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Survey of fungi associated with cassava root rot from different producing regions in Brazil

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ABSTRACT: Although root rot is one of the major diseases affecting Brazilian cassava (*Manihot esculenta* Crantz.), little is known about the diversity of root rot pathogens. In this study, diseased plants exhibiting root rot symptoms were collected from cassava-producing regions in five Brazilian states: Bahia, Sergipe, Paraíba, Maranhão, Tocantins, and Paraná. Seventy isolates were obtained and assigned to species complexes based on rDNA's ITS (internal transcribed spacer of the ribosomal DNA) region (ITS1, ITS2 and 5.8S). A total of seven species complexes was found belonging to the genus *Fusarium* (56/74), followed by *Lasiodiplodia* (8/74), *Neoscytalidium* (6/74), and *Diaporthe/Phomopsis* complex (2/74), *Phytophthora*, and *Corallomycetella* (1/74 each). These species were distributed differently according to sample locations and states, but overall, the *F. solani* species complex (FSSC) was the most prevalent. A number of phylogenetic lineages had not been previously reported as being associated with cassava-root rot disease, such as: *F. graminearum* (FGSC), *F. incarnatum-equiseti* (FIESC) and *F. chlamydosporum* (FCSC) complexes, and a phylogenetic lineage most closely related to *P. phaseolorum*. Results suggest the need to improve knowledge of the species associated with cassava, including multilocus phylogeny for a more specific characterization, and differences in the resistance background associated with these species, as a strategy to incorporate resistance to multiple pathogens in cassava breeding programs.
Keywords: *Manihot esculenta*, ITS barcode, phylogeny, soil-borne diseases

Introduction

Cassava is a major source of carbohydrates for millions of people in several regions, particularly in developing countries. The cassava crop plays a vital role in reducing poverty and rural exodus because the use of technology required is minimal (Akinbo et al., 2012). In addition to the social impact, cassava has attracted the interest of the agriculture business due to its multiple industrial uses of starch (Tonukari, 2004).

In Brazil, different diseases affect cassava production, namely, root rot disease (CRRD), a major constraint responsible for up to 80 % of losses in yield. Symptoms of CRRD vary according to the causal agent and can be divided into dry, soft, and black rot (Bandyopadhyay et al., 2006). In general, dry rot is characterized by the appearance of dark brown streaks in the roots with no aqueous aspect, while soft rot is characterized by the darkening of the affected tissues with liquid exudation and is foul-smelling. On the other hand, black rot is characterized by dark lesions (blackened) in the roots and stems and may evolve into soft rot but without the unpleasant odor.

The main pathogens associated with CRRD in Brazil are *Fusarium* spp. causing dry rot (*F. solani*, *F. oxysporum*, and *F. verticillioides*); *Phytophthora* spp. (*P. nicotianae* and *P. drechsleri*), *Pythium scleroteichum*, associated with soft rot, and *Neoscytalidium hyalinum* and *Lasiodiplodia* spp. causing black rot (Oliveira et al., 2013; Machado et al., 2014a). Knowledge of the diversity and geographical distribution of root rot pathogens may be useful to breeders targeting root rot resistance. This study aimed to identify the main species complex associated with cassava root rot samples in several regions in Brazil based on a phylogenetic approach.

Materials and Methods

Diseased roots and stems of cassava were collected from different producing regions in the states of Bahia (seven fields), Sergipe (two fields), Paraíba (one field), Maranhão (two fields), Tocantins (one field), and Paraná (two fields). Isolates were collected from 1991 to 2014, with the majority of the isolates being collected from 2012 to 2014 (Table 1). The sampled locations were chosen by information of CRRD outbreaks obtained from the plant disease clinic of Embrapa Cassava and Fruit and producers, and based on previous history of CRRD incidence in experimental and/or commercial areas.

In the laboratory, symptomatic tissues were cut into small pieces of approximately 0.5 cm, dipped for 2 min in 70 % ethanol, sterilized for 2 min in 0.5 % sodium hypochlorite solution, and then washed with sterilized distilled water three times. The fragments were laid out to dry on sterile filter paper, and afterwards they were placed on potato dextrose agar (PDA) and incubated at 24 °C for 5-7 days, for a 12-h photoperiod.

The isolates of fungi and oomycetes obtained were grown in a specific medium to verify the morphology of

Table 1 – Cassava root rot pathogens studied, with collection details and GenBank accession number for the ITS rDNA region.

