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COMPOSITION OF ENDOPHYTIC FUNGAL COMMUNITY ASSOCIATED WITH LEAVES OF MAIZE CULTIVATED IN SOUTH BRAZILIAN FIELD

VIVIAN J. SZILAGYI-ZECCHIN[1]*, DOUGLAS ADAMOSKI[2], RENATA RODRIGUES GOMES[3], MARIANGELA HUNGRIA[4], ANGELA C. IKEDA[2], VANESSA KAVA-CORDEIRO[2], CHIRLEI GLIENKE[2] and LYGIA V. GALLI-TERASAWA[2]

[1]Department of Plant Science and Crop Protection, Federal University of Paraná, Curitiba, Brazil
[2]Department of Genetics, Federal University of Paraná, Curitiba, Brazil
[3]Department of Basic Pathology, Federal University of Paraná, Curitiba, Brazil
[4]Soil Biotechnology Laboratory, Embrapa Soja, Londrina, Brazil

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The objective of this study was to conduct a survey about fungi associated with leaves from two different maize plant lineages and to analyze their microbiota diversity. Isolated fungi were identified by morphological analysis and molecular taxonomy was performed using ITS1-5.8S-ITS2 rDNA. About 27 fungi morphotypes were obtained, 15 of them were from the first maize lineage. About 86.7% of the individuals belonged to the Dothideomycetes class (*Phoma sorghina*, *Epicocum nigrum*, *Cladosporium* sp., *Bipolaris zeicola*, and *Alternaria alternata* complex) and 13.3% to the Sordariomycetes class (*Diaporthe*/*Phomopsis* sp. and *Nigrospora* sp.). This ratio was opposite in the other maize lineage with 25.0% of Dothideomycetes (*E. nigrum* and Pleosporales) and 75.0% of Sordariomycetes (*Gibberella fujikuroi* complex, *Fusarium graminearum* complex, *Diaporthe*/*Phomopsis* sp., and *Nigrospora* sp.). By concerning the analyses of morphological characteristics and molecular phylogeny, this study intended to identify the groups of saprophytic, phytopathogenic, and mycotoxin fungi, which differently co-inhabit leaf tissue of maize plants in both tested lineages.

Keywords: fungi, molecular phylogeny, microbiota, ITS1-5.8S-ITS2

*Corresponding author; E-mail: vivian.szilagyi@gmail.com

Introduction

Maize (*Zea mays* L.) crop has a high economic impact on Brazilian agriculture. This is because it is a rising commodity on international market due to its extensive supply chain. Furthermore, it is essential to compose the crop rotation system [1].

Leaves represent one of the most dynamic interfaces of plants. Fungi inhabiting this tissue share the characteristics that allow them to grow and survive in a constantly biochemical changing environment. This constant change is mainly due to the different stages of plant development [2].

Endophytes are fungi growing inside plants without causing symptoms. Normally, this group is ubiquitous, and the associated plant species have few descriptions in the literature. There is a large biological diversity among endophytes, and it is not rare for some plant species to host more than 100 different endophytic species. In addition, different lifestyles occur among endophytic species, and the same endophytic fungi can be either pathogenic (those that cause diseases to plants) or harmless, depending on the host and its health [3]. Besides, some saprophytic fungi (those that obtain nutrients from organic matter of dead plants) found in senescent plants have been isolated as endophytes inhabiting healthy tissues [4]. These endophytic fungi behave as latent saprophytes and are asymptomatic and spatially restricted during the host development, but can grow and unrestrictedly reproduce when the host tissue ages or dies [5].

The mutual dependence between endophytic fungi and plants leads to desirable consequences, such as herbivory reduction, drought resistance increase, plant growth promotion, and insect and pathogenic fungi control [6].

However, fungi are the main microorganisms responsible for yield losses in maize grain production [7], causing seed rot, seedling death [8], and damage during grain storage [9]. Furthermore, if ingested, contaminated seeds can lead to severe poisoning [10].

It is known that interaction between microorganisms and their hosts can be influenced by several causes. As an example, the genetic diversity of symbionts; the ways they are acquired from environment and the ability of individuals to colonize hosts. In addition, direct and indirect interactions between the environment and the evolutionary history of each microorganism should be considered. Genomic architecture is involved in all these aspects and it is associated with pathogenicity or with other kinds of environment interaction (endophytes or saprophytes) [11].

This study aimed to lead a survey about genetic diversity of fungi associated with leaves from two lineages of maize plants, performing the analyses of morphological characteristics and molecular taxonomy.

