Genetic diversity and population structure of *Lasiodiplodia theobromae* from different hosts in northeastern Brazil and Mexico

T. J. S. Rêgo\(^a\), G. Elena\(^b\), K. C. Correia\(^c\), J. M. Tovar-Pedraza\(^d\), M. P. S. Câmara\(^a\), J. Armengol\(^b\), S. J. Michereff\(^c\) and M. Berbegal\(^b^*\)

\(^a\)Departamento de Agronomia, Universidade Federal Rural de Pernambuco, 52171-900 Recife, Brazil; \(^b\)Instituto Agroforestal Mediterráneo, Universitat Politècnica de València, 46022 Valencia, Spain; \(^c\)Centro de Ciências Agrárias e da Biodiversidade, Universidade Federal do Cariri, 63133-610 Crato, Brazil; and \(^d\)Coordinación Culiacán, Centro de Investigación en Alimentación y Desarrollo, 80110, Culiacán, Sinaloa, Mexico

*Corresponding author: Mónica Berbegal - e-mail: mobermar@etsia.upv.es
Telephone number: +34 963879254
Fax number: +34 963879269
Abstract

*Lasiodiplodia theobromae* is one the most frequent fungal pathogens associated with dieback, gummosis, leaf spot, stem-end rot, and fruit rot symptoms, in cashew, mango, papaya and grapevine. In this study, the variation in the genetic diversity of 117 *L. theobromae* isolates from northeastern Brazil (n=100) and Mexico (n=17), which were collected from these four crops, was analyzed using microsatellite markers. The results revealed low genetic diversity among *L. theobromae* populations and the existence of two genetic groups. All Mexican isolates were grouped with Brazilian isolates suggesting a low level of differentiation between these populations. Furthermore, no evident host or climate-based population differentiation was observed for *L. theobromae* in Brazil. The populations studied were mostly clonal, but additional studies are needed to better understand the mode of reproduction of the pathogen. The low genetic diversity of *L. theobromae* populations in northeastern Brazil suggests that resistant cultivars could be used as a durable management strategy to reduce the impact of the diseases caused by this pathogen.

Keywords: Microsatellites, population biology, SSR, variability

Introduction

Brazil ranks third in the world in fruit producing countries with more than 37 million t (FAO 2018), representing US$ 9.5 billion in value (IBGE 2018). Fruit crops are widely grown in the country, but the northeastern states are among the largest producers. In 2016, the northeastern region of Brazil produced 8.7 million t of fruit, corresponding to 23.5% of the national production (IBGE 2018). Of this total, 2.1 million t were obtained from cashew, mango, papaya,
and grapevine, which are the most economically relevant fruit crops grown in the region (IBGE 2018).

In recent years, fungal diseases affecting cashew, mango, papaya, and grapevine crops have been reported in northeastern Brazil, with prevalence of infections caused by members of the family Botryosphaeriaceae (Netto et al. 2014; Correia et al. 2016; Coutinho et al. 2017). Botryosphaeriaceae species are important pathogens of native and non-native plants worldwide (Slippers & Wingfield 2007; Philips et al. 2013). For instance, research on the identification and prevalence of Botryosphaeriaceae spp. in fruit producing orchards and at post-harvest conditions in northeastern Brazil showed that Lasiodiplodia theobromae (Pat.) Griff. & Maubl. (syn. Botryodiplodia theobromae Pat.) is present in all areas studied, being the most frequent species associated with dieback, gummosis, leaf spot, stem-end rot, and fruit rot symptoms (Netto et al. 2014; Correia et al. 2016; Coutinho et al. 2017).

