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Original article

Characterization of indigenous arbuscular mycorrhizal fungi associated with coconut cultivation in Baracoa

Caracterización de hongos micorrícicos arbusculares autóctonos asociados con el cultivo del coco en Baracoa

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ABSTRACT: The 85 % of the national coconut production takes place in Baracoa, which is affected by the low availability of mineral fertilizers, which limits the obtaining of quality seedlings and the development of plantations. This geographical area is located in a Biosphere Reserve, Cuchillas del Toa, characterized by a high endemism, so it is a potential priority agricultural technology that allow its protection while increasing the productivity of the coconut tree. In order to have autochthonous strains with potential for use in the nutritional management of the coconut tree, the isolation and characterization of arbuscular mycorrhizal fungi (AMF) was carried out in four sites. A high abundance of AMF spores was found in Cabacú and Cane, while the lowest was in Playa Duana, with the highest percentage of species and morphotypes observed. A relative specificity of the Orders to which the characterized species and morphotypes belong in relation to the studied sites was observed. The results indicate that both the abundance and the species or morphotypes found depend on factors specific to each ecosystem, which determine the autochthonous community of AMF that is established. It is shown that despite the development of coconut cultivation in these sites, they constitute very well conserved ecosystems, mainly due to the few tasks and applications of fertilizers that are carried out, which preserves their ecological balance.

Key words: Cocos nucifera, mycorrhizae, biodiversity.

RESUMEN: El 85 % de la producción nacional de coco se realiza en Baracoa, la cual es afectada por la baja disponibilidad de fertilizantes minerales, lo que limita la obtención de posturas de calidad y el desarrollo de las plantaciones. Esta área geográfica se encuentra en una Reserva de la Biosfera, Cuchillas del Toa, caracterizada por un alto endemismo, por lo que es una prioridad estimular el uso de tecnologías agrícolas que permitan su protección, al mismo tiempo que permitan incrementar la productividad del cultivo del cocotero. Con el objetivo de contar con cepas autóctonas, con potencial para su empleo en el manejo nutricional del cocotero, se realizó el aislamiento y la caracterización de hongos micorrícicos arbusculares (HMA) en cuatro sitios. Se encontró una elevada abundancia de esporas de HMA en Cabacú y Cane, mientras que la menor fue en Playa Duana, con el mayor porcentaje de especies y morfotipos observados. Se observó una especificidad relativa de los Orden a los cuales pertenecen las especies y los morfotipos caracterizados, en relación con los sitios estudiados. Los resultados indican que, tanto la abundancia como las especies o morfotipos encontrados, dependen de factores propios de cada ecosistema, los que condicionan la comunidad autóctona de HMA que se establece. Se demuestra que, a pesar de desarrollarse el cultivo del coco en estos sitios, constituyen ecosistemas muy bien conservados debido, principalmente, a las pocas labores y aplicaciones de fertilizantes que se realizan al mismo, lo cual conserva su equilibrio ecológico.

Palabras clave: Cocos nucifera, mycorrhizae, biodiversidad.

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INTRODUCTION

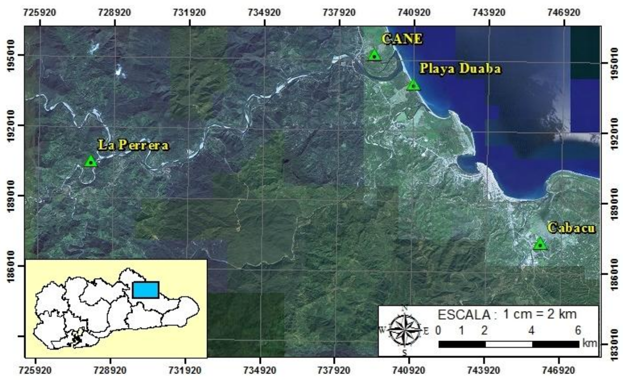
The coconut palm (*Cocos nucifera* L.) is considered one of the most important and useful of all tropical palm crops. It provides sustenance for millions of people in the world and it is cultivated in more than 80 countries in the tropics (1). In Cuba, this species has spread throughout the country, although the largest areas of cultivation are located mainly in Baracoa (Guantánamo), Niquero and Pilón (Granma), as well as in several municipalities from Holguín, Pinar del Río and Sancti Spíritus. This crop is of great economic importance due to the high nutritional value of its products (2); in addition, its by-products are widely used in the production of cosmetics (3-7). Recently, the importance of coconut oil consumption for human health has been demonstrated (8-11), as well as the importance of other by-products in diverse uses (12-14).