Voucher	Species/complex	Year	Location*	GenBank accession number
CBPPR0002	*Corallomycetella repens*	2013	Umbaúba-SE	KT211495
CBPPR2001	*DPMSC*	2013	Cruz das Almas-BA	KT211565
CBPPR2002	*Diaporthe phaseolorum*	2013	Cruz das Almas-BA	KT211566
CBPPR0008	*FCSC*	1991	São Miguel das Matas-BA	KT211501
CBPPR0047	*FCSC*	2014	Cruz das Almas-BA	KT211539
CBPPR0051	*FCSC*	2012	Marechal Cândido Rondon-PR	KT211543
CBPPR0031	*FIESC*	2014	Cruz das Almas-BA	KT211523
CBPPR0032	*FIESC*	2014	Cruz das Almas-BA	KT211524
CBPPR0058	*FIESC*	2013	Umbaúba-SE	KT211550
CBPPR0049	*FGSC*	2012	Diamante do Norte-PR	KT211541
CBPPR0052	*FGSC*	2012	Marechal Cândido Rondon-PR	KT211544
CBPPR0053	*FGSC*	2012	Diamante do Norte-PR	KT211545
CBPPR0003	*FOSC*	2013	Umbaúba-SE	KT211496
CBPPR0004	*FOSC*	2013	Umbaúba-SE	KT211497
CBPPR0005	*FOSC*	2013	Umbaúba-SE	KT211498
CBPPR0006	*FOSC*	1994	São Miguel das Matas-BA	KT211499
CBPPR0007	*FOSC*	1990	Ribeirópolis-SE	KT211500
CBPPR0010	*FOSC*	2013	Cruz das Almas-BA	KT211503
CBPPR0011	*FOSC*	2013	Cruz das Almas-BA	KT211504
CBPPR0013	*FOSC*	2013	Cruz das Almas-BA	KT211506
CBPPR0017	*FOSC*	2013	Cruz das Almas-BA	KT211510
CBPPR0019	*FOSC*	2013	Cruz das Almas-BA	KT211512
CBPPR0021	*FOSC*	2013	Cruz das Almas-BA	KT211513
CBPPR0028	*FOSC*	2013	Cruz das Almas-BA	KT211520
CBPPR0035	*FOSC*	2007	Vitória da Conquista-BA	KT211527
CBPPR0037	*FOSC*	2007	Cachoeira-BA	KT211529
CBPPR0038	*FOSC*	2007	Vitória da Conquista-BA	KT211530
CBPPR0039	*FOSC*	2007	Ribeirópolis-SE	KT211531
CBPPR0041	*FOSC*	2008	Guaratinga - BA	KT211533
CBPPR0043	*FOSC*	2013	Cruz das Almas-BA	KT211535
CBPPR0054	*FOSC*	2012	Diamante do Norte-PR	KT211546
CBPPR0055	*FOSC*	2012	Diamante do Norte-PR	KT211547
CBPPR0056	*FOSC*	2013	Umbaúba-SE	KT211548
CBPPR0015	*FFSC*	2013	Cruz das Almas-BA	KT211508
CBPPR0048	*FFSC*	2012	Cruz das Almas-BA	KT211540
CBPPR0001	*FSSC*	2013	Umbaúba-SE	KT211494
CBPPR0009	*FSSC*	2013	Cruz das Almas-BA	KT211502
CBPPR0012	*FSSC*	2013	Cruz das Almas-BA	KT211505
CBPPR0014	*FSSC*	2013	Cruz das Almas-BA	KT211507
CBPPR0016	*FSSC*	2013	Cruz das Almas-BA	KT211509
CBPPR0018	*FSSC*	2013	Cruz das Almas-BA	KT211511
CBPPR0022	*FSSC*	2013	Cruz das Almas-BA	KT211514
CBPPR0023	*FSSC*	2013	Cruz das Almas-BA	KT211515
CBPPR0024	*FSSC*	2013	Cruz das Almas-BA	KT211516
CBPPR0025	*FSSC*	2013	Cruz das Almas-BA	KT211517
CBPPR0026	*FSSC*	2013	Cruz das Almas-BA	KT211518
CBPPR0027	*FSSC*	2013	Cruz das Almas-BA	KT211519
CBPPR0029	*FSSC*	2013	Cruz das Almas-BA	KT211521
CBPPR0030	*FSSC*	2013	Texeira de Freitas-BA	KT211522
CBPPR0033	*FSSC*	2007	Porto Seguro-BA	KT211525
CBPPR0034	*FSSC*	2007	Humberto de Campos-MA	KT211526
CBPPR0036	*FSSC*	2007	Axixá-MA	KT211528
CBPPR0040	*FSSC*	2008	Guaratinga - BA	KT211532
CBPPR0042	*FSSC*	2008	Cruz das Almas-BA	KT211534

Continue...