Lasiodiplodia theobromae is widely distributed in the tropics and subtropics, causing important diseases in several species of host plants (Philips et al. 2013). This fungus, known as a latent pathogen, has an endophytic infection ability without exhibiting symptoms in colonized plant tissues until conditions are favorable, making its control difficult (Mohali et al. 2005; Slippers & Wingfield, 2007). In Brazil, there are few products registered for the management of L. theobromae, and are restricted to avocado (Persea americana), cotton (Gossypium spp.), Annona spp., banana (Musa spp.), cacao (Theobroma cacao), citrus (Citrus spp.), coconut (Cocos nucifera), and papaya (Carica papaya) crops.

Studies on population genetics often provide information on the biology and evolution of plant pathogens, sometimes contributing directly to disease management (Milgroom 2015). Knowledge obtained from genetic analysis of plant pathogen populations may be relevant for breeding and fungicide resistance management programs (McDonald & Linde 2002). Population genetics can be used to identify genetic patterns and processes of pathogen emergence and reemergence (Grünwald et al., 2017). A better understanding of these processes
is necessary to predict and mitigate their impact. Furthermore, population genetics can also be useful to infer the relative contribution of asexual and sexual reproduction in pathogen populations (Milgroom 1996).

On a global scale, genetics of *L. theobromae* populations have been studied primarily using microsatellite or simple-sequence repeat (SSR) markers (Mohali *et al.* 2005; Begoude *et al.* 2012; Shah *et al.* 2011). SSR markers have become very valuable for population genetic analysis because they are highly polymorphic, codominant, and locus-specific (Milgroom 2015). Rapidly mutating markers like SSRs offer unique advantages since genetic divergence in contemporary populations and within closely related individuals can be observed when it is not visible using other marker systems (Varshney *et al.* 2005). In a preliminary study, Burgess *et al.* (2003) developed eight polymorphic SSR markers for *L. theobromae* and determined that host species seemed to influence the population structure of this pathogen.

Other genetic diversity studies of *L. theobromae* in South Africa, Mexico, Venezuela and Punjab suggested that this species predominantly reproduces asexually (Mohali *et al.* 2005; Shah *et al.* 2011). However, there are still few studies involving the population genetics of this pantropical pathogen (Burgess *et al.* 2003; Shah *et al.* 2011; Begoude *et al.* 2012, Mehl *et al.*, 2017). Although recent research has studied the identity, distribution, and pathogenicity of *L. theobromae* in Brazil (Netto *et al.* 2014; Correia *et al.* 2016; Coutinho *et al.* 2017), currently there is a single study about the genetic diversity and structure of this pathogen isolated from coconut trees (*Cocos nucifera*) populations at a regional level, and compared with other countries.

In the present study, the variation in the genetic diversity of *L. theobromae* in northeastern Brazil and Mexico was analyzed using microsatellite markers with the following objectives: (i) to determine the structure and potential subdivision among and within populations based on the country of origin, and based on host and/or climate type within northeastern Brazil and, (ii) to infer about the relative contribution of the reproduction mode of the pathogen on the genetic
diversity observed. This information will provide critical insights into the pathogen biology and evolution with subsequent implications for disease management.

**Materials and methods**

**Fungal isolates and DNA extraction**

A total of 117 *L. theobromae* isolates from Brazil and Mexico were genotyped in this study (Supplementary Table 1). Isolates from Brazil (n = 100) and Mexico (n = 17) were obtained from the Culture Collection of Phytopathogenic Fungi “Prof. Maria Menezes” (CMM) of the Universidade Federal Rural de Pernambuco, Brazil. The identity of all isolates was confirmed by sequencing a partial region of the translation elongation factor 1-alpha (EF1-α) and the rDNA ITS region as described in previous studies (Netto *et al.* 2014; Correia *et al.* 2016; Coutinho *et al.* 2017). The isolates from Brazil were collected between 2006 and 2013 from cashew (n = 27), mango (n = 21), papaya (n = 29) and grapevine (n = 23), and the isolates from Mexico were collected in 2014 from mango (n = 17) (Supplementary Table 1).