Baracoa municipality is responsible for the national production of coconut, to which 25.10 % of the agricultural surface of the municipality is dedicated. However, the lack of inputs and the little sensitization by the technical scientific services, has favored the aging of the plantations and the reduction of yields and fruit quality (15), reason why a greater emphasis is made in the use of bio-products and organic fertilizers.

A diverse beneficial microflora develops in the roots of coconut palms, including arbuscular mycorrhizal fungi (AMF) (16,17). They form mutualistic associations with most terrestrial plants (18,19), which provide nutrients to the host plant (19,20), and induce important physiological and biochemical changes that allow the plant to overcome biotic and abiotic stresses (21-24). In spite of having the widest geographical distribution of all biofertilizing microorganisms, some species do not adapt to conditions different from the soil from which they were isolated (25). It indicates the need to be able to count on autochthonous strains that allow the management of nutrition, mainly in crops such as coconut, which have very little availability of nutritional sources. Autochthonous AMF ecotypes are already adapted to the soils from which they were isolated, so it is expected that the identification of highly efficient autochthonous AMF strains will improve their exploitation in agricultural programs and provide solutions for obtaining productions that are compatible with humans and the environment (23).

Deepening the functional diversity of autochthonous AMF ecotypes for their impact on crop nutrition, as well as tolerance to different stresses, allows greater use of these beneficial soil microorganisms in sustainable agriculture programs in a scenario influenced by climate change (24,26).

The objective of the present study was to perform the isolation and characterization of indigenous AMF strains associated with coconut cultivation.



(Google Earth, 2021) Cabacú: nursery Cabacú; Playa Duaba: nursery Playa Duaba; CANE: UBPC “Mártires de Angola”; Quibijan (La Perrera): CCSF “Manuel Tames”

Figure 1. Location of study sites

MATERIALS AND METHODS

The research was carried out from January 2009 to September 2016. Sampling was carried out in the ecosystems belonging to Playa Duaba (nursery Playa Duaba), Cabacú (nursery Cabacú), Cane (UBPC "Mártires de Angola") and Quibiján (La Perrera, CCSF "Manuel Tames") (Figure 1, Table 1), linked to coconut plantations. All the study sites belong to the municipality of Baracoa, Guantánamo province, Cuba, and are located within the "Cuchillas del Toa" Biosphere Reserve, which underwent chemical characterization (27) (Table 2).

The sites were selected taking into account their high incidence of coconut production. Playa Duaba and Cabacú are the nurseries where most of the seedlings used to repopulate the production areas are produced. The UBPC "Mártires de Angola", located in the town of Cane, and the CCSF "Manuel Tames" in La Perrera (Toa), are the companies with the highest production of this crop.

Table 1. Cartographic location of the experimental sites, height above sea level and relief

Locality	Nursery/ production form	Cartographic coordinates	Height (m a.s.l.)	Relief
Playa Duaba	Playa Duaba	X=740808 Y=193882 (cartographic sheet 5276-I-b, scale 1:25 000, Baracoa municipality)	6	Plain
Cabacú	Cabacú	X=745830 Y=187732 (cartographic sheet 5376-IV-a, scale 1:25 000, cartographic sheet	6	Plain
Cane	UBPC “Mártires de Angola”	X=739450 Y=195450 (cartographic sheet 77-II-b, scale 1:25 000, Baracoa municipality	6	Plain
Quibijan (La Pèrrera)	CCSF “Manuel Tames"	X=728312 Y=189596 (cartographic sheet 5277-II-c, scale 1:25 000, Baracoa municipality)	236	Premontane

Sampling

Rhizospheric soil (0-20 cm depth) was sampled in the area occupied by the roots of 10 coconut plants from each site, where the highest concentration of AMF propagules is found. The soil was air-dried for further processing.