Materials and Methods

Fungi isolation

Leaf samples were collected from two maize plant lineages (L1 and L2) (*Zea mays* L.), cultivated in the same area. Lesions were observed in both lineages, L1 presented signs of Cercospora leaf spot, whose etiologic agent is *Cercospora zeae-maydis* and L2 presented typical lesion spots of *Exserohilum turcicum*. Leaves were collected when plants were in the phenological stage of grain filling and the lineages were cultivated at the experimental station of Semília Genetics and Breeding LTDA, Campo Largo, Paraná, Brazil. A direct planting system was used without crop rotation.

Fungi isolation was performed by washing the leaves in water and treating them with 70% ethanol (v/v) for 1 min, 3% NaClO (v/v) for 4 min, 70% ethanol (v/v) for 30 s, followed by three consecutive washes in distilled water. Five fragments (5–7 mm) were incubated at 28 °C in Petri dishes containing Potato Dextrose Agar (PDA) medium, supplemented with tetracycline (100 mg/mL).

Morphological characterization

Isolated fungi were grouped into morphotypes according to their micromorphological characteristics (reproductive structures), analyzed by microculture technique and macromorphological characteristics (e.g., colony color and structure and growth rate). They were incubated on Tomato Juice Extract Agar and PDA culture media, at 28 °C during 7, 14, and 21 days. From each colony, a lamina was prepared containing the fungus stained by cotton lactophenol blue. Each lamina was visualized under optical microscopy and, when possible, morphological features were compared with taxonomic patterns [12].

The isolated fungi are classified in the collection of microorganisms from the Microorganisms Genetics Laboratory, Federal University of Paraná, Curitiba, Paraná, Brazil. Fungi colonies are maintained into inclined tubes containing PDA medium, kept at 4 °C. Mycelia are also stored in distilled water at room temperature.

Genomic DNA extraction and ITS1-5.8S-ITS2 rDNA sequencing

One fungus colony of each morphotype was selected for genomic DNA extraction. This extraction was performed using the Microbial ultraclean DNA Isolation Kit (MoBio®, Carlsbad, CA, USA), according to the manufacturer's instructions, and DNA resuspended in 20 µL of ultrapure water. Its integrity was

verified by electrophoresis on agarose gel 0.8% (w/v), stained with GelRed™ (Biotium, USA), observed in an UV transilluminator (Ultraviolet Benchtop transilluminators) and photo-documented (Program Digidoc it). DNA quantification and purity were assessed by the spectrophotometer NanoDrop® 2000 (Thermo Scientific, Wilmington, USA).

Amplification of ITS1-5.8S-ITS2 rDNA was performed by polymerase chain reaction (PCR) using primersV9G [13] and ITS4 [14] with 10 ng of DNA, 1X PCR buffer, 0.5 U Taq polymerase, 0.1 mM of each primer (1.25 pmol/reaction), 0.2 mM of each dNTP, 1.5 mM $MgCl_2$, and 12.5 µL final volume. Initial DNA denaturation was at 94 °C for 2 min, followed by 35 cycles of 30 s at 94 °C, 1 min at 55 °C, 1 min at 72 °C, and final extension at 72 °C for 3 min. DNA fragment integrity was verified as described above on agarose gel 1.5% (w/v). Purification with ammonium acetate 7.5 M was performed according to Menna et al. [15]. Ultrapure water was used to resuspend PCR products and their quantification and purity were measured by spectrophotometry as described above, adjusting final concentration to 10 ng/µL.

Sequencing reaction was performed using 50 ng of purified PCR product added to 1 mM of primer and 2 µL of ET mixture for sequencing kit (ET-DYEnamic Dye Terminator Sequencing Kit for Cycle MegaBace, Amersham Bioscience®). Ultrapure water was added to make up a final volume of 10 µL.

Sequencing reaction was performed with 35 cycles of 30 s at 94 °C, 15 s at 50 °C, and 60 s at 60 °C. After that, fragments were purified by gel filtration on Sephadex™ G-50 medium (GE® Healthcare) and electrophoresis was carried out on an automated DNA sequencer model MegaBACE1000 (Amersham Biosciences®) [16].

Phylogenetic analysis

The quality of sequence fragments obtained was checked by Phred software [17]. Sequence fragments were compared on BLASTn (ftp://ftp.ncbi.nlm.nih.gov/blast/) and sequences of reference were obtained from NCBI database (www.ncbi.nlm.nih.gov). Alignments using the obtained sequences and reference sequences were obtained using the PRANK software [18]. Maximum likelihood trees were set using the program GARLI 2.0 [19]. Bootstrap analyses were performed using 1,000 replicates by DendroPy version 3.8.1 software [20].

Diversity and ecological associations

Fungal diversity was evaluated by the Shannon index, which generally ranges between 1 and 3.5, equivalent to common and rare species [21]. It was also

estimated by the Simpson index considering values between 0 and 1 [22], and it was stable with smaller sample sizes. This estimative was obtained for all isolated fungi using PAST software [23].