The isolates from Brazil were grouped into two populations based on climate types according to Köppen’s climate classification map for Brazil (Alvares *et al.* 2013): tropical with dry summer (As) and semiarid (BSh). The As climate is located in coastal regions with a strong rainfall gradient (east to west), from 1,500 to 700 mm concentrated in a rainy season from May to July and a dry season from September to December. The BSh climate is dry, with high indices of insolation during most of the year with a drought period of nine months and a rainy period from February to April (Alvares *et al.* 2013). Both climate types-based populations included isolates from five states of northeastern Brazil: As climate (Ceará, Paraíba, Pernambuco, and Rio Grande do Norte) and BSh climate (Bahia, Pernambuco, and Rio Grande do Norte).
The Mexican isolates were obtained from mango orchards located in four states (Chiapas, Guerrero, Sinaloa, and Veracruz) (Supplementary Table 1).

Pure cultures of *L. theobromae* were grown in potato-dextrose-agar (PDA, Biokar diagnostics, Beauvais, France) medium and incubated at 25°C in the dark for a period of five days. Mycelium was scraped from the colony surface using a sterile pipette tip and genomic DNA was extracted using the E.Z.N.A. Plant Miniprep Kit (Omega Biotek®, Doraville, GA) following the manufacturer’s instructions.

**Haplotype identification**

Primers for eight previously characterized SSR loci (LAS3&4, Las13&14, LAS15&16, LAS17&18, LAS21&22, LAS27&28, LAS29&30, and LAS 35&36) (Burgess *et al*. 2003) were tested for specific amplification, polymorphism and reproducibility of the allele calls with a selection of 11 *L. theobromae* isolates representing different origins and hosts. Primers were synthesized by Metabion International (Berlin, Germany). All amplifications were performed in 20 μl reaction volume containing 1x PCR buffer, 1.5 mM MgCl₂, 0.2 mM dNTPs, 0.2 μM each primer, 0.05 U Horse-Power™ Taq DNA polymerase (Canvax Biotech, S.L. Córdoba, Spain), and 1 μl of DNA template. PCR amplifications were performed on a Veriti 96 Well Thermal Cycler (Applied Biosystems, Foster City, CA) using the following conditions: an initial step of 5 min at 94°C, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 56°C for 30 s, elongation at 72°C for 30 s and a final extension at 72°C for 6 min. Amplification products were purified (Kit Quickclean, dominion MBL, Córdoba, Spain) and sequenced to validate the variation in the number of SSR repeats at the sequencing service of the Institute of Molecular and Cellular Biology of Plants (IBMCP, UPV-CSIC).

Fluorescent dye labeling of PCR fragments was performed for each for each SSR locus using three primers in each reaction: SSR forward primer with M13 tail...
(TGTAAAACGACGGCCAGT) at its 5′ end, SSR reverse primer, and the fluorochrome 6-carboxyfluorescein (FAM)-labeled M13 primer (TGTAAAACGACGGCCAGT) (Invitrogen, Carlsbad, CA), as described by Schuelke (2000) with minor modifications (Berbegal et al. 2013). PCR reactions (20 µl) were performed with the following final concentrations: 1x PCR buffer, 0.2 mM dNTPs, 0.04 µM M13-SSRf primer, 0.16 µM SSRr primer, 0.16 µM FAM-M13 primer, 0.05 U Horse-Power™ Taq DNA polymerase, and 1 µl of DNA template. The PCR program consisted of an initial step of 2 min for 94°C followed by 35 cycles of denaturation 94°C for 30 s, 56°C for 30 s, 72°C 30 s, and final extension 72°C for 6 min.

Samples were prepared in 96-well plates using 0.1 µl of GeneScanTM500 LIZ® internal standard (Applied Biosystems, Foster City, CA), 10.9 µl of Hi-Di Formamide (Applied Biosystems), and 1 µl of the SSR-PCR product. A negative control was included in each plate. Capillary electrophoresis to determine the sizes of the fluorescent labeled fragments was performed on a 3130xl/Genetic Analyzer® sequencer (Applied Biosystems) at the sequencing service of the Institute of Molecular and Cellular Biology of Plants (IBMCP, UPV-CSIC). Allele sizes were determined using the GeneMarker V2.6.3 software package (Applied Biosystems). The analysis was replicated for a subset of 11 isolates to confirm reproducibility of results.