Characterization of AMF

Spore quantification and morphotype characterization were performed at the Institute of Ecology and Systematics. Samples of 100 g of soil were used and the extraction method described by Gerdeman and Nicholson (1963) and later modified (28), which is based on wet sieving and decantation of the fungal propagules. It employs the deagglomeration of the soil with the use of hydrogen peroxide and domestic blender, for the recovery of the greatest number of mycorrhizal propagules. The spores were collected on two screens, one of 140 and the other of 40 μm aperture, then centrifuged with sucrose and Tween 80, to be observed under an optical stereomicroscope (20-40x), model Stemi 2000-C (28).

For the identification of autochthonous AMF morphotypes, intact spores with abundant lipid content were selected and in each case collected and mounted in PVLG and PVLG/Melzer's reagent (1:1, v/v) for morphological identification. This was based on their color, size, surface ornamentations and wall structure following various taxonomic criteria (29-31) and the International Collection of Vesicular-Arbuscular Mycorrhizal Fungi (INVAM) (32). Spores were photographed under a Carl Zeiss compound microscope model Axioskop 2 with Axiocam coupled camera and processed using AxioVision 3.1 software at 1300 x 1030 dpi plus. The relative abundance of the genera found was calculated according to the formula (33):

$$Relative\ abundance = (number\ of\ species\ or\ isolates\ observed\ for\ each\ genus \div total\ isolates) \times 100\%$$

RESULTS AND DISCUSSION

For the characterization of the autochthonous AMF populations associated with coconut cultivation in Baracoa, the abundance of spores of these fungi was first determined. Differentiation was made by size, evaluating the content in two fractions, larger than 140 μm and between 140 and 40 μm (Table 3). In all the sites, a greater abundance was found in the 140 and 40 μm fraction, which indicates the prevalence of morphotypes characterized by producing smaller spores, but with a high degree of abundance of these structures, in which 97 to 99 percent of the total found in each site are located. The highest contents in both fractions and, consequently, in the totals, were observed in Cabacú, site where one of the nurseries with the highest levels of contribution of seedlings for the repopulation of the production areas is located. The site with the lowest abundance in both fractions was Playa Duaba. In Canes a high prevalence of spores with sizes belonging to the 140 and 40 μm fraction was observed, 99.1 % in relation to the total obtained in this site.

In general, AMF spore abundance at the sites studied was found to be similarly high to that reported in undisturbed natural environments. It is suggested that AMF biodiversity in natural environments is typically higher than that found in agricultural systems, due to the greater diversity of plants and the greater complexity of habitats, which can support a wide variety of microorganisms (34).

In a study of natural ecosystems in different areas of Cuba, a high density of spores and species richness has been found (35-37), with a predominance of Glomeraceae species and morphotypes (38).

In Ciénaga de Zapata Biosphere Reserve, Cuba, when comparing the density of AMF spores in natural and agricultural ecosystems, high levels of spores were found in swampy forests (7124 in 100 g of soil), while in coastal semi-deciduous forest and semi-deciduous forest, periodically flooded. They only reached 2932 and 1848,

Table 2. Characteristics of the arable horizon (0-0.20 m) of the soils belonging to the study sites

Locality	Soil type	pH (H_2O)	OM (g kg^{-1})	P_2O_5 (mg kg^{-1})	Ca	Mg	K	Na	EC	Ca/Mg
					(cmolc kg^{-1})					
Playa Duaba	Typical Arenosol	7.5	23.7	265.00	7.5	3.58	0.09	0.08	11.17	2.14
Cabacú	Fluvic Gleysol	7.6	1.68	57.2	16	11.5	0.03	0.21	27.74	1.39
Cane	Fluvisol, typical eutric	6.75	19.2	57.2	8.07	17.75	0.08	0.07	25.97	0.45
Quibijan (La Perrera)	Ferrallitic Red Leached, humic	6.6	55.8	123.6	6.8	1.6	0.12	0.05	8.57	4.25

Methods of analysis: pH (H_2O) potentiometer with soil ratio: 1:2,5. Organic matter (OM) Walkley Black

P_2O_5 by extraction with H_2SO_4 0.1 mol L^{-1} with soil ratio: 1:25. Exchangeable cations (EC) (cmolc kg^{-1}) extraction with NH_4Ac 1 mol L^{-1} at pH 7, determination by complexometry (Ca and Mg) and flame photometry (K and Na) (27)