Results

Isolation and morphological classification

About 27 different fungi morphotypes were obtained from leaves of two maize plant lineages (L1 and L2). Out of all the morphotypes, 15 were isolated from L1 and 12 from L2. In both lineages, it was not possible to isolate fungi considered as etiologic agents of diseases (*C. zeae-maydis* and *E. turcicum*). Reproductive structures were observed in 92.6% of fungi (Figure 1).

From L1, 86.7% of fungi belonged to Dothideomycetes (*Phoma, Epicoccum*, *Cladosporium*, *Bipolaris/Cochliobolus*, and *Alternaria*) and 13.3% to Sordariomycetes (*Diaporthe*/*Phomopsis* sp. and *Nigrospora* sp.). Proportion ratio was opposite for L2, with 25.0% of Dothideomycetes (*Epicoccum* and

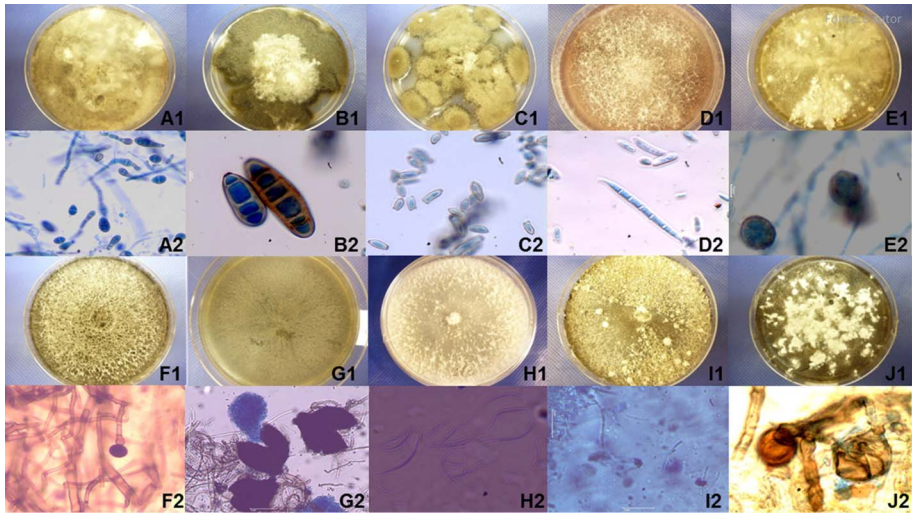

Figure 1. Morphological characterization of isolated fungi grown in PDA medium. A1 and A2: *Alternaria* sp. (LGMF1021); B1 and B2: *Bipolaris* sp. (LGMF1013); C1 and C2: *Cladosporium* sp. (LGMF1020); D1 and D2: *Fusarium* sp. CGF (LGMF1243); E1, E2, J1, and J2 Phoma/*Epicoccum* sp. (LGMF1042, LGMF1016); F1 and F2: *Nigrospora* sp. (LGMF1017); G1 and G2: *Phoma* sp. (LGMF1051); H1 and H2: *Phomopsis* sp. (LGMF1026); I1 and I2: *Diaporthe/Phomopsis* sp. (LGMF1245)

Pleosporales) and 75.0% of Sordariomycetes (*Gibberella*, *Fusarium*, *Diaporthe*/*Phomopsis* sp., and *Nigrospora* sp.) (Table I and Figure 2).

Fungal identification by ITS1-5.8S-ITS2 rDNA analysis

Using BLASTn tool, it was possible to verify the percentage of similarity comparing obtained sequences and sequences from reference strains. Sequences of 417–544 bp length were used. LGMF1041 had the lowest similarity percentage (94%) and the other showed 96%–100% (Table I).

Phylogenetic tree was built using the maximum likelihood model (Figure 3). Among these fungi, one (LGMF1041) was set into Pleosporales order (group 6) since its genus classification was not clear, and it was genetically related to *Ochrocladosporium* and *Leptosphaeria*. It was possible to define the genus of seven fungi: LGMF1026, LGMF1040, and LGMF1054 belong to *Diaporthe*/*Phomopsis* sp. (group 4); LGMF1020 belong to *Cladosporium* (group 3) and LGMF1017, LGMF1038, and LGMF1039 belong to *Nigrospora* sp. (group 2). Seven individuals were placed in groups of three complex species: *Alternaria alternata* complex (AAC) (LGMF1018 and LGMF1021), with 99% of support (group 8); *Fusarium graminearum* complex (FGC) (LGMF1036 and LGMF1037) and *Gibberella fujikuroi* complex (GFC) (LGMF1049, and LGMF1050 LGMF1053) with 93% and 82% of support, respectively (group 1) (Figure 3).