Table 1 – Continuation.

CBPPR0044	*FSSC*	2014	Cruz das Almas-BA	KT211536
CBPPR0045	*FSSC*	2013	Texeira de Freitas-BA	KT211537
CBPPR0046	*FSSC*	2013	Texeira de Freitas-BA	KT211538
CBPPR0050	*FSSC*	2012	Marechal Cândido Rondon-PR	KT211542
CBPPR0057	*FSSC*	2013	Umbaúba-SE	KT211549
CBPPR1002	*LTSC*	2014	Cruz das Almas-BA	KT211551
CBPPR1003	*LTSC*	2014	Cruz das Almas-BA	KT211552
CBPPR1004	*LTSC*	2014	Cruz das Almas-BA	KT211553
CBPPR1005	*LTSC*	2014	Cruz das Almas-BA	KT211554
CBPPR1006	*LTSC*	2012	Palmas-TO	KT211555
CBPPR1007	*LTSC*	2012	Palmas-TO	KT211556
CBPPR1008	*LTSC*	2012	Palmas-TO	KT211557
CBPPR1009	*LTSC*	2012	Palmas-TO	KT211558
CBPPR1001	*Neoscytalidium hyalinum*	1996	Areias-PB	KT211559
CBPPR1011	*N. hyalinum*	2007	Humberto de Campos-MA	KT211560
CBPPR1012	*N. hyalinum*	2007	Areias-PB	KT211561
CBPPR1014	*N. hyalinum*	2007	Axixá-MA	KT211562
CBPPR1015	*N. hyalinum*	2012	Cruz das Almas-BA	KT211563
CBPPR1016	*N. hyalinum*	2012	Cruz das Almas-BA	KT211564
CBPPR3001	*Phytophthora melonis*	2014	Cruz das Almas-BA	KT211567

DPMSC: *Diaporthe* (*Phomopsis*) *melonis* species complex; FCSC: *F. chlamydosporum* species complex; FIESC: *F. incarnatum-equiseti* species complex; FGSC: *F. graminearum* species complex; FOSC: *F. oxysporum* species complex; FFSC: *F. fujikuroi* species complex; FSSC: *Fusarium solani* species complex; LTSC: *Lasiodiplodia theobromae* species complex. *States from the Northeast region of Brasil: BA: Bahia; MA: Maranhão; PB: Paraíba; SE: Sergipe. *State from the Northern region of Brasil: TO: Tocantins. *State from the South region of Brasil: PR: Paraná.

spores and mycelia, PDA + pine acicula for fungi and 'carrot agar' + Beta-sitosterol for oomycetes. Isolates were grouped, based on the morphology of the colony and spores, and then the cassava-root slices (Onyeka et al., 2005b) were inoculated to confirm pathogenicity. Monosporic cultures were preserved in tubes with culture medium according to the Castellani method and with mineral oil. Only those isolates considered as pathogenic, based on the cassava root-slice inoculation, were used in this study.

DNA extraction

Monosporic cultures of each isolate of fungi and oomycetes were placed to grow in sucrose and yeast extract broth (10 g sucrose, 2 g yeast extract, 1 g KH_2PO_4, 0 , 1g $MgSO_4.7H_2O$, 0.44 mg of $ZnSO_4.7H_2O$, and 1000 mL distilled water) for 15 days, and then the mycelium was filtered, arranged so as to dry out, and then macerated with liquid nitrogen and mixed with 700 µ extraction buffer (1 % CTAB, 20 mM Tris, pH 8.0; 10 mM EDTA; 0.7 M NaCl) and 10 µL of β-mercaptoethanol). The mixture was kept at 65 °C for 45 min and homogenized gently every 15 min. Subsequently, 500 µL of Chloroform:Isoamyl Alcohol (24:1 v/v) was added, and the mixture was centrifuged for 10 min at 10,000 rpm. Supernatant was transferred to a new tube containing 500 µL of isopropanol and 100 µL of sodium acetate and was centrifuged at 10,000 rpm for 12 min.

