheric soil subsamples of 100 g through the modified OHM & Jemkins method, based on wet sieving in sucrose gradient. Moreover, the morphological identification of the species was made taking into account the morphological characters of the spore, basal hyphae, number of cell walls and color, among others; micrographs of different spores per species were taken in order to be identified using the monographs published by Redecker, Schüßler, Stockinger, Stürmer, Morton & Walker (2013), and Salmerón-Santiago, Pedraza-Santos, Mendoza-Oviedo and Chávez-Bárcenas (2015). Evaluation of ecological parameters of abundance and frequency, as well as the Shannon-Wiener and the Simpson diversity indexes were quantified using the software BioDiversity Pro®, version 2. The following formula was used for the Shannon-Weiner diversity index (Equation 1) as follows:

$$H = -\sum_{i=1}^{s} pi * \mathrm{Ln}(pi) \qquad pi = \frac{n_i}{N} \quad \text{Equation 1}$$

Where: H is the Shannon-Weiner index, *pi* is relative abundance or number of individuals of species *i*, and *N* is the total number of individuals. In most natural ecosystems, this value ranges between 0.5 and 5, although its normal value ranged from 2 to 3; values lower than 2 are considered low, and higher than 3 are associated with high biodiversity. In this way, this index considers the number of species present in the study area (species richness), and relative number of individuals of each of those species (abundance). To calculate the Simpsons diversity index, the following formula (Equation 2) was used:

$$D = \frac{\sum_{i=1}^{s} ni\,(ni-1)}{N\,(N-1)} \quad \text{Equation 2}$$

Where: *D* is the number of species, *N* is the total number of organisms present (or square units), and *n* is the number of specimens per species; it is considered diverse when *D* is closer to 1.0.

Results

Quantification of AMF species associated with plantain cultivation

The AMF spores found in seven municipalities of the department of Córdoba, Colombia considered in this study and associated with plantain cultivations belong to the genera *Acaulospora, Glomus* and *Scutellospora*, represented in 15 species and eight (8) morphospecies (Table 2).

Table 2. Average number of AMF spores in rhizopheric soil samples from plantain cultivation plots in seven municipalities of the department of Córdoba, Colombia.

Mycorrhizae species	Municipalities							
	A	B	C	D	E	F	G	Total
***Glomus* (number of spores.100 g^{-1} of soil)**								
G. boreale	0.00	0.00	0.00	0.03	0.06	0.00	0.00	0.10
G. deserticola	1.07	1.43	0.34	0.00	0.04	0.00	0.17	**3.04**
G. etunicatum	1.42	0.22	0.02	0.16	0.27	0.02	0.03	2.14
G. fasciculatum	0.33	0.13	0.33	0.44	0.42	0.62	0.02	2.29
G. flavisporum	1.43	0.27	0.00	0.00	0.00	0.00	0.00	1.70
G. clavispora	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01
G. sinuosum	0.00	0.02	0.01	0.00	0.00	0.01	0.00	0.04
G. mosseae	0.36	0.06	0.19	0.01	0.00	0.02	0.00	0.64
G. geosporum	0.13	0.04	0.04	0.22	0.00	0.30	0.00	0.73
G. heterosporum	0.35	0.05	0.04	0.00	0.44	0.24	0.00	1.12
G. intraradices	0.17	0.00	0.00	0.14	0.38	0.01	0.00	0.69
G. taiwanensis	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01
G. morphospecies 1	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.01
***G.* morphospecies 2**	0.24	4.02	0.02	0.14	0.33	0.39	0.02	**5.15**
G. morphospecies 3	0.00	0.16	0.00	0.00	0.00	0.00	0.03	0.18
G. morphospecies 4	0.02	0.00	0.00	0.03	0.00	0.00	0.00	0.05
G. morphospecies 5	0.29	0.08	0.48	0.05	0.03	0.06	0.22	1.19
G. morphospecies 6	0.04	0.00	0.00	0.00	0.00	0.01	0.00	0.05
G. morphospecies 7	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.01
***Acaulospora* (number of spores.100 g^{-1} of soil)**								
A. gdanskensis	0.04	0.06	0.15	0.00	0.00	0.01	0.00	0.26
A. scrobiculata	0.09	0.12	0.34	1.23	0.12	0.05	0.11	2.06
A. mellea	0.08	0.59	0.95	0.21	0.23	0.01	0.03	2.09
***Scutellospora* (number of spores.100 g^{-1} of soil)**								
S. morphospecies 1	1.78	0.00	0.01	0.00	0.03	0.07	0.03	1.92
Total number of spores per municipality.100 g^{-1} of soil	7.84	7.24	2.91	2.67	2.36	1.83	0.64	25.48