Data analysis

Genetic diversity and mode of reproduction. For each population defined either by country of origin or climate within northeastern Brazil, the total number of alleles and allele frequencies at each SSR locus were estimated. A multilocus genotype (MLG) was constructed for each isolate by combining data for single SSR alleles and expected multilocus genotype (eMLG) based on rarefaction were calculated using the R package poppr V.2.3.0 (R Core Team 2017; Kamvar et al. 2015). Given the clonality observed analyses were conducted for the clone-
corrected data set, only one isolate of each MLG for each geographical location and host by population were considered. The Stoddart and Taylor’s diversity index (G) (1988) and evenness index $E_5$ (Grünwald et al. 2003) were calculated using the same R package.

The standardized index of association ($r_{\text{barD}}$) as an estimate of linkage disequilibrium, was calculated to investigate the mode of reproduction (Agapow & Burt 2001; Kamvar et al. 2015). The expectation of $r_{\text{barD}}$ for a randomly mating population is zero and significant deviation from this value would suggest clonal reproduction. The test was not performed in Mexico population with a very low sample size after clone correction due to lack of statistical power (Fincham & Day, 1963). Significance was tested based on 999 permutations and conducted in the R package poppr using the clone corrected data (Kamvar et al. 2015).

**Population structure.** The standardized measure of genetic differentiation $G^\prime$st described by Hedrick (2005) is suitable to estimate subdivision among populations. It ranges from 0 to 1, independent of the extent of population genetic variation and loci mutation rates (Hedrick, 2005). Pairwise $G^\prime$st values within the clone corrected data were calculated using the R packages strataG V.1.0.5 (Archer et al. 2017) and mmod V.1.3.3 (Winter 2012). Statistical significance was calculated based on 1000 permutations. Analysis of Molecular Variance (AMOVA) was performed using the R package ade4 V.1.7-5 (Dray & Dufour 2007; Excoffier et al. 1992). AMOVA was calculated on clone corrected data to estimate the variance explained by populations defined by country of origin or individuals within populations. Statistical significance was tested using 999 permutations.

Discriminant analysis of principal components (DAPC) was performed to infer clusters of populations without considering previous geographic/climate types-based assignment criteria. This approach is based on sequential K-means clustering of principal component analysis (PCA) and discriminant analysis (DA) (Jombart et al. 2010). DAPC was conducted with the R package adegenet V. 2.0.1 (Jombart 2008) using the Bayesian information criterion (BIC) to infer the optimal number of groups. Important advantages of the DAPC are that it maximizes
variation between the groups, minimizing the within-group genetic variability and it does not require assumptions regarding evolutionary models (Jombart et al. 2010).

To assess the relationships among MLGs, minimum spanning network (MSN) was constructed from the clone-corrected dataset including isolates from Brazil and Mexico. Bruvos’s genetic distance matrix and MSN were generated using the R package poppr V.2.3.0 (Kamvar et al. 2015; R Core Team 2017). Genetic distance described by Bruvo et al. (2004), takes SSR repeat number into account being a distance of 0.1 equivalent to one mutational step (one repeat). The neighbor-joining algorithm based on Bruvo’s distance was also run using the same R package with 1,000 bootstrap replicates. In the resulting tree, DAPC clustering was represented to visualize how both clustering patterns hold up.