Finally, the precipitate was washed twice with 1 mL of 70 % ethanol, placed so as to dry at room temperature, resuspended with Tris-EDTA buffer (TE) (10 mM Tris-HCL + 1 mM EDTA) and 2 µL of ribonuclease A [RNAse] (10 mg mL^{-1}), and incubated at 37 °C for 1 h. After extraction, the solution containing DNA was stored at –20 °C. The quality and quantity of total DNA was measured by visual comparison with phage λ DNA at concentrations of 50 and 100 ng electrophoreses in agarose gel (1 % 80V 60 min^{-1}) stained with ethidium bromide (1.5 µL 100 mL^{-1}).

Amplification and sequencing

Amplification of the ITS region of the rDNA from isolates was performed using the universal primers ITS1 (5'TCC GTA GGT GAA CCT GCG G3') and ITS4 (5'TCC TCC GCT TAT TGA TAT GC3'). In 200 µL microtubes the following was added: 50 ng DNA; 1X Tris-HCl / KCl pH 8.3 (10 mM / 50 mM); $MgCl_2$ (1.5 mM); 0.1 µM of each dNTP; 0.2 µM of each primer; 1.5 U Taq polymerase, and ultrapure water to complete the volume of 50 µL. The reactions consisted of an initial denaturation phase at 94 °C for 4 min, followed by 35 cycles at 94 °C for 30 s, 30 s annealing at 50 °C, extension at 72 °C for 1 min, and a final extension of 72 °C for 7 min. Amplification was performed in a 96-Well Thermal Cycler.

The amplified products in the reaction were separated by agarose gel electrophoresis at 1 % to 100V in 0.5X TAE buffer (Tris-acetate-EDTA) for approximately 2 h and stained with ethidium bromide solution 1.5 µL 100 mL^{-1}. The amplified fragments were visualized and photographed under ultraviolet light. The PCR reaction products were purified by a commercial column purification kit and then sent for sequencing in two directions (forward and reverse).

The DNA sequences obtained were evaluated for sequencing quality, aligned pair-to-pair, and manually edited to obtain a consensus sequence for each isolate. The consensus sequences generated were compared with those deposited in the GenBank (National Center for Biotechnology Information website - http://www.ncbi.nlm.nih.gov) through the BLASTn tool for confirmation of the genus of each of the isolates. The verification of the identity of the species causing CRRD was carried out using phylogenetic analyses of Neighbor-Joining (NJ) and Bayesian inference, and was compared to reference sequences deposited in the Genbank.

Phylogenetic analyses

The rDNA ITS regions (comprising the ITS1, 5.8S and ITS2) were aligned with isolates from different species complexes using the "Muscle" algorithm implemented in the software MEGA v.6. Although the ITS may not be appropriate for accurate identification of strains at the species level, it can be used to classify them into species complexes. In the alignment matrices, sequences from the GenBank database were included based on index similarity. Outgroups used for each genus were *Microdochium nivale* - CBS 116205 (*Fusarium*), *Spencermartinsia viticola* - CBS 117009 (*Lasiodiplodia* and *Neoscytalidium*), *Pythium citrinum* - 221 743 (*Phytophthora*), *Diaporthella corylina* - CBS 121124 (*Phomopsis*).

Phylogenetic analyses based on NJ were performed using MEGA v.6 with a "Kimura 2-parameter" model and 10,000 bootstrap replicates to determine the support of the branches. For the Bayesian inference analysis, the evolution model for each genus was chosen using hierarchical likelihood tests considering the Akaike Information Criterion (AIC) using the MrModeltest v.2.3 program. The consensus trees were generated using the MrBayes software v.3.2, with 5,000,000 generations (convergence 0.001) through two independent runs; each used four Markov Monte Carlo Chains (MCMC), two hot and two cold chains, sampling a tree every 1,000 generations, and burn-in of the initial 1,250,000 generations. The trees were visualized and edited using the FigTree software v.1.2.2.

Results

From a total of 115 isolates obtained from diseased cassava plants, only the 74 considered as pathogenic were used. These isolates were obtained from different producing regions (Table 1). Isolates were separated based on the genus defined by BLAST search in the GenBank database (National Center for Biotechnology Information) and phylogenetic analyses. The most frequent species were of the genus *Fusarium* (56), followed by *Lasiodiplodia* (8), *Neoscytalidium* (6), and *Diaporthe/Phomopsis* complex (2), *Phytophthora* and *Corallomycetella* (1 each).