Isolated LGMF1013 and LGMF1022 strongly matched to *Bipolaris zeicola* species (synonymy: *Cochliobolus carbonum*), with 98% of support (group 7). Both showed 99% of similarity with *B. zeicola* ATCC 48129 (Table I). LGMF1014, LGMF1016, LGMF1019, LGMF1024, LGMF1025 LGMF1042, and LGMF1043 were placed in *E. nigrum* group. All of them had 99% of similarity with *E. nigrum* CBS 115825 except LGMB1042, which was 98% (group 5).

LGMF1015, LGMF1023, and LGMF1051 were classified into *Phoma sorghina* group. The isolates LGMF1023 and LGMF1051 are the most similar to *P. sorghina* PD88/549 (99%), and the other one is 96% (Table I).

Diversity and ecological associations

The most representative order was Pleosporales, including 15 morphotypes (55.5%), 12 from L1 and 3 from L2. It was followed by the Hypocreales order (18.5%) with five fungi, all of them from L2. Diaporthales and Trichosphaerales comprehended three morphotypes each, one from L1 and two from L2. Finally,

Table I. Morphological description of fungi isolated and percentage of similarity obtained by BLASTn according to reference species. (L1) Lineage 1 and (L2) Lineage 2

			Reference species related			
Strain	Lineage	Morphological characteristics	Species	ID/ collections	ITS*	Sequence similarity (%)
LGMF1013	L1	Colonies are cottony, surface, and reverse olive green. Hyphae septate and conidia ellipsoid, five to eight septs, and hilum.	*C. carbonum*	NI1243	AF071326	537/538 (99)
LGMF1014	L1	Colonies are floccose, gray with white patches, reverse brown. Hyphae septate, pycnidia hyaline, and multiseptate.	*E. nigrum*	CBS 115825	FJ426998	459/464 (99)
LGMF1015	L1	Colonies dense, gray with orange yellow patches, reverse yellow. Hyphae septate, pycnidia hyaline, and multiseptate.	*P. sorghina*	PD 88/549	FJ427078	476/479 (99)
LGMF1016	L1	Colonies are floccose, gray and green, reverse yellow orange. Hyphae septate, pycnidia hyaline, and multiseptate.	*E. nigrum*	CBS 115825	FJ426998	485/490 (99)
LGMF1017	L1	Colonies are woolly, graphite gray, reverse gray. Hyphae septate, conidia simple, terminal, hyaline, and globose.	*Nigrospora oryzae*	NRRL 54030	GQ28855	463/478 (97)
LGMF1018	L1	Colonies are floccose, gray and white, reverse black. Hyphae septate, conidia with longitudinal and transversal septa.	*A. alternata*	ATCC MYA-4642	HQ263343	544/544 (100)
LGMF1019	L1	Colonies are floccose, gray and white, reverse yellow. Conidia rounded and hyaline.	*E. nigrum*	CBS 115825	FJ426998	476/477 (99)
LGMF1020	L1	Colonies are velvety, olivaceous brown, reverse black. Hyphae septate and conidia ovoid with hilo.	*C. cladosporioides*	CPC 12762	HM148030	540/540 (100)
LGMF1021	L1	Colonies are dense, gray and white, reverse brown with concentric circles. Hyphae septate, conidia with longitudinal and transversal septa.	*A. alternata*	ATCC MYA-4642	HQ263343	537/538 (99)
LGMF1022	L1	Colonies are cottony, surface and reverse olivaceous. Hyphae septate, conidia ellipsoid, five to eight transversal septa, and hilo.	*C. carbonum*	NI1243	AF071326	544/545 (99)

Table I. (cont.)