Table 3. Abundance of AMF spores in 100 g of soil in the studied sites

Locality	140 μm fraction	140 μm fraction	Total of spores
Playa Duaba	40	2300	2340
Cabacú	140	5500	5640
Cane	43.33	4583.33	4626.67
Quibijan	76.67	3386.67	3463.33

respectively; however, in soils cultivated with sweet potato, only 480 spores were found (39). In the San Ubaldo-Sabanalamar Floristic Reserve in Pinar del Río province (40), it has been reported between 2000 and levels higher than 5000 spores per 100 g of soil in semi-natural and covered savannah, both in dry and rainy periods, where it was demonstrated that the levels of propagules in the soil are favored by the rainy period. The rainfall regime could have had an influence on the levels obtained in the present experiment, considering that it was developed in areas of high rainfall well distributed throughout the year.

For the characterization of autochthonous AMF, intact spores with abundant lipid content were selected, from which 18 species and morphotypes could be identified, 8 to genus and 10 to species, all belonging to the type Glomeromycetes (Table 4, Figure 2). The highest representativeness of the characterized isolates was observed in Playa Duaba and the lowest in Quibijan. Interestingly, it was found that Playa Duaba was the site with the lowest spore content; however, these were very diverse, with 44.44 % of the total species or morphotypes identified (Figure 3), while in Cabacú and Cane, where the highest spore contents were reported, only 33.33 and 22.22 % were observed, respectively. Quibijan, with intermediate spore contents, has the lowest percentage of identified species or morphotypes, only 11.11 %.

When analyzing the relative abundance of AMF genera observed in the sites studied (Figure 4), it was observed that the predominance of isolates corresponded to the genus *Glomus*, with 30.77 %, followed by *Acaulospora* (19.23 %), *Ambispora* (15.38 %) and *Claroideoglomus* (11.54 %), the rest of the genera were below 10 % (*Rhizophagus*, *Scutellospora*, *Pacispora*, *Funneliformis* and *Diversispora*).

Relative specificity was observed in relation to the Order to which the species belong, the characterized morphotypes and the sites, so that in Playa Duaba Glomerales predominated (62.5 %), in Canes it was Diversisporales (50 %), while in Cabacú there was an equal distribution of Glomerales and Diversisporales (50 % each) and in Quibijan there was a predominance of Archaeosporales.

Interestingly, although a high predominance of the genus *Glomus* was observed, it was only found at the Playa Duaba site with four morphotypes and the species *G. microaggregatum* at Cabacú, which shows its high adaptability to the physical-chemical conditions of the soils of these sites, which facilitate the establishment and reproduction of this genus. In studies carried out in coconut plantations in different ecosystems of Yucatan, in dry and humid periods, a high number of genera were found, the most abundant being *Glomus*, followed by *Sclerocystis*, *Rhizophagus*, *Redeckera* and *Diversispora* (41). Other authors in different tropical natural ecosystems have found a high predominance of Glomus (38,39), related to different plant species (42,43).

High levels of mycorrhizal propagules could be related to the location within the buffer zone of "Alejandro de Humboldt" National Park, which constitutes the largest remnant of the most conserved mountainous ecosystems in Cuba and the area of greatest endemism in the Antilles (44). Also, favored by the high rainfall regime, well distributed throughout the year, which has been demonstrated by other authors as a favorable factor for obtaining high levels of propagules in the soil (40). On the other hand, they are undisturbed areas, which have not received application of fertilizers, pesticides, or any other chemical product for a period of almost 20 years, which could provoke alterations of the soil microbiota. In soils of a natural, undisturbed savanna, a high diversity of AMF species was found, including genera such as *Gigaspora* and *Scutellospora*, which have been observed to be susceptible to disturbed environments (45).