According to the phylogenetic analysis of ITS rDNA region, the *Fusarium*-like isolates, associated with dry root-rot symptoms, were divided into six groups (Figure 1). Five of them had more than one isolate per group, and one group had a single isolate. 'Group 1' (*F. solani* species complex - FSSC) comprised a total of 24 isolates which were clustered and divided into three clusters inside the FSSC, but all considered as *F. solani* sensu lato (Table 1).

For the isolates clustered in 'Group 2' (*F. oxysporum* species complex – FOSC) (Figure 1), a high posterior probability was found (0.92). This indicated that 21 of the isolates represented lineages of FOSC, from this 15 were from crops in the state of Bahia, six were from Sergipe, and two were from Paraná, corresponding to 50 % of the states sampled.

'Group 3' was composed of six isolates each grouped into *F. incarnatum-equiseti* (FIESC) and *F. chlamydosporum* (FCSC) complexes (Figure 1). In contrast, 'Group 4' was composed of three isolates belonging to *F. graminearum* complex (FGSC), and two isolates were placed in 'Group 5' with species from *F. fujikuroi* complex (FFSC). In Group 6 an isolate relating to the species *Corallomycetella repens* (teleomorph = *Nectria mauritiicola*) was identified.

Seven lineages associated with dry root rot symptoms with different frequencies were identified: FSSC (24/56), FOSC (21/56), FGCS (3/56), FCSC (3/56), FIESC (3/56), FFSC (2/56), plus one isolate characterized as *Corallomycetella repens* (teleomorph = *Nectria mauritiicola*) and not *Fusarium* (Figure 1).

A total of 15 isolates associated with black root rot were classified within the Botryosphaeriaceae family, being nine isolates clustered as *Lasiodiplodia theobromae* species complex and six isolates were identified as *Neoscytalidium hyalinum* (Figure 2), originating from the states of Bahia, Maranhão, Paraiba and Tocantins.

Two of the strains obtained showed a high similarity to *Phomopsis* sp. in a BLASTn search. These sequences came from fields located in the state of Bahia and the city of Cruz das Almas. According to phylogenetic analysis of ITS-barcoding, they were clustered in a single clade within the *Diaporthe/Phomopsis* complex (posterior probability = 0.63), and the CBPPR2001 isolate had a 99 % identity to *Diaporthe* (*Phomopsis*) *phaseolorum* (Figure 3A). The CBPPR2002 isolate was set in a different branch in the same clade, but it was not possible to determine the species to which it belonged because additional molecular sequences from other regions would have been required.

The CBPPR3001 isolate was obtained in the state of Bahia (Cruz das Almas), causing soft root rot symptoms, and was grouped together with the species *P. melonis* (99 % similarity), according to the phylogenetic analysis based on the ITS region of rDNA (Figure 3B).

The same tree topology was obtained for all phylogenetic analyses based on the NJ and Bayesian inference, for all genera evaluated.