Results

Six of the eight primer pairs successfully amplified SSR loci for *L. theobromae* from Brazil and Mexico. Allele sizes determined by DNA sequencing of 11 representative isolates were consistent with allele sizes determined by fragment analysis. In addition, fragment analysis replication for these 11 isolates showed consistent results, confirming reliability and reproducibility of the method. Three loci (Las3&4, Las21&22, and Las35&36) were polymorphic for the 117 isolates genotyped. The number of observed alleles per locus was 4 resulting in a total of 11 MLGs (Table 1). The Brazilian population exhibited 5 MLGs, the Mexican population 4 MLGs, and both populations shared 2 MLGs (Figure 1A).

A clone correction of data was performed to remove the bias of resampled MLG in the analysis, resulting in 42 representative isolates of the two climate types (17 in BSh and 25 in As) of northeastern Brazil and 10 representative isolates of Mexico. Of the 6 MLGs observed in the Mexican population, the most frequent one was MLG11 (40%), followed by MLG7 (20%); the remaining 4 MLGs were observed once (10%) (Figure 1A). In Brazil, 6 (MLG1,
MLG3, MLG4, MLG5, MLG6, and MLG7) of the 7 MLGs were present in the two climate types studied (Figure 2B). The most frequent MLG was MLG7 (47.6%), followed by MLG6 (21.4%), and the less frequent MLGs were MLG1 and MLG2 (4.8%), with genotype MLG2 exclusive of the As climate population (Figure 2B).

In general, populations showed low genetic diversity. The genotypic richness (R) for the Mexican population was higher (0.56) than was observed for the Brazilian population (0.15) (Table 1). Evenness (E5) values were 0.66 and 0.79 for Brazilian and Mexican populations, respectively (Table 1). A high level of genotypic diversity (G) was observed for the Mexican population (4.17), whereas the Brazilian population exhibited lower diversity (3.42) (Table 1). When considering the Brazilian populations defined by climate types, R and E5 values were low and similar for both populations, ranging from 0.31 to 0.25 and from 0.71 to 0.66, for BSH and As populations respectively (Table 1). Genotypic diversity values observed were also low for populations of different climate types in Brazil (3.32 and 3.42 for BSh and As populations, respectively) (Table 1).

Global population from Brazil showed significant deviation in rbarD value from the null hypothesis of recombination supporting clonal reproduction (Table 1). However, when considering populations defined by climate types in Brazil, As population showed consistent results with the global population but for BSh population the null hypothesis could not be rejected (P = 0.016) indicating linkage equilibrium or presence of sexual reproduction (Table 1).

The minimum spanning network (MSN) revealed two main groups when considering the global population of Brazil and Mexico. The first group included MLGs of both countries and the second group only two MLGs exclusive of Brazil (MLG3 and MLG4) (Figure 1A). The MSN performed between the isolates belonging to the two different climate types of northeastern Brazil showed one main group which was present in both populations including
the most frequent MLGs (MLG6 and MLG7), and a second small group with two MLGs being one MLG exclusive of the As population (MLG2) (Figure 1B).

The Bruvo’s distance-based dendrogram using Neighbor-Joining grouped the 11 genotypes of the global population into two groups. The cluster I consisted of two MLGs and cluster II included nine MLGs. Multivariate analysis showed the presence of five subgroups represented by colors in the dendrogram, where cluster I grouped one subgroup (green) and cluster II grouped four (purple, blue, black and red) (Figure 2A). For northeastern Brazil populations, two main groups were also identified. Cluster I was represented by two MLGs (MLG1 and MLG2), whereas the rest of the MLGs were grouped in the Cluster II. The multivariate analysis showed four subgroups in the dendrogram, where cluster I grouped one subgroup (blue) and cluster II grouped three (red, green, and purple) (Figure 2B).

Pairwise G’st values calculated on the clone corrected data showed very low genetic differentiation among Mexico and Brazil populations and within Brazilian populations (G’st = 0.104 and G’st = 0.014, respectively, $P > 0.01$). Results of population subdivision analysis based on G’st were consistent with those obtained by the AMOVA. Analysis of molecular variance on the clone corrected data revealed only 3.4% of variation between Mexican and Brazilian populations ($P = 0.16$). None of the calculated values were statistically significant showing that further sampling is likely needed.