Strain	Lineage	Morphological characteristics	Reference species related			
			Species	ID/ collections	ITS*	Sequence similarity (%)
LGMF1023	L1	Colonies are velvety, surface and reverse olivaceous. Hyphae septate, simple chlamydospores, cylindrical conidia, pycnidia multiseptate, and hyaline.	*P. sorghina*	PD 88/549	FJ427078	476/479 (99)
LGMF1024	L1	Colonies are floccose, gray and green, reverse orange yellow. Hyphae septate and multiseptate pycnidia.	*E. nigrum*	CBS 115825	FJ426998	423/427 (99)
LGMF1025	L1	Colonies are floccose and white, reverse yellow. Hyphae septate and multiseptate pycnidia.	*E. nigrum*	CBS 115825	FJ426998	487/489 (99)
LGMF1026	L1	Colonies are spreading and sparse aerial mycelium granulose, surface and reverse white. Hyphae septate and beta conidia.	*Diaporthe stewartii*	699518	FJ889448	423/441 (96)
LGMF1051	L1	Colonies with mycelium immersed, branched, and velvety black. Hyphae septate, pycnidia multiseptate, hyaline, and conidia ovoid.	*P. sorghina*	PD 88/549	FJ427078	443/461 (96)
LGMF1036	L2	Colonies are dense, cottony, white, and reverse red. Hyphae septate and chlamydospores globose in chains.	*Gibberella zeae*; *Fusarium brasilicum*	NRRL38371 NRRL31281	DQ459830 DQ459861	456/456 (100)
LGMF1037	L2	Colonies are dense, cottony, white, and reverse yellow. Hyphae septate and chlamydospores globose in chains.	*G. zeae*; *Fusarium boothii*	NRRL38371 NRRL29105	DQ459830 DQ459848	514/514 (100)
LGMF1038	L2	Colonies woolly and gray, reverse white. Hyphae septate and conidia simple, terminal, globose, and hyaline.	*Nigrospora oryzae*	NRRL 54030	GQ28855	458/457 (97)
LGMF1039	L2	Colonies woolly and gray, reverse white. Hyphae septate and conidia simple, terminal, globose, and hyaline.	*N. oryzae*	NRRL 54030	GQ28855	501/529 (96)
LGMF1040	L2	Colonies are spreading and sparse aerial mycelium granulose, surface yellow, and reverse yellow–orange. Hyphae septate.	*Phomopsis camptotheca*	280481	AY622996	529/538 (98)
LGMF1041	L2	Colonies with mycelium immersed, cottony, and olivaceous gray. Reverse pale green.	*Ochrocladosporium frigidarii*; *Ochrocladosporium elatum*	CBS 103.81 CBS 146.33	EU040234 EU040233	417/444 (94)

LGMF1042	L2	Colonies are floccose, yellowish gray, and reverse yellow orange. Hyphae septate and pycnidia and multiseptate pycnidia.	*E. nigrum*	CBS 115825	FJ426998	458/466 (98)
LGMF1043	L2	Colonies are floccose, yellowish gray, reverse yellow orange. Hyphae septate and pycnidia multiseptate, hyaline, and simple chlamydospores.	*E. nigrum*	CBS 115825	FJ426998	438/443 (99)
LGMF1049	L2	Colonies are cottony and white, reverse pink, and white in edge. Hyphae septate, microconidia cylindrical, and abundant.	*Fusarium verticillioides*	7600	X94166	472/472 (100)
LGMF1050	L2	Colonies are cottony and white, reverse yellow with centre pink. Hyphae septate, conidia ovoid, and abundant.	*F. verticillioides*	7600	X94166	423/423 (100)
LGMF1053	L2	Colonies are cottony and white, reverse pink and white in edge. Hyphae septate, microconidia cylindrical, and abundant.	*F. verticillioides*	7600	X94166	476/476 (100)
LGMF1054	L2	Colonies are spreading and sparse aerial mycelium granulose, surface and reverse white. Hyphae septate and Beta conidia.	*Phomopsis chimonanthi*; *Phomopsis micheliae*	280478 280249	AY622993 AY620820	513/528 (97)

*GenBank Accession numbers.

