

Are rot-causing Botryosphaeriaceae species surviving in healthy *Manihot esculenta* propagative material in Brazil?

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Abstract

The study aimed to verify the presence of latent pathogens from the *Botryosphaeriaceae* family, associated with black root rot and stem dry rot, in healthy propagative material of *Manihot esculenta* in Brazil. To this end, we investigated the composition of endophytic fungal communities present in fresh healthy stems collected from *M. esculenta* plantations. Forty-one genera, two families and one subclass of fungi were identified in the collected samples. We detected the species of *Botryosphaeriaceae* - *Lasiodiplodia euphorbiaceicola*, *L. theobromae* and *Neoscytalidium dimidiatum* - composing fungal endophytic communities in *M. esculenta* plantations close to the coast of the state of Pernambuco (Brazil). Pathogenicity tests carried out on seedlings and roots of *M. esculenta* confirmed the capacity of the *Botryosphaeriaceae* specimens obtained to cause lesions in this host. *L. euphorbiaceicola*, *L. theobromae* and *N. dimidiatum* are recognized as pathogenic for *M. esculenta*, responsible for causing black root rot and stem dry rot in this crop in Brazil and other countries. However, this is the first study to isolate them as endophytes in healthy propagative material, showing evidence that these *Botryosphaeriaceae* can survive as pathogens or endophytes in this crop in Brazil.

INTRODUCTION

Botryosphaeriaceae is one of the six families currently accepted in *Botryosphaeriales* (*Dothideomycetes*, *Ascomycota*) comprising 22 genera that are distributed in different ecosystems, with the exception of the polar zones (Phillips et al., 2019; Batista et al., 2021). These fungi can be found in dead woody tissues as saprobes, in a wide variety of plants as pathogens and endophytes and associated with human infections such as onychomycosis and dermatomycosis (Gonçalves et al., 2016; Gil-González et al., 2021).

Some *Botryosphaeriaceae* species, such as *Lasiodiplodia theobromae*, are recognized as latent pathogens, given that symptoms of diseases caused by them, such as rot, cankers and necrosis, tend to manifest soon after some type of stress is suffered by their host, with water stress being the most common factor (Stanosz et al., 2001; Mohali et al., 2005; Slippers & Wingfield, 2007). The ability of these fungi to colonize plants as endophytes and their lack of host specificity allow them to establish themselves asymptotically in seeds and propagation materials and be transported anywhere in the world (Slippers & Wingfield, 2007; Batista et al., 2021). Such characteristics are worrying, especially in crops such as *Manihot esculenta* that are vegetatively propagated, since in this type of propagation there are greater chances of maintenance, increase and dissemination of diseases (Filho & Mattos, 2006).

Manihot esculenta Crantz, popularly known as cassava, is cultivated in more than 100 countries, in tropical and subtropical zones, with Brazil being one of the largest producers (FAO, 2017). The crop can be affected by several diseases that can be transmitted through its propagation material (stem fragments known as stem cuttings), including stem dry rot and black root rot caused by *Botryosphaeriaceae* (*Lasiodiplodia* spp. and *Neoscytalidium dimidiatum*) (Brito et al., 2020). Stem dry rot is a poorly studied (neglected) in which it is not always possible to clearly visualize the symptoms of the disease in the field, except in the most severe cases. This can lead to a more serious situation, since the same group of pathogens responsible for stem dry rot has also been identified causing symptoms of black rot in *M. esculenta* roots indicating a correlation between these diseases (Msikita et al., 2005; Brito et al., 2020; Sangpueak et al., 2023).

Studies involving the prospection of endophytic fungi in *M. esculenta* are scarce. However, in a recent study carried out with roots of this plant in China, more than half of the endophytic fungi present in the samples belonged to *Botryosphaeriaceae*, with *Lasiodiplodia* being the most abundant (Li et al., 2020). Although the pathogenicity of these isolates has not been tested, it is already known the ability of these fungi, isolated as endophytes, to act as aggressive pathogens when artificially inoculated in plants, whether they are their own hosts or not (Gonçalves et al., 2016).

Knowing that species of *Botryosphaeriaceae* have already been identified as pathogens and endophytes in this host (Machado et al., 2014; Brito et al. 2020; Li et al., 2020), combined with the fact that several members of this family are considered latent pathogens, it is important to carry out studies to identify whether this group of fungi is present in the endophytic community in *M. esculenta* plants in Brazil, and subsequently whether they are related to diseases development in this host. The present study aimed to verify the presence of latent pathogens from the *Botryosphaeriaceae* family, associated with black root rot and stem dry rot, in healthy propagative material of *M. esculenta* in Brazil.

MATERIAL AND METHODS

Sample collection

Between March 2019 and April 2022, healthy stems (propagative material) of *M. esculenta* plants were collected in 12 cultivation areas distributed in the Zona da Mata (ZM), Agreste (AG) and Sertão (SE) mesoregions of the state of Pernambuco, Northeast region of Brazil (Fig. 1). The visited areas in the ZM mesoregion is located within the Atlantic Forest biome and, according to the Köppen classification, have a tropical climate type As. The AG and SE mesoregions are located in an ecoregion of Brazilian tropical dry forests known as Caatinga, where the climate is tropical semiarid type Bsh according to the Köppen classification. The annual temperature average in the collection areas varies between 23.1–25.6°C in ZM, 21.4–24.3°C in AG and 23.2°C in the city of Araripina in SE. Regarding annual precipitation values, the averages in the areas were 1,014–1,536 mm in the ZM, 738–997 mm in the AG and 713 mm in the SE (<https://koppenbrasil.github.io/>). From each plantation, 10 healthy stems were randomly collected from the cultivation area and sent to the Laboratório de Micologia Ambiental, located in the Departamento de Micologia of the Universidade Federal de Pernambuco (Recife, Brazil) for isolation of endophytic fungi.

Isolation and morphological identification of endophytic fungi

The methodology used to isolate endophytic fungi was adapted from Sun et al. (2011) as will be described below. Fifteen fragments (1 cm²) were cut from the inner part of each stem (sample) collected. The fragments were superficially disinfected with 70% ethanol (1 min), 2% NaClO (1 min), 70% ethanol (30s) and washed in sterilized distilled water. Excess moisture from the fragments was removed with sterilized filter paper. For each sample, the 15 fragments were divided into three Petri dishes containing Potato Dextrose Agar (PDA) culture medium supplemented with 0.05 g/L chloramphenicol and observed daily for 30 days for fungal isolation. After growth, the fungi were transferred to Petri dishes with PDA medium and maintained at $\pm 25^{\circ}\text{C}$. The fungal isolates were purified using the hyphal tip technique as described in Brito et al. (2020) and preserved in cryogenic tubes with sterilized distilled water (Castellani, 1967).

Endophytic fungi were identified at the genus level based on the morphological characters of the cultures (appearance, colour, texture, colony size, growth time) and their reproductive structures (ascoma, conidioma, ascospores, conidia, conidiogenic cells, presence of chlamydospores). For this purpose, the fungi were kept on Petri dishes with PDA medium in the dark at $\pm 25^{\circ}\text{C}$ until they sporulated. The slides were mounted with lactoglycerol between the slide and the coverslip for observation under an optical microscope. When necessary, slide cultures were also carried out in Water Agar (WA) culture medium to better visualization of the reproductive structures.

DNA extraction, PCR and sequencing

DNA extractions were performed with isolates that did not sporulate in culture medium and for those that presented cultures with characteristics similar to those of the *Botryosphaeriaceae* family (fast growth, aerial mycelium with grey to black colour and reverse of the Petri dish indigo-grey or black). The genomic DNA of the fungi was extracted from the mycelium of fresh cultures (seven days old), grown in PDA ($\pm 25^{\circ}\text{C}$, in the dark), using the Wizard Genomic DNA Purification Kit (Promega), following the manufacturer's protocol.

Amplification and sequencing of the internal transcribed spacer (ITS) was performed for these fungi using the ITS1/ITS4 primer pair (White et al., 1990). For *Botryosphaeriaceae* isolates, in addition to the ITS region, part of the genic regions of translation elongation factor 1 alpha (*TEF 1- α*) and RNA polymerase II second largest subunit (*RPB2*) were also amplified and sequenced using the EF1F/EF2R (Jacobs et al., 2004) and rpb2-LasF/rpb2-LasR primer pairs (Cruywagen et al., 2017), respectively.

PCR amplifications were performed in 12.5 μL reaction medium containing 6.25 μL of Taq DNA polymerase Master Mix (Cellco®), 0.5 μL of each forward and reverse primer, 4.25 μL of ultrapure water, and 1 μL of DNA sample. PCR conditions for ITS, *TEF 1- α* and *RPB2* are described in Table 1. To observe the purity and size of PCR amplicons, electrophoresis was performed on 1% agarose gels stained with GelRed™ in Tris- EDTA acetate (1x TAE) and the results were visualized under ultraviolet light. DNAClean up Exo + SAP (Cellco®) was used for amplicon purification. Sequencing was performed at Centro de Biociências at the Universidade Federal de Pernambuco (Recife, Brazil). Nucleotide sequences were edited in MEGA v.7 (Kumar et al., 2016) and compared to NCBI sequences using the *BLASTn* tool.