Table 4. Presence of AMF species and morphotypes observed in the studied sites

	AMF species and morphotypes	Playa Duaba	Cabacú	Cane	Quibijan
1	*Acaulospora morrowiae*	x			
2	*Acaulospora kentinensis*		x	x	
3	*Acaulospora scrobiculata*		x		
4	*Ambispora* sp. 1	x			
5	*Ambispora* sp. 2			x	
6	*Claroideoglomus claroideum*	x			
7	*Claroideoglomu etunicatum*			x	x
8	*Diversispora spurca*		x		
9	*Funneliformis. geosporum*	x			
10	*Glomus* sp. 1	x			
11	*Glomus* sp. 2	x			
12	*Glomus* sp. 3	x			
13	*Glomus sp. 4*		x		
14	*Glomus microaggregatum*		x		
15	*Pacispora* sp.	x			
16	*Rhizophagus aggregatum*		x		
17	*Rhizophagus intraradices*				x
18	*Scutellospora* sp. 1			x	

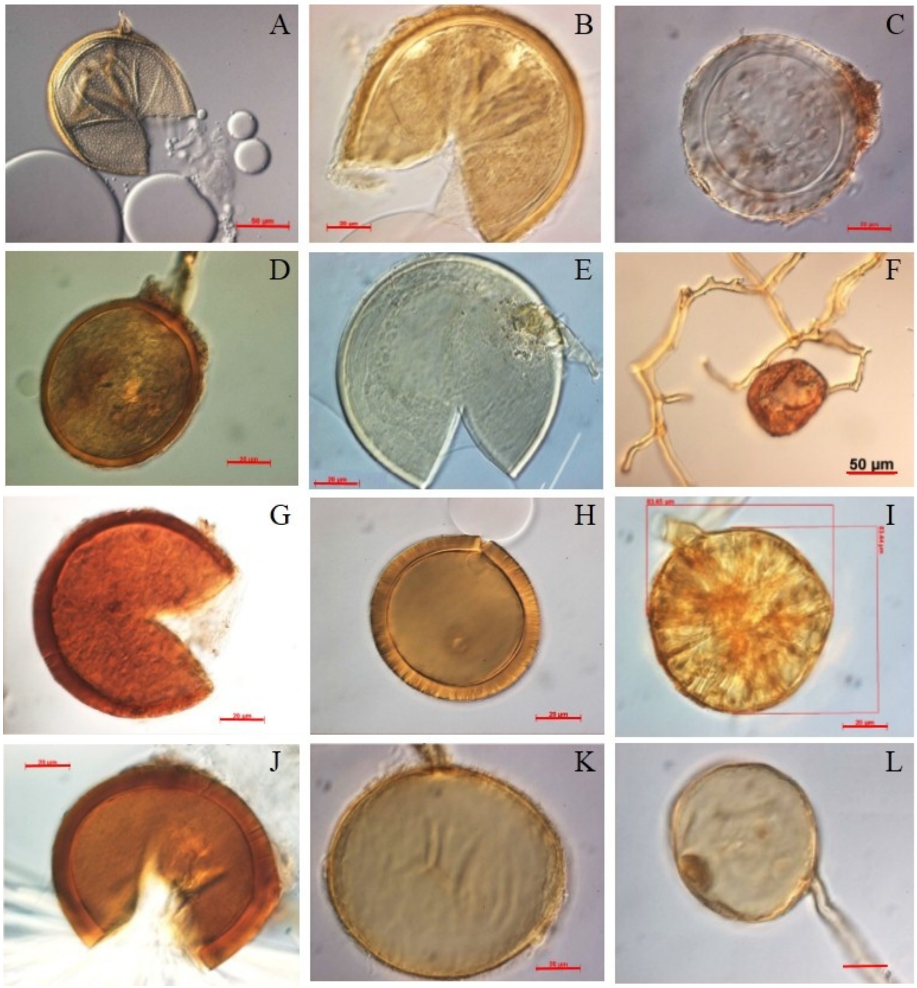


Figure 2. Some AMF morphotype species identified in the sites studied, associated with coconut cultivation. (A) *Acaulospora kentinensis*. Bar = 50µm. (B) *Acaulospora morrowae.* Bar = 20µm. (C) *Ambispora* sp1. Barra = 20µm (D) *Claroideoglomus claroideum.* Bar = 20µm. (E) *Claroideoglomus etunicatum.* Bar = 20µm. (F) *Glomus microaggregatum.* Bar = 50µm. (G) *Glomus* sp1. Bar = 20µm. (H) *Glomus* sp2. Bar = 20µm. (I) *Glomus* sp4. Bar = 20µm. (J) *Funneliformis geosporum.* Bar = 20µm. (K) *Rhizophagus aggregatus.* Bar = 20µm. (L) *Rhizophagus intraradices*. Bar = 20µm

Studies carried out on AMF populations associated to this crop, report having found a high density and species diversity of mycorrhizal fungi (*Glomus*, *Gigaspora*, *Sclerocystis* and *Acaulospora*) forming part of mycorrhizal associations with coconut. The highest colonization rate was observed in the rhizosphere of tall varieties and in intercropping system, with a range of 40.4 to 154.5 spores in 10 g^{-1} of soil (16).