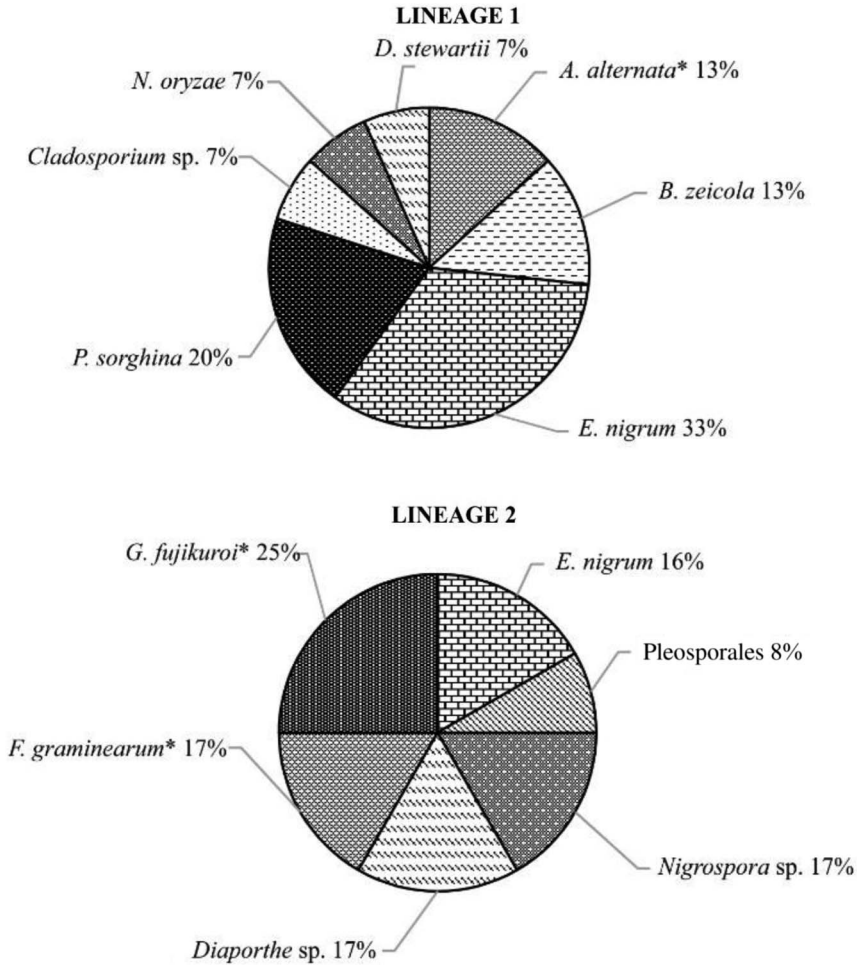

Figure 2. Fungi genera distribution in two lineages of maize according to morphological characterization. *refers to species complex

Capnodiales was the least represented order, with just one morphotype isolated from L1 (Figure 3).

Predominant genera in L1 and L2 are, respectively, *Epicoccum* (33%) and *Fusarium* (anamorfo – *Gibberella*) (42%). L1 totaled six genera and L2 totaled four (Table I and Figure 3).

Shannon-Wiener and Simpson diversity index were $H' = 2.067$ and $1\text{-}D = 0.855$. Comparing L1 and L2 diversity: ($H' = 1.767$ and $1\text{-}D = 0.80$) for L1 and ($H' = 1.594$ and $1\text{-}D = 0.7934$) for L2.