Phylogenetic analyses of *Botryosphaeriaceae*

Botryosphaeriaceae sequences generated in the present study were compared with a set of *Lasiodiplodia* and *Neoscytalidium* sequences obtained from GenBank (<http://www.ncbi.nlm.nih.gov>) (Table 2). Single locus alignments were performed using the MUSCLE® tool (Edgar, 2004) in the MEGA v.7 (Kumar et al., 2016), and then concatenated into the same matrix using MESQUITE v.3.7 (Maddison & Maddison, 2021). The resulting alignments were deposited into TreeBASE (<http://www.treebase.org/>; Submission ID 30285).

Concatenated matrices of the ITS, *TEF1- α* , *RPB2* (*Lasiodiplodia* analysis) and ITS, *TEF1- α* (*Neoscytalidium* analysis) sequences were used to construct phylogenetic trees based on Bayesian Inference (BI) analysis. Nucleotide substitution patterns were selected using MrMODELTEST v.2.3 (Posada & Buckley, 2004) and estimated separately for each genic region based on the Akaike Information Criterion (AIC). The Markov Monte Carlo Chain (MCMC) method was applied for BI analysis over 10,000,000 generations with a tree sampled every 1,000 generations, resulting in 10,000 trees. The first 2,500 trees with the lowest probability values were

discarded from the analyses and later probability values were determined in the consensus tree among the remaining 7,500 trees (Rannala & Yang, 1996). Bayesian Inference analyses were performed using MrBayes v.3.2.7a (Ronquist & Huelsenbeck, 2003) on the CIPRES Science Gateway (<https://www.phylo.org/>) (Miller et al., 2010). *Lasiodiplodia* tree was rooted with *Neodeightonia phoenicum* CBS 122528 and *Neoscytalidium* with *Cophinforma tumefaciens* IMI 76762 and CBS 117451. Representative *Botryosphaeriaceae* isolates were deposited in the public culture collection *Micoteca URM*.

Statistical and ecological analysis

For the analysis of endophytic fungal communities, the difference in the number of genera between the mesoregions studied (ZM, AG and SE) was determined. For the calculation, the isolates were distributed by genus and counted. Data were tested for normality and homogeneity of variance using the Shapiro-Wilk and Bartlett tests, respectively. When the data did not meet these assumptions, even after being transformed, the analysis was performed using the non-parametric Kruskal-Wallis test.

The relative abundance was also calculated by mesoregion, being defined as the ratio between the number of isolates of a given genus by the total number of isolates. For these analyses, the data represented an average of 10 repetitions ($n = 10$) that refer to the 10 samples (= stems) collected in each visited area. The frequency of occurrence (FO) of the genera was estimated according to the equation: $FO = J_i/k$ where, FO = frequency of occurrence of the genus; J_i = number of samples where the genus occurred; k = total number of samples. The following categories were considered based on the frequency of occurrence: dominant with FO > 50%, very common with FO between 31 and 50%, common with FO between 10 and 30%, and rare with FO < 10% (Zhang et al., 2004).

A Permanova analysis, based on the Bray-Curtis distance, was applied using the “*adonis*” function of the “*vegan*” package, to verify if there was a difference in the fungal communities between the mesoregions studied. For this analysis, three samples per area were used, totalling 12 samples per mesoregion. The P value was adjusted by Bonferroni correction for multiple comparisons. Analyses were performed using the R v.3.5.3 system, with a significance level of 5%.

Pathogenicity tests with cassava

The pathogenicity of the *Botryosphaeriaceae* isolates obtained during collections was evaluated in seedlings and *M. esculenta* roots of the Santo Estevão variety (= Recife variety). The tests aimed to verify whether these endophytic fungi were capable of causing lesions when inoculated in their host (*M. esculenta*). For this, the isolates were initially cultivated in Petri dishes with PDA medium for seven days. Then, mycelium discs were removed from the margin of the colonies and transferred to Erlenmeyer flasks containing 50g of parboiled rice and 10 mL of previously autoclaved distilled water (Claudino & Soares, 2014). For the control treatment, Erlenmeyer flasks prepared in the same way were used, but without the mycelium discs. The Erlenmeyer flasks were kept at $\pm 25^\circ\text{C}$ for 15 days to carry out the tests.

Pathogenicity tests on seedlings and roots were performed separately and conducted as described in Brito et al. (2020) with minor modifications. Freshly harvested *M. esculenta* stem cuttings were disinfected in 0.5% NaClO (1 min), washed twice with sterilized distilled water for 1 min each, and planted in autoclaved soil. Seven *M. esculenta* seedlings were used per treatment, which were inoculated close to the basal part, with a colonized rice grain and sterile rice (control) 30 days after the sprouting of the seedlings. These remained in a greenhouse

under natural light for 45 days. The pathogenicity test on *M. esculenta* roots was carried out in the laboratory. For this, five fresh roots previously disinfected in 1% NaClO (5 min) and washed with sterilized distilled water (5 min) were used per treatment. After inserting the colonized rice grain and sterile rice (control) in the central region of the roots with the aid of sterilized tips (Brito et al., 2020), the roots were kept in plastic boxes with moistened cotton for six days.

To evaluate the pathogenicity of the tested isolates, at the end of each test performed the stem of the seedlings and the inoculated *M. esculenta* roots were cut longitudinally to observe the absence/presence of internal lesions. Measurements of the lesions (length) were performed and the results compared using the Scott-Knott test at 5% probability in the R v.4.2.3 software. To confirm that the lesions were caused by the inoculated isolates, fragments of the injured stems and roots were cut and disinfected in 70% ethanol (1 min), 2% NaClO (1 min) and washed in sterilized distilled water. Excess moisture from the fragments was removed with a sterilized filter paper. The fragments were transferred to Petri dishes with PDA + antibiotic (0.05g chloramphenicol) for re-isolation of the fungi used in the tests and closure of Koch's postulates.

RESULTS

Isolation of endophytic fungi

One hundred and sixty-two fungal isolates were obtained from *M. esculenta* healthy stems (78 in ZM, 49 in AG, 35 in SE mesoregion). The identification of the fungi was carried out up to the genus level, thus, it was possible to identify 100 isolates based on morphological characters of the cultures and their reproductive structures, and 57 through DNA sequences of the ITS region using the *BLASTn* tool in the NCBI database. For five isolates it was only possible to identify them in higher taxa than the genus based on ITS region sequences. As fungi belonging to the *Botryosphaeriaceae* family were the focus of the present study, phylogenetic analyses to elucidate the species were carried out only with this group of fungi.

Phylogenetic analyses of Botryosphaeriaceae

Six *Botryosphaeriaceae* isolates were obtained in the present study, four *Lasiodiplodia* and two *Neoscytalidium*. The substitution models selected for BI analysis in *Lasiodiplodia* were HKY + I, HKY + G and GTR + G for ITS, *TEF1-α* and *RPB2*, respectively. For *Neoscytalidium* were HKY for ITS and HKY + I for *TEF1-α*. The concatenated alignment of *Lasiodiplodia* contained 58 taxa with 1273 characters including gaps (460 ITS; 304 *TEF1-α*; 509 *RPB2*), while *Neoscytalidium* included 20 taxa with 683 characters including gaps (413 ITS; 270 *TEF1-α*).

Three isolates of *L. theobromae*, one of *L. euphorbiaceicola* and two of *N. dimidiatum* were identified inhabiting healthy stems in the ZM mesoregion (Figs. 2, 3). The isolates clustered well with species ex-type, supported with high posterior probability values above 0.99. It is possible to observe that the endophytic *Botryosphaeriaceae* isolates from this study also grouped well with the pathogenic *M. esculenta* isolates - *L. theobromae* (ARM28, ARM30, URM8033), *L. euphorbiaceicola* (URM8027, ARM179, ARM191, ARM204, CMM4018, CMM3973, CMM3897) and *N. dimidiatum* (CMM4022, ARM178, ARM203, ARM214, ARM425) - indicating that the isolates are phylogenetically identical according to the studied genic regions.

Pathogenicity tests with *Manihot esculenta*

All *Botryosphaeriaceae* isolates obtained in the present study were evaluated. Thus, seven treatments were performed on *M. esculenta* seedlings and roots: control treatment (CT), *Lasiodiplodia theobromae* ARM 598 (LT_598), *L. theobromae* ARM 599 (LT_599), *L. theobromae* ARM 602 (LT_602), *L. euphorbiaceicola* ARM 601 (LE_601), *Neoscytalidium dimidiatum* ARM 626 (ND_626) and *N. dimidiatum* ARM 628 (ND_628).