A: Lórica, B: Los Córdobas, C: Puerto Escondido, D: San Bernardo del Viento, E: Moñitos, F: Valencia, G: Tierralta, G: Valencia.

Mycorrhizal genera with highest number of species were *Glomus, Acaulospora,* and *Scutellospora* with 19, 3 and 1 species, respectively, being *Glomus* the dominant genus with highest relative abundance showing a value of 19.14 spores.100 g^{-1} of rhizospheric soil, while *Acaulospora* and *Scutellospora* only registered 4.42 and 1.92 spores.100 g^{-1} in rhizospheric soils, respectively (Figure 1).

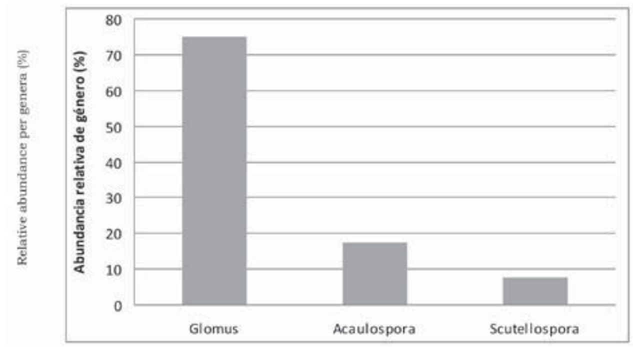

Figure 1. Relative abundance per AMF genus that forms symbiosis with plantain crops in the department of Cordoba, Colombia.

Within the genus *Glomus*, the most abundant species was *Glomus* morphospecies 2 with a relative abundance of 26.9 % within the genus, and a total relative abundance of 20.21 % (Figure 2); owing to this high percentage, it was considered dominant, not only regarding the *Glomus* genus, but also within all the AMF species found in the study. Furthermore, the Shannon index in the study area showed a value of 2.5 which shows a middle range (2-3). Additionally, the most frequent species found in the *Glomus* genus was *Glomus* morphospecie 2, and in a much lower relative abundance, *Glomus deserticola* (Figure 2).

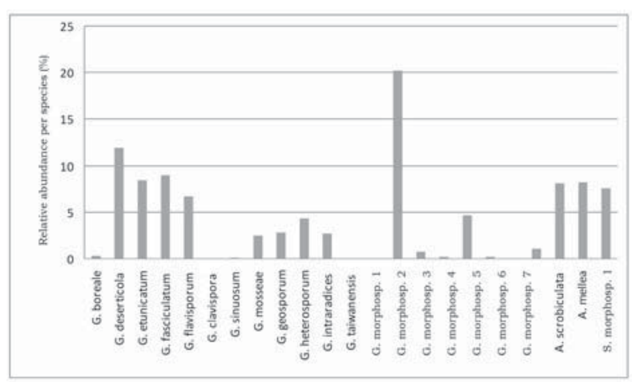

Figure 2. Relative abundance per AMF species in the rhizosphere of plantain cultivation plots in seven municipalities of the department of Córdoba.

