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Inter- and intraspecific functional variability of tropical arbuscular mycorrhizal fungi isolates colonizing corn plants

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ABSTRACT

For a single plant species under the same environmental conditions, the interaction with arbuscular mycorrhizal fungi (AMF) and their contribution to plant growth varies among AMF isolates, with both inter and intraspecific variability. The present study evaluated the functional variability of 41 isolates of 20 species and eight genera of AMF for root colonization, growth promotion, and *P* uptake of corn and observed the relationship of this functional variability with the isolates genetic variability revealed by PCR-RFLP analysis. All the isolates abundantly colonized the corn roots, but only 23 promoted higher shoot dry mass and *P* leaf content. The cluster analysis based on functional variability data separated the isolates *Acaulospora morrowiae* (Am2), *Acaulospora* sp. (Aca), *A. colombiana* (Ac3, Ac4, and Ac5), *Gigaspora albida* (Gia1), *Gi. margarita* (Gim4 and Gim5), *Gi. rosea* (Gir), *Rhizophagus clarus* (Rc2, Rc3, Rc4, Rc5, and Rc6), *Claroideoglomus etunicatum* (Ce4), *R. manihotis* (Rm), *Scutellospora calospora* (Sc), *S. heterogama* (Sh2, Sh3, Sh4, and Sh5) and *S. pellucida* (Sp3) from the others at the distance of 80% functional similarity. These were considered efficient in promoting functional symbiosis in corn while the other isolates were considered inefficient. The cluster analysis obtained by the PCR-RFLP technique was partly coherent with the species classification based on spore morphology. The isolates of *R. clarus* fell into one cluster and the isolates of the *Gigaspora* and *Scutellospora* genera (Gigasporaceae family) were clustered in a second cluster, without the ability to separate the species of these genera.

1. Introduction

The arbuscular mycorrhizal fungi (AMF) form symbiotic relationships with the majority of land plant species and are distributed throughout the tropics. This association is characterized by the penetration of the fungal mycelium between and within the cells of the radicular cortex, without causing visible morphological change to the naked eye, but that modifies the plant's physiology, enhancing its capacity to absorb nutrients from the soil, especially those with low soil mobility such as phosphorus. For the same plant species, the effects and contribution of AMF vary according to the fungal isolates, reflected in differences in the symbiotic efficiency of the fungus (van der Heijden and Kuyper, 2001). This variation is well documented, but the mechanisms that regulate these interactions and effects are poorly understood. This leaves doubt about the role of AMF in crop yields and recuperation of degraded ecosystems hampering the definition of strategies for the application of these fungi.

At present 230 AMF species are known (Schüßler and Walker, 2010), a number considered low compared to the number of plant species with which these fungi can associate. This situation induces the view that AMF form a homogenous group of symbionts in which all the species perform identical biological functions in the ecosystem (Husband et al., 2002).

The use of molecular techniques based on PCR-RFLP (polymerase chain reaction – restriction fragment length polymorphism) has allowed verification that the AMF community in the rhizosphere varies among plant species (Babu and Reddy, 2011; Martínez-García and Pugnaire, 2011) and among soils with different concentrations of nutrients (Martinez-García et al., 2011). It has been found that the community variability is greater in the rhizosphere than in the roots (Martinez-García et al., 2011; Miras-Avalos et al., 2011), suggesting some degree of preference or specificity.

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According to Wagg et al. (2011), due to the seemingly limitless possibilities for complementarity or selection effect, it is difficult, if not impossible, to determine all the functional and resource-based niches that each AMF species within a more AMF-rich community can occupy to influence, and thus direct, the richness-plant productivity relationship.

Great functional variability of AMF has been detected, both regarding species and isolates of the same species defined based on spore morphology (Avio et al., 2006; Munkvold et al., 2004; Smith et al., 2004). However, these studies evaluated a small number of isolates of a single genus (*Glomus*). The present study is the first to assess a large number of isolates, encompassing various species and genera from tropical ecosystems, regarding their functional variability in relation to a single plant species.