In a greenhouse, 45 days after inoculations, it was possible to visualize external lesions on *M. esculenta* seedlings with yellowish to brownish edges around areas with necrosis, close to the point of inoculation in all treatments with *Lasiodiplodia*. In the same period, seedlings treated with *Neoscytalidium* showed large and extensive lesions with ill-defined borders with a yellowish to reddish-brown colour around a conspicuous necrosis.

Treatments LT_599 and LT_602 caused significant brownish internal lesions in all replicates, with the exception of one replicate LT_602, where there was no invasion of the internal part by the fungus. Signs of internal lesions were observed in three replications in seedlings treated with LE_601. Treatments with *Neoscytalidium* ND_626 and ND_628 caused extensive dark brown internal lesions and necrosis. Among all treatments, LT_598 practically did not cause signs of injury to cassava seedlings. Only two repetitions of this treatment showed a very small lesion limited to the point of inoculation. The control treatment remained healthy throughout the experiment (Fig. 4).

The *M. esculenta* roots were evaluated in the laboratory, six days after the inoculations. All CT repeats remained intact with no signs of contamination. In the LT_598 treatment, fungal growth was limited to the region of the inoculated rice, where it caused dark brown to black lesions inside the roots. In the other treatments with *Lasiodiplodia* (LT_599, LT_601, LT_602), the root bark was colonized and the formation of fungal reproductive structures could be observed in some repetitions of the treatments. Inside the roots, yellowish and brownish spots with no defined pattern were observed. In the roots treated with *Neoscytalidium* (ND_626 and ND_628) it was possible to observe a pattern of internal lesion from the points of inoculation. The lesions were uniform with a dry appearance and brownish colour. Mycelial growth was also observed on the root bark with formation of fungal reproductive structures.

The statistical result of the pathogenicity test on *M. esculenta* seedlings showed that all treatments, with the exception of LT_598 and LE_601, differed from CT. *Neoscytalidium dimidiatum* ARM 626 was the treatment that showed the greatest extent of lesions on the seedlings differing significantly from the other treatments (Fig. 5). Due to the lack of lesion pattern in the treatments with *Lasiodiplodia* in roots, it was not possible to statistically measure and compare all treatments to evaluate which was the most aggressive and if there were significant differences between them. All isolates used in the pathogenicity tests on *M. esculenta* seedlings and roots were re-isolated at the end of the experiments, with the exception of the isolate *L. theobromae* ARM 598 (LT_598).

Life cycle of *Botryosphaeriaceae* in *Manihot esculenta* plantations

From the confirmation of the presence of *Botryosphaeriaceae* species – *L. euphorbiaceicola*, *L. theobromae* and *N. dimidiatum* – as endophytes in healthy *M. esculenta* plants and their background as pathogens responsible for black root rot and stem dry rot in this crop, we propose a life cycle of these species in *M. esculenta* plantations in Brazil based on existing information on the ecology of this group of pathogens in other economically important crops (Fig. 6). The conidia formed in the pycnidia are released into the environment and dispersed by rainwater and wind, reaching healthy *M. esculenta* plants and starting the infection process in their

new host. After the infection process, these fungi may begin to develop symptoms of stem dry rot (cracks in the stem with black masses, brownish lesions progressing to black in the inner part) and black root rot (roots with dry, yellowish-brown to black internal lesion) or establish themselves latently (asymptotically) in stems of *M. esculenta*.

As fragments of the stems are used as propagative material for this crop, these fungi can be disseminated during the *M. esculenta* planting season on the property or be transported to new cultivation areas asymptotically in the propagative material. When healthy plants latently infected with *L. euphorbiaceicola*, *L. theobrome* and *N. dimidiatum* undergo water stress conditions, disturbing the physiological balance of this plants, these species leave the state of latency and begin to develop symptoms of the diseases already mentioned.

Crop residues (stems and diseased roots) left in the field serve as a source of inoculum for these pathogens, allowing them to survive in the plantation and infect weeds and other hosts present in the area, which also begin to act as a source of inoculum *L. euphorbiaceicola*, *L. theobrome* and *N. dimidiatum*. Insects and sharp materials used in cultivation can also disperse these fungi in the planting area. Whether surviving on cultural remains or on other hosts, these species can reinfect *M. esculenta* plants, restarting the cycle of these fungi and the diseases stem dry rot and black root rot in the crop. Black root rot in *M. esculenta* is often not observed in the field, so apparently healthy roots will be sold at fairs and markets, however, in this post-harvest phase it is possible to observe signals and symptoms of these fungi on the roots (dark grey to black mycelium growing on root tips, necrotic areas on the bark, black dots or yellowish-brown lesions inside the tuber).

Ecological analysis

Forty-one genera, two families and one subclass were identified as endophytic fungi in *M. esculenta* healthy stems. For five isolates it was only possible to identify them in higher taxa than the genus based on ITS region sequences. For this reason, these isolates were not used in the ecological analyses. Most of the isolates obtained are inserted in *Ascomycota*, distributed mainly between the classes *Sordariomycetes* and *Dothideomycetes*. *Diaporthe* and *Nigrospora* were the most abundant genera followed by *Preussia* and *Xylaria*. In addition to being more abundant, *Diaporthe*, *Nigrospora* and *Xylaria* were the only genera present in the samples from the three mesoregions studied.

Zona da Mata had the highest number of endophytic fungal genera, differing significantly from AG and SE ($p < 0.05$). By mesoregion *Nigrospora* (26%) and *Diaporthe* (35%) were the most abundant genera in ZM and AG, respectively. In SE, *Microascus* (12%) and *Purpureocillium* (12%) were the most abundant. As for the frequency of occurrence, *Nigrospora* was considered very common (50%) in the ZM, *Diaporthe* was very common (42.5%) in the AG and *Microascus* and *Purpureocillium* were found only in SE stems and their frequencies were considered common (10%). The frequency of occurrence of *Botryosphaeriaceae* in ZM mesoregion was common for *Lasiodiplodia* (10%) and rare for *Neoscytalidium* (5%) (Table 3). By Permanova analysis, it was possible to verify that there was a significant difference in the composition of the fungal genera of the endophytic communities between the ZM and the SE ($p < 0.05$).

DISCUSSION

Manihot esculenta Crantz is one of the main sources of staple food in the world and serves as a raw material for different industrial sectors (Howeler, 2011; Aguiar et al., 2013). Despite its relevance in the national scenario, information on the composition of endophytic fungal communities in Brazil is scarce (Pereira et al., 2022). For the first time *Lasiodiplodia* and *Neoscytalidium* (both genera belonging to the *Botryosphaeriaceae* family) are being identified as part of endophytic communities in *M. esculenta* plantations in Brazil.

Among *Botryosphaeriaceae*, only *Lasiodiplodia* has already been described in *M. esculenta* endophytic communities on roots in China and Indonesia (Li et al., 2020; Hartanti et al., 2021). In the present study, *L. theobromae*, *L. euphorbiaceicola* and *N. dimidiatum* were isolated as an endophytes in healthy stems. These three species are associated with stem dry rot and black root rot in this plant in Brazil and other countries (Msikita et al., 2005; Vilas Boas et al., 2017; Brito et al., 2020; Sangpueak et al., 2023). Probably these diseases are related to each other, as both are caused by the same group of pathogens *Botryosphaeriaceae* (*Lasiodiplodia* spp. and *N. dimidiatum*) and they can lead to serious productivity losses in *M. esculenta* crop (Vilas Boas et al., 2017; Brito et al., 2020; Sangpueak et al., 2023).

The identification of these species in healthy stems of *M. esculenta* is relevant because even isolated as endophytes, these species had their pathogenicity proven when inoculated in *M. esculenta* seedlings and roots in the present study as observed in pathogenicity tests performed with species of this family in other hosts (Gonçalves et al., 2016). In addition, stem cuttings are the propagation material for *M. esculenta* and can be easily transported to areas other than its point of origin. As human action is primarily responsible for the long-distance dispersal of *Botryosphaeriaceae* (Batista et al., 2021), these species can easily be taken to new *M. esculenta* areas and, under unfavourable conditions for the plant, develop diseases in this crop (Slippers & Wingfield, 2007), such as stem dry rot and black root rot.

Despite the identification of the causal agents, the epidemiology of these disease as well as the factors that contribute to the appearance of these *Botryosphaeriaceae* in *M. esculenta* still needs to be better understood. In view of this and the identification of *Lasiodiplodia* spp. and *N. dimidiatum* as endophytes in *M. esculenta* in the present study and knowing the ability of *Botryosphaeriaceae* species to behave as latent pathogens in their hosts we proposed a life cycle of *L. theobromae*, *L. euphorbiaceicola* and *N. dimidiatum* in *M. esculenta* plantations based on information obtained in previous studies about the behaviour of this group of pathogens in other economically relevant hosts (Bragard et al., 2023).