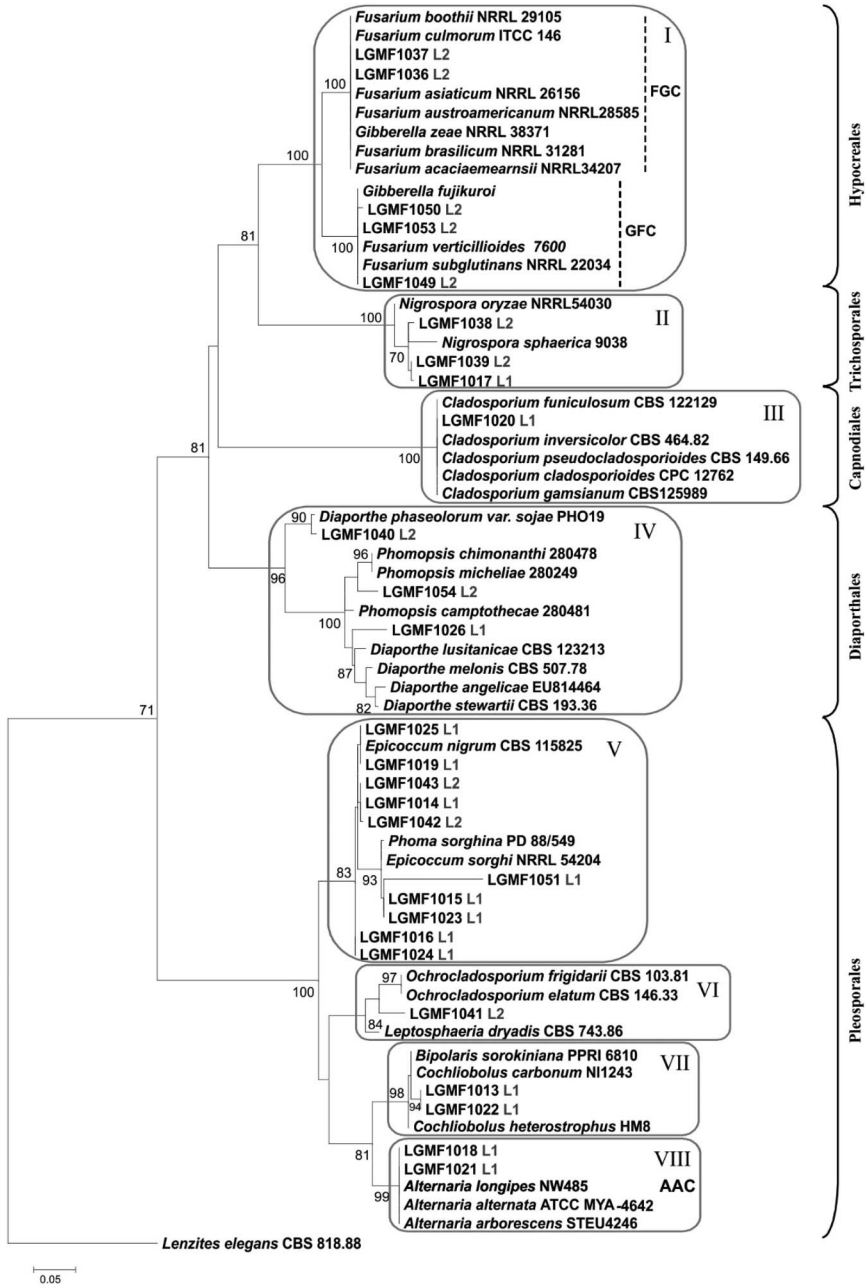

Figure 3. Phylogenetic tree based on ITS1-5.8S-ITS2 sequences concerning fungi isolated in this study and sequences from reference strains obtained from NCBI. Eight groups were set using fragments of 545 bp length. Data were generated using maximum likelihood model and *Lenzites elegans* CBS 818.88 as outgroup. L1 and L2: lineages of maize; AAC, *Alternaria alternata* complex; GFC, *Gibberella fujikuroi* complex; FGC, *Fusarium graminearum* complex

Discussion

Usually, fungi identification involves the analysis of morphological characteristics by microcultures and phylogenetic analysis. These techniques provide more accurate identification results [24]. In this context, ITS1-5.8S-ITS2 region of rDNA sequencing analysis has been successfully used for some taxa. However, some fungi isolated in this study belong to species whose sequences are not available in scientific databases. It may be not an effective identification tool at the species level, but it is very efficient for identification at genus level [25].

We analyzed the fungi diversity (Shannon-Wiener and Simpson diversity index) from two different lineages of maize plant cultivated in the same area. L1 is more diverse in morphotypes, more than half (53%) belongs to *Phoma*/*Epicocum* group. This same group appears in a minor amount L2 (17%). While members of the group *Fusarium* (FGC and GFC) together account for 42% of the total morphotypes in L2 and surprisingly are not present in L1. These differences may be related to the plant genotype and the types of damage caused by diseases present on the leaves, because the two lineages were grown at the same time on the same type of soil and environment.

In this study, the most abundant species was *E. nigrum* (Punith., MC and CM Leach Tulloch 1972), ubiquitous fungus that can present an endophytic lifestyle [26], but is also associated with the primary decomposition of plant tissues [27]. *E. nigrum* has been used as a biological control agent on peaches and nectarines orchards against *Monilinia* spp. [28] and *Pythium cotton* [29].

In addition to *E. nigrum*, two more genera, *Diaporthe/Phomopsis* sp. and *Nigrospora* sp., were found in both L1 and L2. *Nigrospora* genus has not enough sequences deposited in databases like Genbank, where only two species were found with described sequences (http://www.ncbi.nlm.nih.gov). This fact hinders the consolidation of phylogenetic studies about this genus. Some other groups require multigene analysis in order to clearly distinguish their taxonomic position, such *Diaporthe*/*Phomopsis* [3]. In this study, those funguses appear as endophytic, colonizing both lineages of maize plants. Other group of fungi requires multigene analysis to clarify their taxonomy, such as *Cladosporium* genus [30], found in L1, and especially for complex species such as *Cladosporium cladosporioides* [12].

Furthermore, we found *P. sorghina*, *B. zeicola*, and AACs in L1. It has been reported that specimens of *P. sorghina* are morphological and phylogenetically diverse and probably represent multiple species [31]. This is also the agent of *Phaeosphaeria* leaf spot in maize, along with *Phaeosphaeria maydis* [32]. Bipolaris spot caused by *B. zeicola* is common in Brazil with low to medium severity. Currently, in some areas of the Midwest and Northeast, an outbreak of considerably severe diseases is taking place in susceptible cultivars [33]. It is

possible to differentiate *Cochliobolus heterostrophus*, *C. carbonum*, *Cochliobolus victoriae*, *Burkholderia sacchari*, and *Bipolaris sorghicola* species by ITS region sequencing to corroborate our data [34].

AAC is a group with few data on morphological and molecular characteristics, which would allow a clear discrimination between taxa [35]. It is not possible to discern members of this group by the analyses of major mitochondrial ribosomal sub-unit, beta-tubulin, actin, calmodulin, chitin synthase, elongation factor alpha, and 1,3,8-trihydroxynaphthalene reductase [36]. Consequently, it is still unclear how this branch should be phylogenetically classified [35]. *A. alternata* is commonly associated with plants for food production, and may cause fruit deterioration during shipping and storage, for example. This species is responsible for the production of a variety of mycotoxins, like alternariol; alternariol monomethyl ether; altenuene; altertoxins I, II, and III; and L-Tenuazonic acid [10]. *Fusarium* (anamorfo: *Gibberella*) genus, despite being extremely widespread in at least 80% of cultivated plants, is associated with at least one disease caused by one of its species [37] and was found only in L2. Asymptomatic maize plants, showing stalk rot, are infected by *Fusarium moniliforme* and may decrease up to 50% of their photosynthetic capacity. This causes reduction of electron transport components and consequently reduces carbohydrate synthesis, which is possibly caused by toxins produced by fungi [38].