G. = Glomus; A. = Acaulospora; S. = Scutellospora.

According to AMF richness by municipality, Lorica and Los Córdobas were the ones that showed the highest mycorrhizal richness, with tenors of 7.84 and 7.24 spores.100 g^{-1} in rhizospheric soil. On the contrary, the municipalities of Puerto Escondido, San Bernardo del Viento and Moñitos showed an average of 2.65 ± 0.27 spores.100 g^{-1} and Tierralta showed the lowest number, *i.e.* 0.64 spores.100 g^{-1} in rhizospheric soil (Table 2).

The genus *Glomus* was dominant in the municipalities of Los Córdobas, Moñitos, Valencia and Tierralta, with its species *G.* morfoespecie 2, *G. heterosporum, G. fasciculatum* and *G.* morfoespecie 5, respectively, according to the results showed by the Shannon-Weiner and Simpson indexes, as shown in Table 3.

Table 3. Diversity and dominance indexes showing the dominant AMF species per municipality

Municipality	Parameter			
	Diversity (Shannon-Weiner)	Diversity (Simpson)	Dominance (Simpson)	Dominant species
Lórica	2.19	0.85	0.15	*S.* morfoespecie 1
Los Córdobas	1.51	0.64	0.36	*G.* morfoespecie 2
Puerto Escondido	1.96	0.82	0.18	A. mellea
San Bernardo del Viento	1.74	0.73	0.27	*A. scrobiculata*
Moñitos	2.1	0.86	0.14	*G. heterosporum*
Valencia	1.87	0.79	0.21	*G. fasciculatum*
Tierra Alta	1.76	0.77	0.23	*G.* morfoespecie 5
Total	2.5	0.9	0.1	***G.* morfoespecie 2**

The genus *Acaulospora* was dominant in the municipalities of Puerto Escondido and San Bernardo, i.e. soils that have expandable characteristics, while *Scutellospora* was dominant in the municipality of Lorica. It is worth noting that in all municipalities there is high AMF diversity, i.e. above 70% (Simspon index). Nevertheless, the highest diversity was found in the municipality of Lorica with a Shannon index of 2.19, followed by Moñitos with 2.10, indicating a moderate diversity; however, as this value is placed in the middle range (2-3) this indicates normality in the diversity found.

Discussion

The mycorrhizal genus with highest number of species found in the study area was *Glomus,* that agrees with several published reports by Gañan, Bolaños and Asakawa (2011), Bolaños, Rivillas and Suárez (2000), Becerra-Encinales, Castaño-Zapata and Villegas-Estrada (2010), and Barrera-Violeth, Oviedo-Zumaque and Barraza-Alvarez (2012), among others. Moreover, this high presence also coincides with studies carried out in plantain cultivations in other regions in Colombia, as the coffee growing zone. In addition to the above mentioned, this genus shows to be prevalent in acid soils of Llanos Orientales (Gañan *et al.,* 2011; Serralde & Ramírez, 2004).

According to the diversity indexes, the municipalities have a moderate AMF diversity, which gives this region the potential to be used as sources of inoculum for the production of biofertilizers based on these fungi.

Differences in the number of spores in rhizopheric soils is possibly due to differences in soils, as well as to their management practices, which is consistent with the assertions stated by Gañan *et al.* (2011), in relation to the fact that the effective colonization of AMP depends precisely on the type of soil and management practices.

Conclusion

Soils used for plantain cultivation in the department of Córdoba, Colombia are characterized by having a medium AMF diversity that includes species of the genera *Glomus, Aculospora* and *Scullepospora* isolated from these soils and that have formed symbiotic associations with the evaluated plantain cultivations. Moreover, *Glomus* was the most abundant and dominant genus in the soils studied, so it has potential to be used for different sustainable agricultural applications associated to plantain cultivations and based on AMF.

According to diversity indexes, the municipalities of Lorica and Moñitos are the most diverse areas in AMF associated to plantain cultivations.

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