**Discussion**

To the best of our knowledge, this is the first study that aims to investigate the genetic structure and the mode of reproduction of a collection of *L. theobromae* isolates obtained from different hosts in Brazil and Mexico. These populations were defined based on the country of origin and
climate types within northeastern Brazil, and the analysis was performed using the microsatellite markers developed by Burgess et al. (2003).

Minimum spanning network analysis and discriminant analysis of principal components identified two distinct genetic clusters in the Brazilian and Mexican populations of *L. theobromae*, the first one with nine MLGs including the two most common MLGs identified in this study (MLG11 and MLG10), and the second one containing two MLGs only found in Brazil (MLG3 and MLG4). A limitation of this work that need to be stated is the unbalanced number of isolates from Mexico, which ideally should have represented more hosts than only mango. Studies based on culture collections are sometimes associated with this kind of issues. However, the ideal strategy of sampling and sample size are often difficult to put in practice and depend on many factors including the questions being addresses, the type of genetic markers used and the resources available to support the project (Milgroom, 2015)

All Mexican isolates were grouped with Brazilian isolates suggesting the existence of a low level of differentiation between these populations. These results agree with those obtained by Burgess et al. (2003) indicating that geographical location did not determine the genetic relationship between isolates of *L. theobromae*. No evident population differentiation based on host or climate was observed for *L. theobromae* in Brazil. These results are consistent with those found in previous studies where the relationships between *L. theobromae* isolates from different hosts and geographical origins did not contribute in the differentiation of pathogen haplotypes (Begoude et al. 2012; Mohali et al. 2005).

*Lasiodiplodia theobromae* spores develop in a sticky matrix, are relatively large (21.5–31.5 × 13–17 μm) (Phillips et al. 2013), and can be dispersed by wind or rain splash (Úrbez-Torres 2011). In this context, the spores are not likely to be spread over large distances (Mehl et al. 2017). A likely explanation for the genetic similarity among the studied populations would be the movement of infected plant material (Begoude et al. 2012). *Lasiodiplodia theobromae* is recognized as a latent pathogen that could be dispersed when seeds or asymptomatic plant
material are moved between different regions (Burgess et al. 2016; Mohali et al. 2005). In a recent study, Mehl et al. (2017) concluded that *L. theobromae* isolates collected from different hosts and countries of the world represent a single species distributed worldwide, without apparent phylogeographic structure, and suggested that the most likely explanation for this result is the long-distance human dispersal of this pathogen.

Seven MLGs were obtained using the SSR markers in the populations of *L. theobromae* from Brazil. The lack of diversity found in this study emphasizes the low genetic diversity of this fungus in northeastern Brazil. This is contrast with results about population genetics of *L. theobromae* from coconut trees in Brazil and other countries based on EF1-α sequences analysis (Santos et al., 2017). In this study, relatively high levels of genetic diversity were observed in population from northeastern and southeast Brazil. However, for this specific and according to authors, the variability of *L. theobromae* haplotypes would be associated to a selection process experienced by the pathogen due high host diversity and control management strategies adopted by different regions in Brazil (Santos et al., 2017).

Some other studies have analyzed the genetic structure of *L. theobromae* populations from different countries and hosts with variable results. Begoude et al. (2012) reported high to moderate levels of genetic diversity of *L. theobromae* collected from *Theobroma cacao* and *Terminalia* spp. in Cameroon. Mohali et al. (2005) reported low levels of genotypic diversity of *L. theobromae* collected from *Pinus caribaea* var. *hondurensis*, *Eucalyptus urophylla* and *Acacia mangium* in Venezuela, and Shah et al. (2011) reported high levels of genetic diversity of *L. theobromae* collected from *Pyrus* sp. in India. These variable results might be related to the intrinsic characteristics of the different countries and hosts used in each study.