From the infection of these species in plants of *M. esculenta*, depending on, for example, the susceptibility of the plant to diseases and your physiological condition, these fungi may begin to exhibit symptoms of stem dry rot and black root rot visible in the aerial part of the plant in the field or establish themselves as "endophytes" thus remaining in latency until the plant undergoes water stress to the point that allows the proliferation of these fungi and begin to cause damage to the plant. In the case of stem dry rot, it is possible to see the sporulation of these *Botryosphaeriaceae* through cracks that open in the stem, through which the reproductive structures of these fungi, covered by black stroma, appears. Black root rot, on the other hand, is more difficult to be seen in the field (Msikita et al., 2005), so in non-severe attacks the roots are harvested and marketed because they have a healthy appearance on the outside. However, when opened it is possible to observe signs of infection caused by these fungi.

One of the reasons to be aware of *L. theobromae* in endophytic communities in *M. esculenta* is that this species is considered a latent pathogen, capable of establishing itself as an endophyte in its hosts, and in conditions of plant stress (drought and heat) this can activate its pathogenic behaviour causing “sudden” outbreaks and the rapid development of some diseases (Mohali et al., 2005). Studies revealed a high expression of pathogenicity-related genes acting on the degradation of the plant cell wall, carbohydrate catabolism, necrosis-inducing proteins, among others, during the infection process of *L. theobromae* in its hosts, as well as the mechanisms used by the species to evade the defence of its hosts (Gonçalves et al., 2019; Ali et al., 2020).

In addition to their ability to survive as endophytes and cause *M. esculenta* rots demonstrated in this study, another reason to be aware of the presence of *L. theobromae*, *L. euphorbiaceicola*, *N. dimidiatum* as part of endophytic fungal communities on plants, is related to the range of genes related to pathogenicity that these fungi can express induced by increasing temperature, as observed for *L. theobromae* (Félix et al., 2019; Nagel et al., 2022). The modulation of the pathogenicity of this species by temperature can represent a potential risk to *M. esculenta* plants, in view of the increase in global temperature and climate change. Therefore, the behaviour of these endophytic *Botryosphaeriaceae* in scenarios of drought and increased temperature needs to be studied in *M. esculenta* cultivation in Brazil to better understand the epidemiology of stem dry rot and black root rot caused by these fungi in this crop.

Due to the morphological characteristics of the species - it presents two types of conidia morphology (one *Fusicoccum*-like and the other *Scytalidium*-like) - for many years in Brazil *N. dimidiatum* was mistakenly identified as *Scytalidium lignicola* (*Helotiales*, *Leotiomyces*) in *M. esculenta* roots and stems with black root rot and stem dry rot, respectively (Machado et al., 2014). In the present study, the endophytic isolates of *N. dimidiatum* grouped well with the ex-type of the species (CBS 499.66) and other isolates previously identified as pathogenic to *M. esculenta*. Despite the formation of a clade/subclade with posterior probability value equal to or greater than 0.98, all isolates were considered as *N. dimidiatum* due to recent synonymization that reduced the genus to a single species (Zhang et al., 2021). In addition to our study, little information was found about *N. dimidiatum* composing endophytic communities (Aghdam & Fotouhifar, 2017).

The pathogenicity tests showed that all *Botryosphaeriaceae* endophytic species were pathogenic to *M. esculenta* seedlings and/or roots, reinforcing the theory that these species are latent pathogens in this plant. Although lesions caused by *N. dimidiatum* were larger, only *N. dimidiatum* ARM 626 differed statistically from two treatments with *L. theobromae* (ARM 599, ARM 602). In a pathogenicity test with *Botryosphaeriaceae* pathogens related to black root rot and stem dry rot conducted by Brito et al. (2020), *N. dimidiatum* was one of the most aggressive species, even invading and necrosing the main stem cutting of the seedlings. In the same way as in Brito et al. (2020), it was also not possible to measure the length of the lesions on the roots in the present study, as the *Lasiodiplodia* isolates quickly colonized the external part and caused yellowing in the internal part of the roots. This symptom may be related to the expression of genes involved in the catabolism of starch, a carbohydrate that *M. esculenta* roots have in large quantities (Yan et al., 2018). Roots treated with *N. dimidiatum* presented internally standardized lesions that varied only in length between repetitions.

In the present study, we identified 41 genera of fungi composing endophytic communities of healthy stems of *M. esculenta* in plantations distributed across the state of Pernambuco (Brazil). Despite the use of DNA sequences from the ITS region, five isolates remained unidentified at the genus level. Comparing our sequences with those deposited in the GenBank database, it was only possible to identify the family or class of these fungi. This result

shows that such isolates are probably taxonomic novelties within the *Phaeosphaeriaceae* family and the *Xylariaceae* order.

The number of isolates recovered from *M. esculenta* stems gradually decreased as the collection areas moved away from the ZM, located in the Atlantic Forest ecoregion, towards the SE mesoregion, in the Caatinga ecoregion of the state of Pernambuco. Higher humidity and precipitation may have favoured sporulation and fungal infection in *M. esculenta* plantations located in ZM mesoregion, while ultraviolet radiation together with lower rainfall and humidity may have negatively influenced fungi in SE mesoregion, decreasing sporulation and infection of endophytic fungi in plants in this area as observed in previous studies with other hosts in the same Brazilian state (Pádua et al., 2019; Silva et al., 2023).

This work is part of a larger study on species of the *Botryosphaericeae* family in *M. esculenta* plantations in Brazil, seeking to better understand the ecology of these species and the epidemiology of the diseases they cause and that impact culture in the country and around the world. In this way, the identification of latent pathogens of *Botryosphaeriaceae* - *L. theobromae*, *L. euphorbiaceicola*, *N. dimidiatum* - in communities of endophytic fungi in Brazilian *M. esculenta* plantations indicates the need for a more detailed look at the fungal communities associated with this crop, in view of the relationship between these species with cassava diseases (stem dry rot/black root rot).

Declarations

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are deposited in GenBank (<https://www.ncbi.nlm.nih.gov/genbank/>), concatenated alignment is deposited in TreeBASE (<http://www.treebase.org/>). The representative isolates of the *Botryosphaeriaceae* species have been deposited in public culture collection *Micoteca URM* located at the Universidade Federal de Pernambuco (Recife, Brazil).

Compliance with ethical standards

Disclosure of potential conflicts of interest: The authors declare that they have no conflict of interest.

Financial interests: The authors declare they have no financial interests.

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References

1. Aghdam, S.A. & Fotouhifar, K. B. (2017). Introduction of some endophytic fungi of sour cherry trees (*Prunus cerasus*) in Iran. *Rostaniha*, 18, 77-94.
2. Aguiar, J. L. P., Sousa, T. C. R., Lôbo, C. F. (2013). A importância da mandioca. In: Fialho, J. F. & Vieira, E.A. (Eds.), *Mandioca no Cerrado: orientações técnicas* (2th ed., pp. 14 – 26). Brasília: Embrapa Cerrados.