Species from FGC can be characterized by morphological structures and ITS1-5.8S-ITS2 rDNA sequencing but sometimes a more accurate identification is needed [39]. Therefore, species determination has been done by multi-gene analysis, considering up to 13 genes discriminating 13 species: *Fusarium acacia mearnsii*, *Fusarium aethiopicum*, *Fusarium asiaticum*, *Fusarium austroamericanum*, *Fusarium boothii*, *Fusarium brasilicum*, *Fusarium cortaderia*, *Fusarium gerlachii*, *F. graminearum sensu stricto*, *Fusarium meridionale*, *Fusarium mesoamericanum*, *Fusarium ussurianum*, and *Fusarium vorosii* [39]. Members of FGC group cause rotting tip on corn spikes [40]. In addition, *F. graminearum* and *Fusarium culmorum* cause stem rot [41]. All FGC species produce trichothecenes type B toxin [39] and zearalonas (*F. culmorum* and *Fusarium cerealis*) [42]. Most foliar endophytes species belong to Dothideomycetes and Sordariomycetes classes [43]. This represents over 75% of the endophytes described, spread from Arctic to Tropics. Their abundance varies depending on the latitude [44], genetic diversity, host genotype, and on how they are acquired from the environment and on the ability of their individuals to co-colonize hosts [11]. All genera of fungi reported in this study have been associated with maize. Some in vegetative tissues such as *Cochliobolus, Epicoccum, Phoma*, and *Diaporthe* [45] and other in seeds such as *Nigrospora* sp., besides fungi already mentioned [46]. *Alternaria*, *Cladosporium*,

and *Fusarium* are considered soil living fungi, which invade grains at pre- or post-harvest stage and can cause damage in corn before threshing [47]. *Epicoccum* and *Nigrospora* sp. grow up in grains near post-harvest or at storage period, altering grain health. Some problems caused by these fungi are loss of weight/discoloration/necrosis of grains and mycotoxin production, and they are decisive factors for international trade of corn. The presence of mycotoxins, besides being harmful to human and animal health, leads to significant economic losses for farmers [48].

These groups of fungi may also be found in other hosts as medicinal plants [29], trees (*Acer truncatum*) [25]. These reports corroborate with the pogo stick hypothesis, postulating that host-specific fungal pathogens frequently show the ability to colonize non-host tissue, enabling them to disperse further, in an attempt to find the host on which they are pathogenic [3, 49].

Abundance, diversity, and species composition of endophytes are influenced by microhabitat, microclimatic conditions [50], and dynamics of horizontal and vertical transmission of microorganisms [26]. Horizontal transfer may be detected by studying seedlings cultivated under sterile conditions [51]. Vertical transfer was observed in *Fusarium* spp. colonizing seeds of cowpea [52]. In addition, host genotype may influence biodiversity of endophytic fungi and can affect genetic variation of these endophytes [53].

Some endophytes are generalists, being able to infect a wide range of hosts, while others are specialists, limited to one or a few hosts [5]. Some species have been described as either non-harmful exclusive endophytes, or phytopathogenic. However, studies suggest that a significant number of fungi may represent multiple ecological roles. Fungal pathogens may inhabit different hosts at different stages of life, as distinct modes of interaction [54]. Endophytes can take place when leaves reach senescence [55]. For example, *Fusarium verticillioides* is commonly isolated from maize plants, even in the absence of visible symptoms. In this condition, as latent pathogens (endophyte transitory), it can contribute to mycotoxins accumulation [56]. Plants may develop typical disease symptoms only when they go through biotic and abiotic stress situations [57]. In this same context, *Chaetomium globosum* is known as endophyte, saprophytic, or pathogen [43]. Therefore, for some species, the distinction between endophytic, phytopathogenic, and opportunistic microorganisms is purely theoretical. There is a slight threshold dividing them, so it is difficult to establish the limits to separate each category [58].

In this study, it is possible, by molecular phylogeny analysis of the ITS1-5.8S-ITS2 region of rDNA, to identify saprophytic, phytopathogenic, and micotoxic fungal species co-inhabiting leaf tissue of maize plants considering two homozygous genotypes (lineages). It also indicated the necessity to expand studies

about endophytic microbiota associated with this culture. It is essential to better understand the dynamics and risks associated with changes in saprophytic condition that can enable a fungus manifestation as phytopathological, a phenomenon usually triggered by environmental fluctuations.

Conflict of Interest

The authors declare that they have no conflict of interest.

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