*Botryosphaeria rhodina* (Berk. & M. A. Curtis) Arx. was reported as the teleomorph of *L. theobromae*. However, the connection between *L. theobromae* and its sexual morph has not been proven conclusively (Phillips et al. 2013). Furthermore, in recent years, cryptic species have been described from isolates previously identified as *L. theobromae* (Burgess et al. 2006;
Alves et al. 2008; Cruywagen et al. 2017), leading to uncertainty on the accuracy of information regarding the sexual status of *L. theobromae* (Begoude et al. 2012). In the present study, linkage disequilibrium analysis showed evidence of recombination in *L. theobromae* population of the BSh climate type. However, in nature, it is difficult to observe sexual structures of *L. theobromae* and the fungus appears to exist predominantly in an asexual form (Mohali et al. 2005).

In Brazil, the BSh climate type is characterized by annual mean precipitation of less than 800 mm (Alvares et al. 2013). The conditions of temperature, luminosity and relative humidity in this climatic region, as well as the use of technologies, such as irrigation management, facilitate an intensive fruit production throughout the year. However, it is likely that stress conditions experienced by plants are extended to plant pathogens by forcing them to recombine. Thus, our results suggest that *L. theobromae* may develop sexual state in its life cycle under such conditions. In a previous study performed by Begoude et al. (2012), using SSR markers with populations of *L. theobromae* and *L. pseudotheobromae* from *Terminalia* spp. and *T. cacao*, these authors observed that these two species develop regular sexual reproduction in humid forests areas with bimodal rainfall in Cameroon. Considering the results obtained, it is difficult to reach conclusions about the species mode of reproduction and further research is needed to analyze such question.

Overall, in the present study, the population structure and mode of reproduction of *L. theobromae* were explored using the microsatellite markers developed by Burgess et al. (2003). The results showed low genetic diversity, and two genetic clusters were identified, without structure based on the geographic region, host of origin or climate type within Brazil. Populations studied were mostly clonal but further studies are needed to better understand the mode of reproduction of the pathogen. Our results suggest that the management of *L. theobromae* infections will not likely be so challenging as for other Botryosphaeriaceae species. For example, development of resistant cultivars for commercial cultivation could be used to
reduce the impact of diseases caused by *L. theobromae*. Since populations of this latent pathogen present low diversity in northeastern Brazil, it is possible that the durability of resistant cultivars would be relatively long-lived (McDonald & Linde 2002).

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**Conflicts of Interest:** The authors declare no conflicts of interest.

**References**


Figure 1 Minimum spanning network from the clone corrected data showing the relationships among the individual multilocus genotypes (MLGs). A. The populations from Brazil (BR) and Mexico (MX). B. The northeastern Brazil population of Lasiodiplodia theobromae defined by climate types (Alvares et al. 2013). Each node represents a different MLG. Distances and thickness of the lines between nodes are proportional to Bruvo’s distance (Bruvo et al. 2004). Node colors and sizes correspond the population studied and number of individuals, respectively.

Figure 2 Neighbor-Joining dendrogram of Bruvo’s genetic distance (Bruvo et al. 2004) among: A, seven observed multilocus genotypes in Brazil (MLG11, MLG10, MLG5, MLG6, MLG9, MLG3, MLG4) and six MLGs in Mexico (MLG11, MLG10, MLG7, MLG1, MLG2, MLG8), and B, seven observed MLGs in northeastern Brazil. The isolates are colored according to PCA analyses results: A, in green, MLGs assigned to cluster I, in purple, blue, black and red, MLGs assigned to cluster II; B, in blue, MLGs assigned to cluster I, in red, green and purple, isolates assigned to cluster II. Support values greater than 70% using 1,000 bootstrap samples are shown.
Figure 1A
Figure 1B

POPULATION
- BSh
- As

DISTANCE

0.107  0.25  0.34  0.51  0.90