3. Ali, S. S., Asman, A., Shao, J., Balidion, J. F., Strem, M. D., Puig, A. S., Meinhardt, L. W. & Bailey, B. A. (2020). Genome and transcriptome analysis of the latent pathogen *Lasiodiplodia theobromae*, an emerging threat to the cacao industry. *Genome*, v. 63(1), 37-52.
4. Batista, E., Lopes, A. & Alves, A. (2021). What Do We Know about *Botryosphaeriaceae*? An Overview of a Worldwide Cured Dataset. *Forests*, 12, 313.
5. Bragard, C., Baptista, P., Chatzivassiliou, E., Di Serio, F., Gonthier, P., Jaques Miret, J.A., Justesen, A.F., MacLeod, A., Magnusson, C.S., Milonas, P., Navas-Cortes, J.A., Parnell, S., Potting, R., Stefani, E., Thulke, H-H, Van der Werf, W., Civera, A.V., Yuen, J., Zappala, L., Migheli, Q., Vloutoglou, I., Maiorano, A., Streissl, F. & Reignault, P.L. (2023). Scientific Opinion on the pest categorisation of *Lasiodiplodia pseudotheobromae*. *EFSA Journal*, 21(1), 7737. <https://doi.org/10.2903/j.efsa.2023.7737>
6. Brito, A.C.Q., Mello, J.F., Câmara, M.P.S., Vieira, J.C.B., Michereff, S.J., Souza-Motta, C.M.S. & Machado, A. R. (2020). Diversity and pathogenicity of *Botryosphaeriaceae* species associated with black root rot and stem cutting dry rot in *Manihot esculenta* in Brazil. *European Journal of Plant Pathology*, 157, 583-598.
7. Castellani, A. (1967). A maintenance and cultivation of the common pathogenic fungi of man in sterile distilled water. Further researches. *The American Journal of Tropical Medicine and Hygiene*, 70, 181–184.
8. Claudino, M.R. & Soares, D.J. (2014). Pathogenicity and aggressiveness of *Macrophomina phaseolina* isolates to castor (*Ricinus communis*). *Tropical Plant Pathology*, 39, 453–456.
9. Cruywagen, E.M., Slippers, B., Roux, J. & Wingfield, M.J. (2017). Phylogenetic Species Recognition and hybridisation in *Lasiodiplodia*: A case study on species from baobabs. *Fungal Biology*, 121(4), 420–436.
10. Edgar, R.C. (2004). MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research*, 32, 1792–1797.
11. FAO (2017) *Food outlook: Biannual report on global food markets November 2017*. Available at: <http://www.fao.org/3/a-l8080e.pdf>. [Accessed 9 may 2018].
12. Félix, C., Meneses, R., Gonçalves, M.F.M., Tilleman, L., Duarte, A.S., Jorrín-Novo, J.V., Van de Peer, Y., Deforce, D., Nieuwerburgh, F. V., Esteves, A. C. & Alves, A. (2019). A multi-omics analysis of the grapevine pathogen *Lasiodiplodia theobromae* reveals that temperature affects the expression of virulence and pathogenicity-related genes. *Sci Rep*, 9, 13144.
13. Filho, J.R.F. & Mattos, P.L.P. (2006). Seleção e Preparo do Material de Plantio. In: Mattos, P.L.P, Farias, A.R.N. & Filho, J.R.F. (Eds.), *Mandioca: o produtor pergunta, a Embrapa responde* (pp. 73–78). Brasília: Embrapa Informação Tecnológica.
14. Gil-González, M., Gómez-Velásquez, J.C., Loaiza-Díaz, N., Florez-Muñoz, S.V., Hernández-Herrera, G.N., Mesa-Arango, A.C. (2021). Onychomycosis caused by the environmental mold *Neoscytalidium dimidiatum* in Colombia, and *in vitro* antifungal susceptibility evaluation. *Medical Mycology*, 59(6), 634–637.
15. Gonçalves, F.J.T., Freire, F.C.O., Lima, J.S., Melo, J.G.M., Câmara, M.P.S. (2016). Patogenicidade de espécies de *Botryosphaeriaceae* endofíticas de plantas da Caatinga do estado do Ceará em manga e umbu-cajá. *Summa Phytopathol.*, 42(1), 43-52.
16. Gonçalves, M.F.M., Nunes, R.B., Tilleman, L., Van de Peer, Y., Deforce, D., Nieuwerburgh, F.V., Esteves, A. C. & Alves, A. (2019). Dual RNA Sequencing of *Vitis vinifera* during *Lasiodiplodia theobromae* Infection Unveils Host–Pathogen Interactions. *Int. J. Mol. Sci.*, 20(23), 6083.

17. Hartanti, A. T., Susanti, F. N., Prasasty, V. D., Radiastuti, N. (2021). Culturable endophytic fungal diversity in cassava tubers of Indonesia. *Biodiversitas*, 22, 1250-1260.
18. HOWELER, R. H. Recent trends in production and utilization of cassava in Asia. (2011). In: Howeler, R. H (Ed.), *The Cassava Handbook. A Reference Manual based on the Asian Regional Cassava Training Course, held in Thailand* (pp.1 – 22). Centro Internacional de Agricultura Tropical (CIAT).
19. Jacobs, K., Bergdahl, D.R., Wingfield, M.J., Halik, S., Seifert, K.A., Bright, D.E. & Wingfield, B. D. (2004). *Leptographium wingfieldii* introduced into North America and found associated with exotic *Tomicus piniperda* and native bark beetles. *Mycological Research*, 108, 411–418.
20. Kumar, S., Stecher, G. & Tamura, K. (2016) MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for Bigger Datasets. *Molecular Biology and Evolution*, 33(7), 1870–1874.
21. Li, H., Yan, C., Tang, Y., Ma, X., Chen, Y., Chen, S., Lin, M. & Liu, Z. (2020). Endophytic bacterial and fungal microbiota in different cultivars of cassava (*Manihot esculenta* Crantz). *Journal of Microbiology*, 58, 1–10.
22. Machado, A.R., Pinho, D.B., Oliveira, S.A.S. & Pereira, O.L. (2014). New occurrences of *Botryosphaeriaceae* causing black root rot of cassava in Brazil. *Tropical Plant Pathology*, 39(6), 464-470.
23. Maddison, W. P. & Maddison, D. R. (2021). *Mesquite: a modular system for evolutionary analysis v. 3.70*. Available at: <http://www.mesquiteproject.org>. [Accessed 14 October 2022].
24. Miller, M.A., Pfeiffer, W. & Schwartz, T. (2010). Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In: *Proceedings of the Gateway Computing Environments Workshop (GCE)* (pp 1–8). New Orleans.
25. Mohali, S., Burgess, T.I. & Wingfield, M.J. (2005). Diversity and host association of the tropical tree endophyte *Lasiodiplodia theobromae* revealed using simple sequence repeat Markers. *For. Path.*, 35, 385–396.
26. Msikita, W., Bissang, B., James, B. D., Baimey, H., Wilkinson, H. T., Ahounou, M. & Fagbemissi, R. (2005). Prevalence and severity of *Nattrassia mangiferae* root and stem rot pathogen of cassava in Bénin. *Plant Dis.* 89, 12-16.
27. Nagel, J.H., Wingfield, M.J. & Slippers, B. (2021). Increased abundance of secreted hydrolytic enzymes and secondary metabolite gene clusters define the genomes of latent plant pathogens in the *Botryosphaeriaceae*. *BMC Genomics*, 22(1), 589.
28. Pádua, A.P.S.L, Freire, K.T.L.S., Oliveira, T.G.L., Silva, L.F., Araújo-Magalhães, G.R., Agamez-Montalvo, G.S. et al. (2019). Fungal endophyte diversity in the leaves of the medicinal plant *Myracrodruon urundeuva* in a Brazilian dry tropical forest and their capacity to produce L-asparaginase. *Acta Bot. Bras.* 33(1). <https://doi.org/10.1590/0102-33062018abb0108>
29. Pereira, V. M., Diniz, F. V., Costa, N. A., Vasconcelos, J.M., Araujo, A. V. & Carvalho, C. M. (2022). Community Of Cultivable Endophytic Fungi Of Cassava *Manihot esculenta* Crantz. *Desafios - Revista Interdisciplinar Da Universidade Federal Do Tocantins*, 9(1), 42–51. <https://doi.org/10.20873/uftv9-10244>
30. Phillips, A.J.L., Hyde, K.D., Alves, A. & Liu, J-K. (2019). Families in *Botryosphaeriales*: a phylogenetic, morphological and evolutionary perspective. *Fungal Diversity*, 94, 1–22.
31. Posada, D. & Buckley, T.R. (2004). Model selection and model averaging in phylogenetics: advantages of Akaike information criterion and Bayesian approaches over likelihood ratio tests. *Systematic Biology*, 53, 793–808.