To be able to count on characterized autochthonous AMF strains is a very useful tool for the use of these fungi in the agroecological nutritional management of coconut, to which few or no fertilizers or pesticides are applied.

It has been proposed that the use of native AMF consortia, composed of several taxa already adapted to extreme conditions and with different symbiotic behaviors, may, in fact, be more efficient in preserving ecosystems by mitigating the vulnerability of horticultural crops to water scarcity and soil salinity, thus allowing the viability of crops in extreme environments (24). The abundance and number of indigenous AMF taxa have been found to be sensitive to soil management in intensive agriculture (46,47).

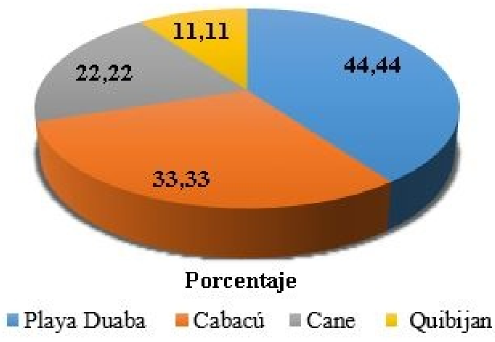


Figure 3. Percentage of AMF species and morphotypes observed in the sites studied

The results indicate that both the abundance and the species or morphotypes found depend on factors specific to each ecosystem, which condition the autochthonous AMF community that is established. It is demonstrated that in spite of the development of coconut cultivation in these sites, they constitute very well conserved ecosystems, mainly due to the little work and fertilizer applications that are made to them, which preserves their ecological balance.

In future works, it is necessary to evaluate the growth promoting and pathogen protection activity of the isolated strains, so that one or more can be selected for the preparation of a bioproduct for crop agroecological management.

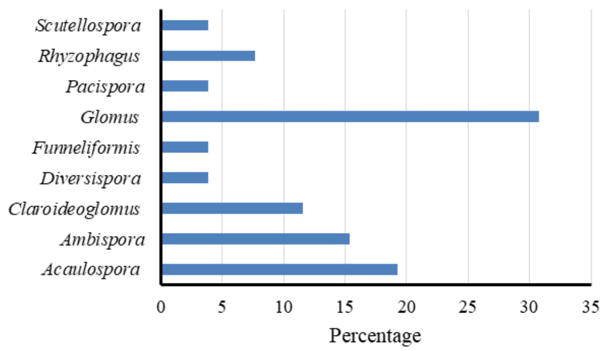


Figure 4. Relative abundance of AMF genera observed in the sites studied

CONCLUSIONS

- For the first time, autochthonous AMF morphotypes are reported in ecosystems associated with coconut cultivation in Cuba.
- A high abundance of AMF spores is observed in Cabacú and Cane, being lower in Playa Duana, with the highest percentage of species and morphotypes observed.
- There is a relative specificity of the Orders to which the characterized species and morphotypes belong in relation to the studied sites, Playa Duaba (Glomerales), Canes (Diversisporales), Cabacú (Glomerales and Diversisporales) and Quibijan (Archaeosporales).

IN MEMORIAM

A Eduardo Furrazola Gómez, Investigador Auxiliar del Instituto de Ecología y Sistemática. Reconocido taxónomo y ecólogo cubano, vinculado a la caracterización e identificación de especies de hongos micorrizógenos arbusculares, en ecosistemas naturales y agroecosistemas diversos, tanto en Cuba como en otros países de la región, cuyo trabajo ha sido reconocido y premiado en varias ocasiones. Reciba nuestro agradecimiento por haber sido sus alumnos, colaboradores y amigos.

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