In general, the symbiotic efficiency of an AMF isolates is attributed to two main factors: the ability to abundantly colonize the roots and the ability to promote beneficial responses for the host plant's nutrition and growth. The difference in symbiotic performance observed among different AMF isolates is considered as a measure of the functional variability of mycosymbionts (Pouyu-Rojas et al., 2006). Corn shows high growth rate, high uptake of nutrients, and high level of mycotrophy making it an ideal host for studies to assess the functional variability of AMF. Therefore, the aim of this work was to evaluate the functional variability, both among the species of different genera and different isolates of the same species of AMF, regarding root colonization, growth effects, and phosphorous uptake of corn, *Zea mays* L. (Poaceae).

2. Material and methods

2.1. Fungal material

A total of 42 AMF isolates belonging to 20 species and 8 genera were used in this study (Table 1). Initially, to reduce the

Table 1
List of the AMF isolates studied in this work.

Family[a]	Species[a]	Code of origin[b]	Code[c]	Plant of origin	Location of origin	Biome of origin	Institution of origin[d]
Acaulosporaceae	*Acaulospora colombiana*	A87 (CNPAB 043)	Ac1	*Gleichenia* sp.	Itumirim, MG	Cerrado	CNPAB
Acaulosporaceae	*Acaulospora colombiana*	A15 (CNPAB 015)	Ac2	*Zea mays*	Barra do Piraí, RJ	Atlantic forest	CNPAB
Acaulosporaceae	*Acaulospora colombiana*	ALCOA-CCB2	Ac3	–	Minas Gerais	Cerrado	UFLA
Acaulosporaceae	*Acaulospora colombiana*	06-UFLA	Ac4	–	–	–	UFLA
Acaulosporaceae	*Acaulospora colombiana*	AMZ570A	Ac5	–	Benjamin Constant, AM	Amazon forest	FURB
Acaulosporaceae	*Acaulospora morrowiae*	401-UFLA	Am1	Native grasses	Três Marias, MG	Cerrado	UFLA
Acaulosporaceae	*Acaulospora scrobiculata*	A38 (IES-33)	As1	–[g]	IES[e] (Cuba)	–	CNPAB
Acaulosporaceae	*Acaulospora scrobiculata*	ALCOA29	As2	–	Poços de Caldas, MG	Atlantic forest	UFLA
Acaulosporaceae	*Acaulospora spinosa*	95-UFLA	Asp	–	–	Cerrado	UFLA
Acaulosporaceae	*Acaulospora* sp.	A88 (CNPAB 044)	Aca	*Gleichenia* sp.	Itumirim, MG	Cerrado	CNPAB
Claroideoglomeraceae	*Claroideoglomus etunicatum*	365-UFLA	Ce1	*Coffea arabica*	Patrocínio, MG	Cerrado	UFLA
Claroideoglomeraceae	*Claroideoglomus etunicatum*	URM-FMA 03	Ce2	–	–	–	UFPE
Claroideoglomeraceae	*Claroideoglomus etunicatum*	–	Ce3	–	Santa Catarina	–	ESALq
Claroideoglomeraceae	*Claroideoglomus etunicatum*	SCT101A	Ce4	*Malus domestica*	Santa Catarina	Pinhais forest	FURB
Glomeraceae	*Glomus formosanum*	A20 (CNPAB 020)	Gf	*Imperata brasiliensis*	Barra do Piraí, RJ	Atlantic forest	CNPAB
Glomeraceae	*Rhizophagus clarus*	A5 (CNPAB 005)	Rc1	–	INVAM[f]	–	CNPAB
Glomeraceae	*Rhizophagus clarus*	URM-FMA 08	Rc2	–	–	–	UFPE
Glomeraceae	*Rhizophagus clarus*	CMM-306	Rc3	Native grasses	Três Pontas, MG	Cerrado	UFLA