32. Rannala, B. & Yang, Z. (1996). Probability distribution of molecular evolutionary trees: a new method of phylogenetic inference. *Journal of Molecular Evolution*, 43, 304–311.
33. Rivera, M. F., Laberry, R. & Lozano, J. C. (1993). Evidences for Endophytes Parasiting Traditional Clones of Cassava (*Manihot esculenta* Crantz). In: Roca WM, Thro AM (Eds.), *Proceedings of the first international scientific meeting of the cassava Biotechnology network, Cartagena, Colombia 25–28 August 1992* (pp. 335–339). Centro Internacional de Agricultura Tropical (CIAT).
34. Ronquist, F. & Heulsenbeck, J.P. (2003). MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, 19, 1572–1574.
35. Sangpueak, R., Duchanee, S., Saengchan, C., Papathoti, N. K., Hoang, N. H., Thanh, T.L., Phansak, P. & Buensanteai, N. (2023). Identification of cassava black stem and root rot agents in Thailand. *Chilean Journal Of Agricultural Research*, 83(1), 70-82.
36. Silva, R. M. F., Neto, W. P., Oliveira, R. J., Bezerra, J. D. P, Bezerra, J. L., Lima, V. X., Vieira, L. C., Tabosa, J. N., Souza-Motta, C. M. & Silva, G. A. (2023). Effect of climate and phenological stage on fungal endophytes community in *Sorghum bicolor* leaves. *Mycol Progress* 22, 19. <https://doi.org/10.1007/s11557-023-01870-z>
37. Slippers, B. & Wingfield, M. J. (2007) *Botryosphaeriaceae* as endophytes and latent pathogens of woody plants: diversity, ecology and impact. *Fungal Biology Reviews*, 21(2), 90–106.
38. Stanosz, G.R., Blodgett, J.T., Smith, D.R. & Kruger, E.L. (2001). Water stress and *Sphaeropsis sapinea* as a latent pathogen of red pine seedlings. *New Phytologist*, 149, 531–538.
39. Sun, X., Guo, L.D. & Hyde, K.D. (2011). Community composition of endophytic fungi in *Acer truncatum* and their role in decomposition. *Fungal Diversity*, 47, 85–95.
40. Vilas Boas, S.A., Oliveira, S.A.S, Bragança, C.A.D., Ramos, J.B & Oliveira, E.J. (2017). Survey of fungi associated with cassava root rot from different producing regions in Brazil. *Sci. Agric.*, 74(1), 60-67.
41. White, T.J., Bruns, T., Lee, S. & Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M.A., Gelfand, D.H., Sninsky, J.J. & White, T.J. (Eds.) *PCR protocols: a guide to methods and applications*. Academic Press, San Diego, USA, pp. 315–322.
42. Yan, J.Y., Zhao, W.S., Chen, Z., Xing, Q.K., Zhang, W., Chethana, K.W.T. et al. (2018). Comparative genome and transcriptome analyses reveal adaptations to opportunistic infections in woody plant degrading pathogens of *Botryosphaeriaceae*. *DNA Research*, 25, 87–102.
43. Zhang, Y., Gui, L.D. & Liu, R.J. (2004). Survey of arbuscular mycorrhizal fungi in deforested and natural forest land in the subtropical region of Dujiangyan, southwest China. *Plant Soil*, 261, 257–263.
44. Zhang, W., Groenewald, J.Z., Lombard, L., Schumacher, R.K., Phillips, A.J.L. & Crous, P.W. (2021). Evaluating species in *Botryosphaeriales*. *Persoonia*, 46, 63–115.

Tables

Table 1. Primer combinations and PCR conditions used in the present study for amplification and sequencing.

<i>Locus</i>	<i>Primers</i>	<i>Primers reference</i>	<i>PCR conditions</i>
ITS	ITS1 ITS4	White et al. (1990)	Initial denaturation at 94 °C (5 min); 30 cycles of denaturing at 94 °C (30s), annealing at 52 °C (40s), and elongation at 72 °C (40s); final extension at 72°C (5 min).
<i>TEF1-α</i>	EF1F EF2R	Jacobs et al. (2004)	Initial denaturation at 95 °C (5 min); 40 cycles of denaturation at 94°C (50s), annealing at 56°C (50s), and elongation at 72°C (50s); final extension at 72 °C (5 min).
<i>RPB2</i>	RPB2-LasF RPB2-LasR	Cruywagen et al. (2017)	Initial denaturation at 95 °C (2 min); 35 cycles of denaturation at 94 °C (30s), annealing at 54 °C (30s), and elongation at 72 °C (1 min); final extension at 72°C (8 min).

Table 2. GenBank accession numbers of DNA sequences used in phylogenetic analyses.

Botryosphaeriaceae isolates obtained in the present study are highlighted in bold. Asterisks (*) indicate ex-type species.

Species (current name)	Species (previously name)	Isolate	GenBank accession numbers		
			ITS	<i>TEF1-α</i>	<i>RPB2</i>
<i>Botryosphaeria corticis</i>		CBS 119047*	DQ299245	EU017539	–
		ATCC 22927	DQ299247	EU673291	–
<i>Botryosphaeria dothidea</i>		CBS 115476*	KF766151	AY236898	–
		CBS 110302	AY259092	AY573218	–
<i>Cophinforma tumefaciens</i>		IMI 76762*	MW810287	MZ073950	–
	<i>C. atrovirens</i>	CBS 117451	KX464086	KX464556	–
<i>Lasiodiplodia avicenniae</i>		CMW 41467*	KP860835	KP860680	KU587878
		LAS 199	KU587957	KU587947	KU587880
<i>L. brasiliense</i>		CMM4015*	JX464063	JX464049	–
		URM 8035	MK480489	MK495398	MN886578
		ARM 353	MK480490	MK495399	MN886579
		ARM 356	MK480491	MK495400	MN886580
		ARM 357	MK480492	MK495401	MN886581
<i>L. bruguierae</i>		CMW 41470*	KP860833	KP860678	KU587875
		CMW 42480	KP860832	KP860677	KU587876
<i>L. crassispota</i>		CBS 118741*	DQ103550	DQ103557	KU696353
		CMW 13488	DQ103552	DQ103559	KU696352
12. euphorbiaceicola		CMM3609*	KF234543	KF226689	–
		URM 8027	MK480479	MK495377	MN935461
		ARM 179	MK480482	MK495381	MN935463
		ARM 191	MK480484	MK500928	MN935465
		ARM 204	MK480485	MK495385	MN886574
		CMM4018	KF369268	KF553901	–
		CMM3973	KF369267	KF553900	–
		CMM3897	KF369266	KF553899	–
		URM 8641 = ARM 601	OP832620	OP866772	OP866778

<i>L. hormozganensis</i>		CBS 124709*	GU945355	GU945343	KU696361
		URM 8029	MK480481	MK500926	MN935462
		ARM410	MK480494	MK495408	MN886583
<i>L. huangyanensis</i>		CGMCC3.20380*	MW880674	MW884177	MW884151
		CGMCC3.20381	MW880675	MW884178	MW884152
<i>L. iranensis</i>		CBS 124710*	GU945348	GU945336	KU696363
		URM 8026	MK480473	MK500923	MN886570
<i>Lasiodiplodia laeliocattleyae</i>		CBS 130992*	JN814397	JN814424	KU696354
		ARM 29	MK480475	MK495373	MN935459
		URM 8028	MK480480	MK495378	MN886573
<i>L. linhaiensis</i>		CGMCC 3.20386*	MW880677	MW884180	MW884154
		CGMCC 3.20383	MW880678	MW884181	MW884155
<i>L. mahajangana</i>	<i>L. caatinguensis</i>	CMM 1325*	KT154760	KT008006	–
	<i>L. caatinguensis</i>	URM 8031	MK480483	MK500927	MN935464
	<i>L. caatinguensis</i>	ARM 207	MK480486	MK500929	MN886575
<i>L. margaritacea</i>		CBS 122519*	EU144050	EU144065	KU696367
		CBS 122065	EU144051	EU144066	–
<i>L. mediterranea</i>		CBS 137783*	KJ638312	KJ638331	KU696368
		CBS 137784	KJ638311	KJ638330	KU696369
<i>L. parva</i>		CBS 456.78*	EF622083	EF622063	KU696372
<i>L. parva</i>		ARM 33	MK480477	MK495375	MN935460
		ARM 432	MK480495	MK495415	MN886584
		ARM 459	MK480496	MK495419	MN886585
<i>L. pseudotheobromae</i>		CBS 116459*	EF622077	EF622057	KU696376
		URM 8034	MK480488	MK500931	MN886577
		CMM3894	KF369264	KJ452244	–
<i>L. rubropurpurea</i>		CBS 118740*	DQ103553	DQ103571	KU696380
		WAC12536	DQ103554	DQ103572	KU696381
<i>L. theobromae</i>		CBS 164.96*	AY640255	AY640258	KU696383
		ARM 28	MK480474	MK495372	MN886571

	ARM 30	MK480476	MK495374	MN886572
	URM 8033	MK480487	MK495388	MN886576
	ARM 598	OP832618	OP866770	OP866776
	ARM 599	OP832619	OP866771	OP866777
	URM 8642 = ARM 602	OP832621	OP866773	OP866779
<i>L. venezuelensis</i>	CBS 118739*	DQ103547	DQ103568	KU696384
	WAC 12540	DQ103548	DQ103569	–
<i>Neodeightonia phoenicum</i>	CBS 122528*	EU673340	EU673309	KX463999
<i>Neoscytalidium dimidiatum</i>	CBS 499.66*	AY819727	EU144063	–
	URM 8643 = ARM 626	OP832622	OP866774	–
	ARM 628	OP832623	OP866775	–
	CMM4022	KF369269	KF553902	–
	CMM3895	KF369265	KF553898	–
	URM 8030	MK461880	MK464022	–
	ARM 178	MK461881	MK464023	–
	ARM 203	MK480467	MK495384	–
	ARM 214	MK480469	MK495387	–
	ARM 219	MK480470	MK495389	–
	ARM 364	MK480471	MK495404	–
	ARM 425	MK480472	MK495413	–
<i>N. novahollandiae</i>	CBS122070	EF585539	EF585579	–
<i>N. novahollandiae</i>	CBS122071*	EF585540	EF585580	–

Table 3. Taxa identified in the present study as *M. esculenta* endophytes. *Botryosphaeriaceae* genera are highlighted in bold.