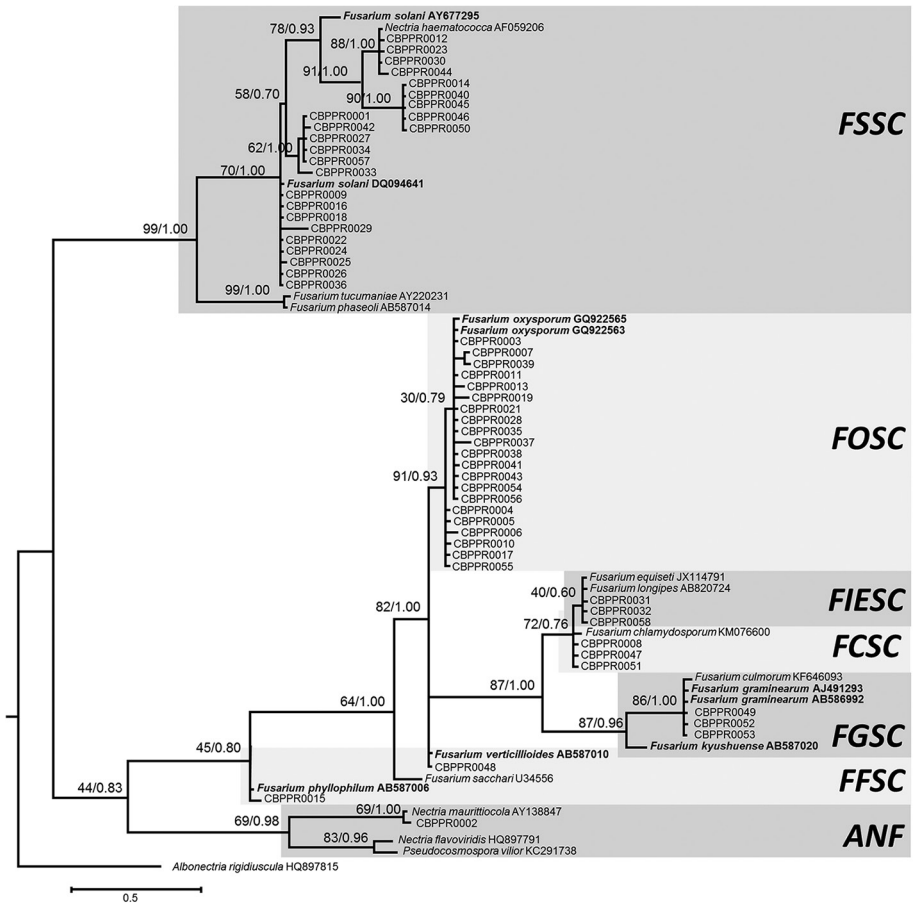

Figure 1 – A Bayesian inference phylogenetic tree of the genus *Fusarium* of the ITS rDNA region. Bootstrap values ≥ 50 and Bayesian posterior probability ≥ 0.5 are shown at the nodes (Neighbor Joining/Bayesian). Ex-type cultures are emphasized in bold font. *Microdochium nivale* (AB58698) was used as outgroup. The scale bar indicates the number of expected changes per site. FSSC = *Fusarium solani* species complex; FOSC = *F. oxysporum* species complex; FIESC = *F. incarnatum-equiseti* species complex; FCSC = *F. chlamydosporum* species complex; FGSC = *F. graminearum* species complex; FFSC = *F. fujikuroi* species complex; ANF = species where the "Anamorph is not Fusarium". Isolates beginning with "CBPPR" are from this study.

Discussion

Isolates in *F. solani* (FSSC) and *F. oxysporum* (FOSC) complexes are often reported as the most prevalent associated with CRRD, FSSC being the most widely distributed among the different producing regions worldwide. *Fusarium solani* sensu lato was also reported as a cassava pathogen in Colombia. Based on morphological characteristics, this fungus is considered to be more common than the *F. oxysporum* as a causal agent of CRRD (Onyeka et al., 2005a).

FSSC and FOSC showed a frequency of 45 % in a survey of pathogens occurring in Nigeria in 1998 and 1999. They occurred more frequently in areas with more than 1,600 mm of rain per year (Bandyopadhyay et al., 2006). In addition to Colombia, *F. solani* has also been reported in association with cassava in India, Malaysia, Nigeria, and New Guinea (Adisa, 1983; Shaw, 1984; Cheari et al., 2014.). In fact, in several African countries, different *Fusarium* spp. are commonly associated with cassava root rot disease. The *F. oxysporum* is one of the most widespread species and is associated with large losses in the productivity of cassava (Bandyopadhyay et al., 2006).

C. repens is a saprophytic species, but it can also be parasitic in plant roots. Generally, this is a problem in soils with drainage problems, causing yellowing and leaves with decay accompanied by a bad odor (Seifert, 1985). Another synonym of *C. repens* is *Sphaerostilbe repens*, which has also been observed in African countries causing CRRD (Obilo and Ikotun, 2008).

The species *L. theobromae* has been described previously in cassava in different countries, but other species are also found in cassava fields associated with CRRD, such as *L. parva L. pseudotheobromae*, and *L. euphorbicola* (Marques et al., 2013; Machado et al., 2014a; Machado et al., 2014b; Netto et al., 2014). As a consequence of the high genetic identity (> 78 %) among dif-