Glomeraceae	*Rhizophagus clarus*	–	Rc4	–	Florianópolis, SC	–	ESALq
Glomeraceae	*Rhizophagus clarus*	351-UFLA	Rc5	–	Flórida, EUA	–	UFLA
Glomeraceae	*Rhizophagus clarus*	A90 (CNPAB 046)	Rc6	*Canavalia rosea*	Recife, PE	Planície costeira	CNPAB
Glomeraceae	*Rhizophagus manihotis*	A83 (CNPAB 041)	Rm	*Manihot esculenta*	Seropédica, RJ	Atlantic forest	CNPAB
Gigasporaceae	*Gigaspora albida*	URM-FMA 01	Gia1	–	–	–	UFPE
Gigasporaceae	*Gigaspora albida*	03-UFLA MG	Gia2	Native grasses	Três Marias, MG	Cerrado	UFLA
Gigasporaceae	*Gigaspora candida*	A36 (IES-29)	Gic1	–	IES[e] (Cuba)	–	CNPAB
Gigasporaceae	*Gigaspora candida*	BEG17[h]	Gic2	–	Taiwan	Tropical agricultural	BEG
Gigasporaceae	*Gigaspora margarita*	A49 (Inóculo 138)	Gim1	*Coffea arabica*	Patrocínio, MG	Cerrado	CNPAB
Gigasporaceae	*Gigaspora margarita*	A1 (CNPAB 001)	Gim2	–	Flórida, EUA	–	CNPAB
Gigasporaceae	*Gigaspora margarita*	A41 (IES-42)	Gim3	–	IES[e] (Cuba)	–	CNPAB
Gigasporaceae	*Gigaspora margarita*	279-UFLA	Gim4	–	–	–	UFLA
Gigasporaceae	*Gigaspora margarita*	R1 UFLA	Gim5	*Zea mays*	–	–	UFLA
Gigasporaceae	*Gigaspora rosea*	A35 (IES-19)	Gir	–	IES[e] (Cuba)	–	CNPAB
Gigasporaceae	*Racocetra gregaria*	200-UFLA	Rg	*Gossypium hirsutum*	Flórida, EUA	–	UFLA
Gigasporaceae	*Scutellospora calospora*	A80 (CNPAB 038)	Sc	Native plant	Oriximiná, PA	Amazon forest	CNPAB
Gigasporaceae	*Scutellospora heterogama*	A34 (IES-16)	Sh1	–	IES[e] (Cuba)	–	CNPAB
Gigasporaceae	*Scutellospora heterogama*	A2 (CNPAB 002)	Sh2	*Coffea arabica*	Campinas, SP	Atlantic forest	CNPAB
Gigasporaceae	*Scutellospora heterogama*	312-UFLA	Sh3	–	Flórida, EUA	–	UFLA
Gigasporaceae	*Scutellospora heterogama*	URM-FMA 05	Sh4	–	–	–	UFPE
Gigasporaceae	*Scutellospora heterogama*	–	Sh5	–	–	–	ESALq
Gigasporaceae	*Scutellospora pellucida*	464-UFLA	Sp1	–	Lavras, MG	Cerrado	UFLA
Gigasporaceae	*Scutellospora pellucida*	A70 (CNPAB 029)	Sp2	*Ipomoea batatas*	Seropédica, RJ	Atlantic forest	CNPAB
Gigasporaceae	*Scutellospora pellucida*	128-UFLA	Sp3	–	Lavras, MG	Cerrado	UFLA

[a] According to the species list available in http://schuessler.userweb.mwn.de/amphylo/accessed in June, 26th 2013.
[b] Code of the isolates used in the institution of origin.
[c] Code of the isolates used in this work.
[d] Institutions that donated the isolates, FURB = Blumenau Regional University; CNPAB = Embrapa–National Center for Agrobiology Research; UFLA = Lavras Federal University; ESALQ = Luiz de Queiroz Superior School of Agriculture; UFPE = Pernambuco Federal University.
[e] Instituto de Ecologia y Sistematica de Cuba.
[f] International culture collections of (Vesicular) arbuscular fungi.
[g] Information not available on the institution of origin.
[h] This isolate was used only in the characterization, due to the unavailability of spores.