Taxa	ZM mesoregion		AG mesoregion		SE mesoregion		Ta
	Ra (%)	Fo (%)	Ra (%)	Fo (%)	Ra (%)	Fo (%)	
<i>Acremonium</i>	-	-	4	5	-	-	2
<i>Agaricus</i>	-	-	-	-	3	2.5	1
<i>Alternaria</i>	1	2.5	2	2.5	-	-	2
<i>Arcopilus</i>	1	2.5	-	-	-	-	1
<i>Aspergillus</i>	-	-	-	-	3	2.5	1
<i>Botrytis</i>	-	-	4	5	-	-	2
<i>Cercophora</i>	1	2.5	-	-	-	-	1
<i>Chaetomium</i>	1	2.5	-	-	-	-	1
<i>Cladosporium</i>	3	5	14	17.5	-	-	9
<i>Colletotrichum</i>	4	7.5	4	5	-	-	5
<i>Coniochaeta</i>	-	-	-	-	3	2.5	1
<i>Curvularia</i>	-	-	-	-	3	2.5	1
<i>Daldinia</i>	-	-	2	2.5	-	-	1
<i>Diaporthe</i>	14	27.5	35	42.5	3	2.5	29
<i>Epicoccum</i>	3	5	-	-	-	-	2
<i>Fusarium</i>	4	7.5	-	-	6	5	5
<i>Grammothele</i>	-	-	-	-	3	2.5	1
<i>Humicola</i>	5	10	-	-	-	-	4
<i>Hypomontagnella</i>	-	-	-	-	3	2.5	1
<i>Hypoxylon</i>	-	-	6	7.5	6	5	5
<i>Knufia</i>	-	-	-	-	3	2.5	1
<i>Lasiodiplodia</i>	5	10	-	-	-	-	4
<i>Meyerozyma</i>	1	2.5	-	-	3	2.5	2
<i>Microascus</i>	-	-	-	-	12	10	4
<i>Montagnula</i>	-	-	-	-	3	2.5	1
<i>Mucor</i>	-	-	-	-	3	2.5	1
<i>Neoscytalidium</i>	3	5	-	-	-	-	2
<i>Nigrospora</i>	26	50	10	12.5	9	7.5	28
<i>Penicillium</i>	-	-	-	-	3	2.5	1

<i>Pestalotiopsis</i>	-	-	2	2.5	-	-	1
<i>Phaeocystostroma</i>	1	2.5	-	-	-	-	1
<i>Phaeosclera</i>	-	-	-	-	3	2.5	1
<i>Phialophora</i>	1	2.5	-	-	-	-	1
<i>Phoma</i>	-	-	-	-	6	5	2
<i>Preussia</i>	14	27.5	2	2.5	-	-	12
<i>Purpureocillium</i>	-	-	-	-	12	10	4
<i>Simplicillium</i>	3	5	2	2.5	-	-	3
<i>Stagonosporopsis</i>	-	-	2	2.5	-	-	1
<i>Talaromyces</i>	1	2.5	-	-	-	-	1
<i>Trichoderma</i>	1	2.5	-	-	-	-	1
<i>Xylaria</i>	4	7.5	8	10	9	7.5	10

Figures

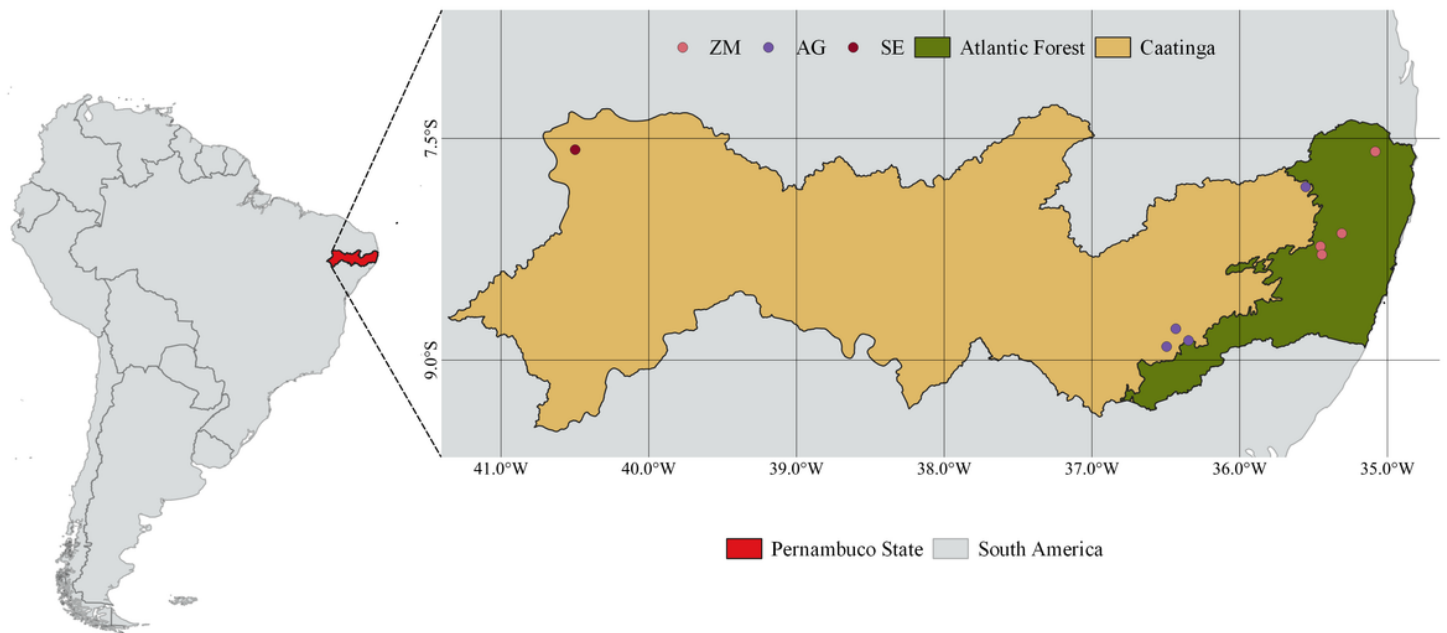


Figure 1

Collection areas of healthy stems of *Manihot esculenta* in the state of Pernambuco, Brazil. Mesoregions: ZM (Zona da Mata), AG (Agreste), SE (Sertão).