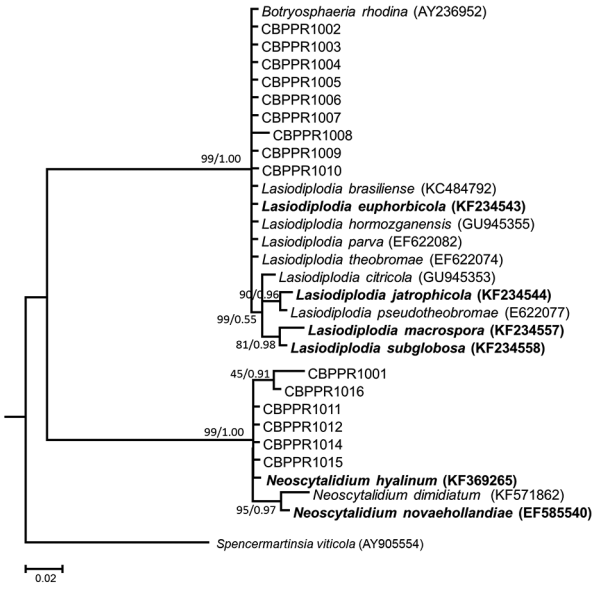

Figure 2 – A Bayesian inference phylogenetic tree of the family Botryosphaeriaceae of the ITS rDNA region. Bootstrap values ≥ 40 and Bayesian posterior probability ≥ 0.5 are shown at the nodes (Neighbor Joining/Bayesian). Ex-type strains are emphasized in bold font. *Spencermartinsia viticola* (AY905554) was used as outgroup. The scale bar indicates the number of expected changes per site. Isolates beginning with "CBPPR" are from this study.

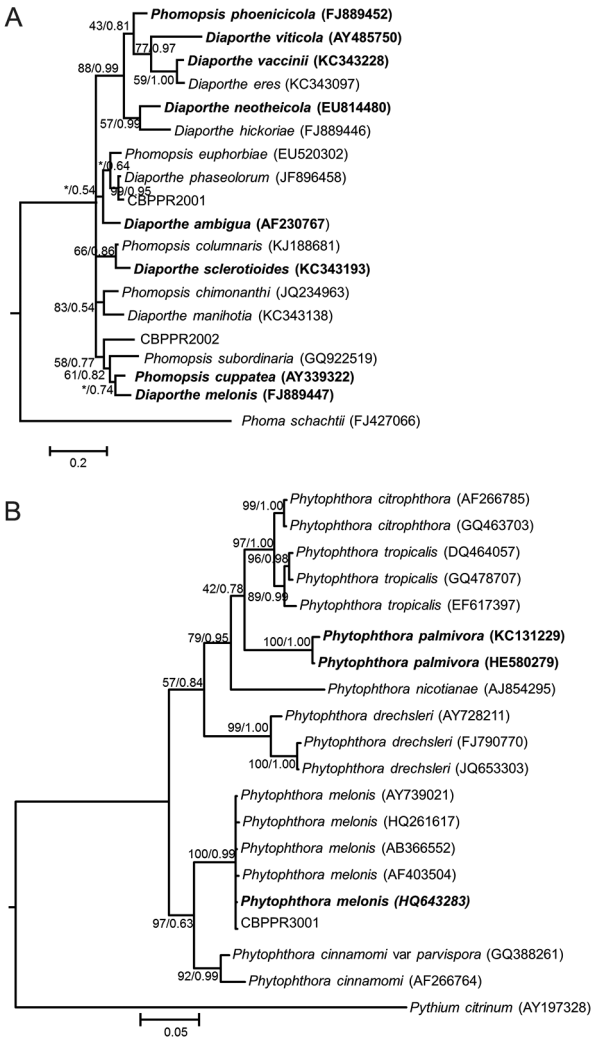

Figure 3 – A Bayesian inference phylogenetic tree of the *Diaporthe/Phomopsis* complex (A) and *Phytophthora* (B) of the ITS rDNA region. Bootstrap values ≥ 40 and Bayesian posterior probability ≥ 0.5 are shown at the nodes (Neighbor Joining/Bayesian). Ex-type strains are emphasized in bold font. *Phoma schachtii* (FJ427066) and *Pythium citrinum* (AY197328) were used as outgroup, for A and B respectively. The scale bar indicates the number of expected changes per site. Isolates beginning with "CBPPR" are from this study.

ferent species and the isolates classified in this work as *L. theobromae*, additional studies should be conducted to more assertively identify the species found through polyphasic characterization, including the sequencing of other genetic regions such as the elongation factor alpha (EF1-α) and beta tubulin (βt).

The name *Scytalidium lignicola* is applied to certain pathogens causing cassava root rot in Brazil (Machado et al., 2014a). However, several species of *Scytalidium* are now classified under the genus *Neoscytalidium*, whose morphology is similar to *Scytalidium* but differs in the presence or absence of pycnidia and conidia between genera and belongs to different orders of Ascomycota (Crous et al., 2006; Seifert et al., 2011; Phillips et al., 2013; Machado et al., 2014a).