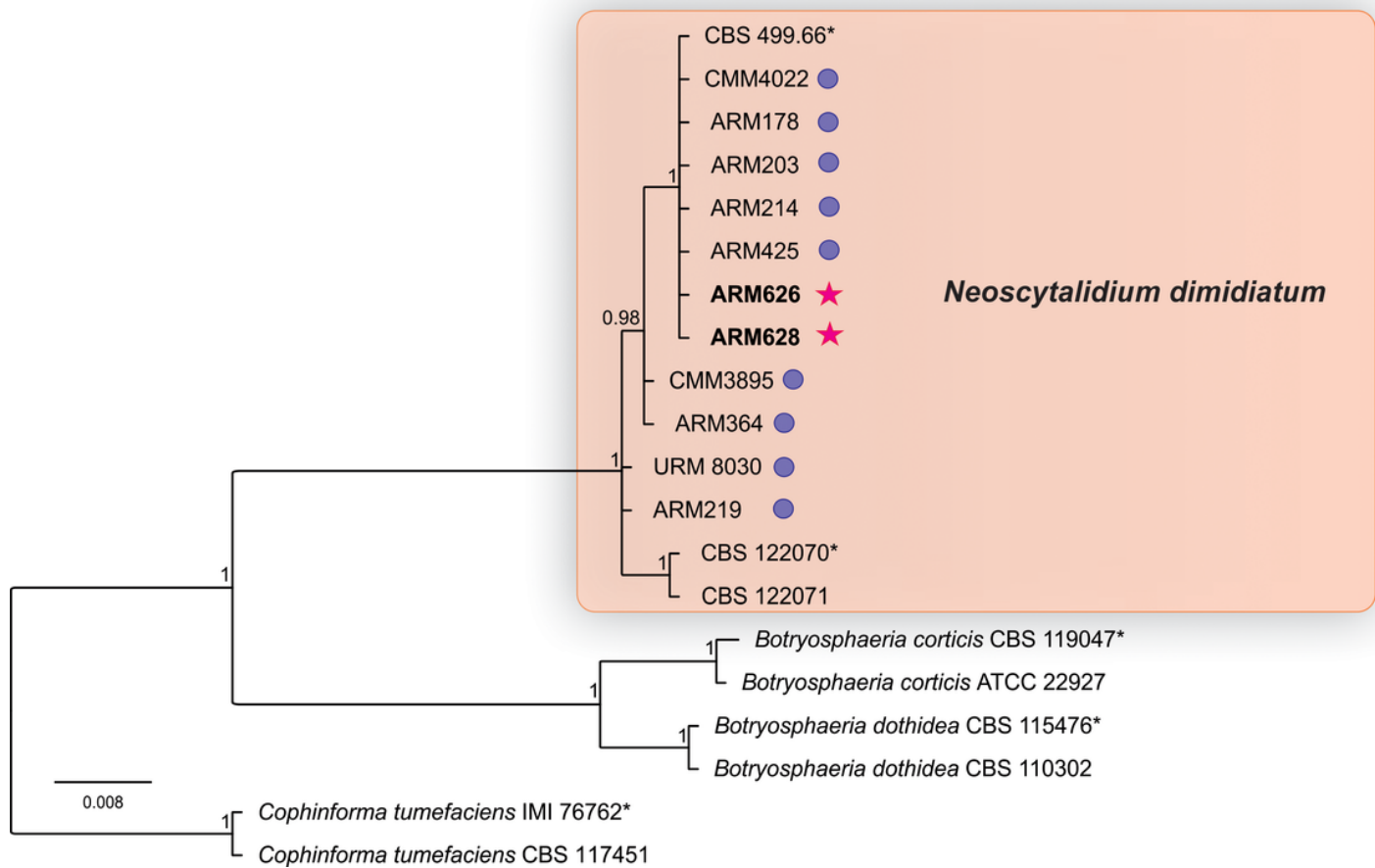


Figure 3

Neoscytalidium concatenated tree using ITS and *TEF1-a* genic regions. Posterior probability values above 0.95 are behind the nodes. The isolates obtained in the present study are highlighted in bold. Stars indicate the endophytic isolates obtained in this study and the circles isolates pathogenic to *Manihot esculenta* identified in previous works (Machado et al., 2014; Brito et al., 2020). Asterisks (*) indicate the ex-type. *Cophinforma tumefaciens* isolates IMI 76762 and CBS 117451 were used as outgroup.



Figure 4

Pathogenicity test in *Manihot esculenta* seedlings and roots. **(a-c)** Lesions caused by *Lasiodiplodia theobromae* isolates. **(a1-a4)** ARM 598, **(b1-b4)** ARM 599, **(c1-c4)** ARM 602. **(d)** Lesions caused by *L. euphorbiaceicola* ARM 601. **(e-f)** Lesions caused by *Neoscytalidium dimidiatum* isolates. **(e1-e4)** ARM 626, **(f1-f4)** ARM 628. **(g)** Control.

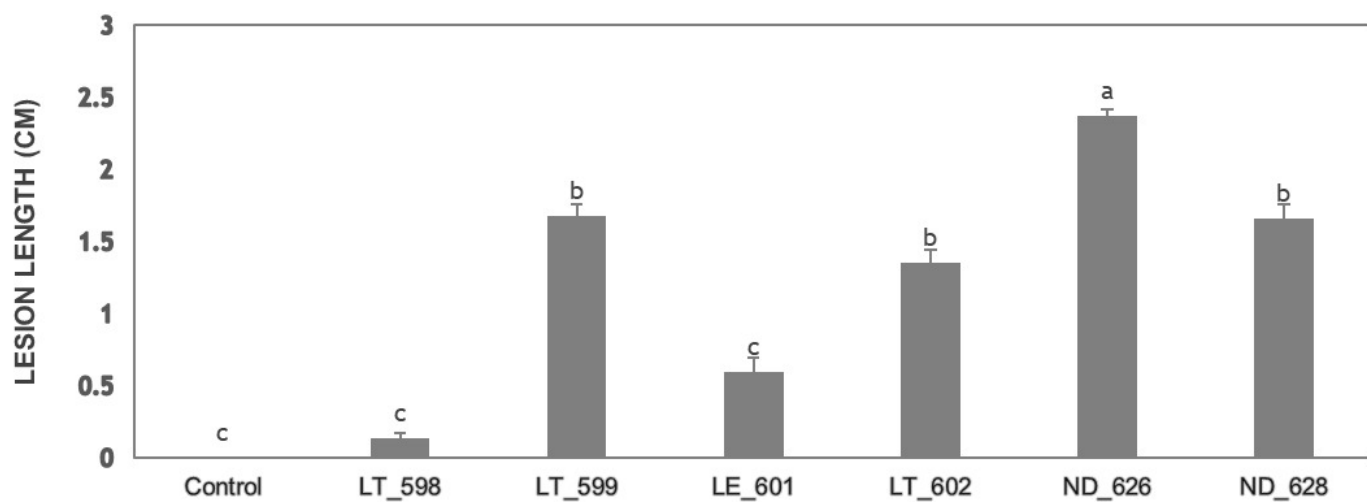


Figure 5

Comparison of lesion length between treatments performed on *Manihot esculenta* seedlings. Means followed by the same letter do not differ from each other by the Scott-Knott test at the 5% significance level. Vertical bars represent the standard error of the means.

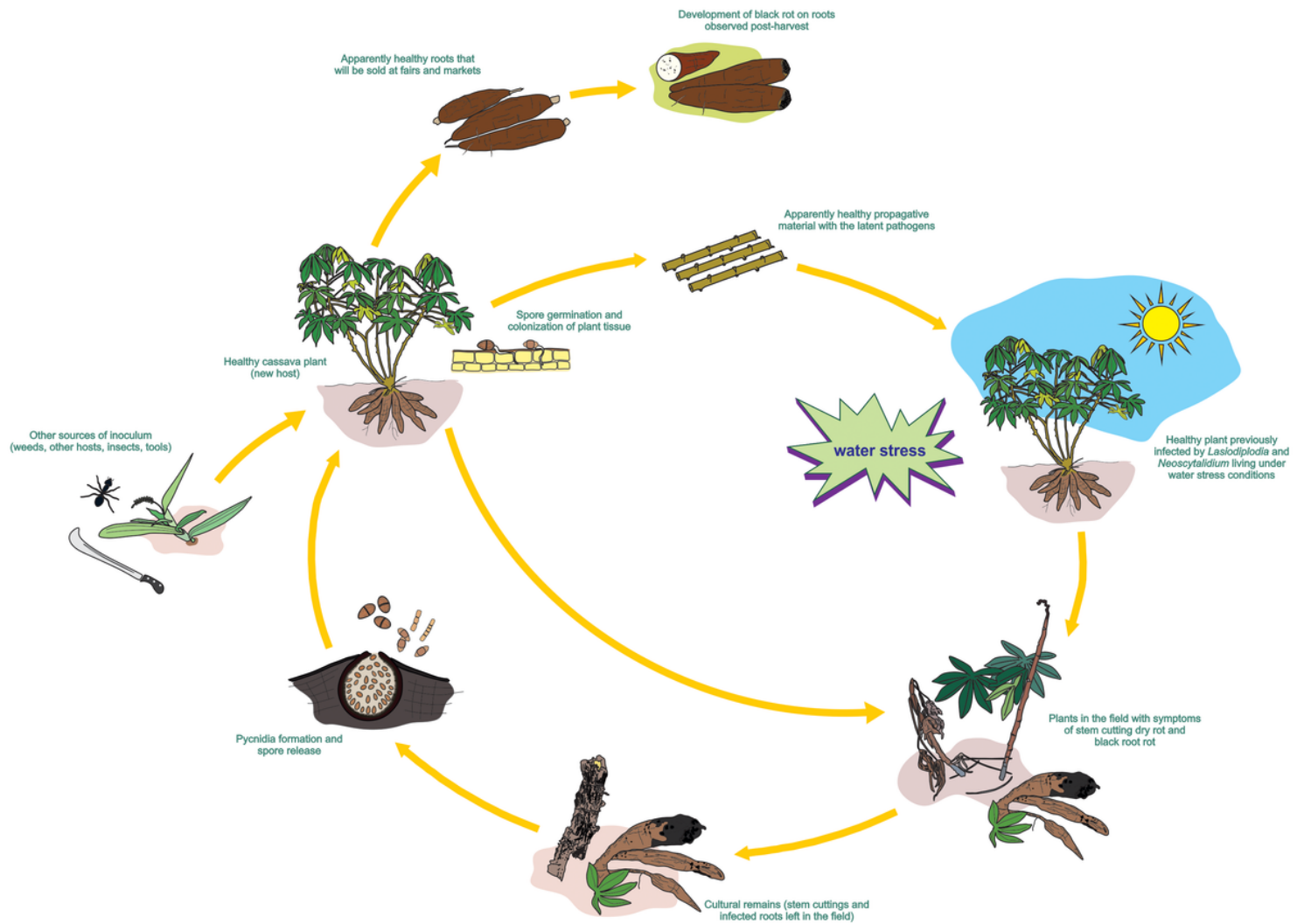


Figure 6

Life cycle of *Lasiodiplodia theobromae*, *L. euphorbiaceicola* and *Neoscytalidium dimidiatum* in *Manihot esculenta* plantation.