The species *N. hyalinum* previously classified as *Neoscytalidium dimidiatum* refers to a Botryosphaeriaceae fungus family, which includes several pathogenic fungi, endophytes, and saprophytes found in all geographic and climatic areas of the world, except for the polar regions (Crous et al., 2006; Phillips et al., 2013). *N. hyalinum* has previously been reported in association with physic nut (*Jatropha curcas* L.), mango (*Mangifera indica* L.), and cassava in Brazil (Machado et al., 2012; Marques et al., 2013; Machado et al., 2014a; Machado et al., 2014b).

The *Diaporthe* genus includes plant pathogenic species in many woody and herbaceous hosts that are found mainly in its anamorphic state *Phomopsis*. Many of its species cause major losses to infected plants of economic interest such as fruit, and cause cancers, rot, diebacks, and necrosis, among other pathologies. These fungi can survive as endophytes, without causing any damage to the host, and they may become pathogenic when the host is senescent or under stress conditions; there are a lot of species with saprophytic behavior.

Examples of pathogens are *P. amygdali* (Delacr.) JJ Tuset & MT Portilla, which are responsible for stem discoloration and death of peach (Farr et al., 1999), *D. helianthi* Munt.-Cvetk., Mihaljč & M. Petrov, that cause *Pho-*

mopsis stem canker in sunflower (Muntanola-Cvetkovic et al., 1991), *D. phaseolorum* (Cooke and Ellis) Sacc., which is the causal agent of soybean stem canker (Black et al., 1996), and *P.viticola* (Sacc.) Sacc., is an important pathogen of grape (Mostert et al., 2001). However, even though *D. phaseolorum* is a notorious rot and stem pathogen in other plant species, this is the first report of its kind associated with CRRD.

Species of the genus *Phytophthora* have been associated with soft root rot in cassava such as *P. palmivora*, which was recently reported to have attacked cassava plants in China and whose symptoms were characterized by widespread wilting in the upper leaves and death of the plant (Guo et al., 2012). In Brazil, *P. drechsleri* and *P. melonis* species have also been associated with CRRD, and *P. drechsleri* is considered the main causal agent of soft root rot.

Species of the genus *Fusarium* were found in almost all locations sampled, except for Areia, the state of Paraíba and Palmas, and the state of Tocantins, where the species *N. hyalinum* and *L. theobromae* were predominant, respectively. Therefore, in general, there was a wide distribution of *Fusarium* species. On the other hand, different compositions of species were found, in some cases restricted to a geographical region such as the occurrence of species of the complex *F. graminearum* in the municipalities of the state of Paraná (Diamante do Norte and Marechal Cândido Rondon).

Fungi that cause black root rot disease such as *N. hyalinum* and *L. theobromae* were mainly identified in the municipalities in the north and northeast of Brazil. According to Aigbe et al. (2008), there is an association between species causing root rot and edaphoclimatic parameters as well as the stage of development of the culture, and in the early stages of plant development there is a common occurrence of *Fusarium* species, while in the later stages, there is a predominance of black rot pathogens.

CRRD is difficult to manage because is caused by a complex of soil-borne fungi/oomycete species, which makes chemical control infeasible due to environmental damage and its high treatment costs for producers. These limitations require the identification of alternative ways to managing the disease including sustainable practices that are more accessible to farmers and less harmful to the environment. Different studies showed that the use of resistant varieties, together with crop rotation and changes in the cropping systems, enabled a reduction of about 60 % in CRRD (Bezerra and Sousa, 2003) and contribute significantly to increasing productivity, thus improving the quality and the aspect of cassava, and obtaining a better final product.

Data from this study support the need for a deeper understanding of the genetic resistance of cassava accessions in germplasm banks. Likewise, they indicate the need for evaluation based on observation of the reaction of cassava genotypes from different species of pathogens individually and based not only on observations in the field, since the pathogens can vary according to location.

Problems related to the selection of plants resistant to CRRD were reported by Bandyopadhyay et al. (2006), who considered that the geographical differences related to the diversity of the populations of root rot fungi pose a challenge to the genetic improvement since that genotypes considered resistant in one region may not be in another.

Thus, because of the diversity of lineages found in this study, breeding programs must include selection for multiple resistant cassava root rot pathogens, mainly for the FSSC and FOSC and for the isolates associated with black root rot (*Lasiodiplodia* spp. and *Neoscytalidium hyalinum* lineages), either in individual evaluations (each species complex versus each of access) or by directly challenge by different groups of pathogens (e.g.: dry and black root rot pathogens). Consequently, the results obtained in this study will be used to conduct experiments aiming to select genotypes resistant to cassava root rot disease through the cassava breeding program, and for other Brazilian research companies and/or universities.

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