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Genetic diversity of *Mycosphaerella fijiensis* in Brazil analyzed using an ERIC-PCR marker

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ABSTRACT. The Enterobacterial repetitive intergenic consensus (ERIC) marker was used to analyze the genetic variability of *Mycosphaerella fijiensis*, the causative agent of Black Sigatoka disease in banana plants. A total of 123 isolates were used, which were divided into populations based on their original hosts and collection sites in Brazil. A total of 9 loci were amplified, 77.8% of which were found to be polymorphic. The genetic diversity found in the population was 0.20. Analysis of molecular variance (AMOVA) demonstrated that the highest level of genetic variation is within populations. Cluster analysis revealed three main groups in Brazil, with no correlation between geographic and genetic distance.

Key words: Black Sigatoka; *Pseudocercospora fijiensis*; Banana plant; *Musa* spp; Genetic variability

INTRODUCTION

Black Sigatoka, which is caused by the fungus *Mycosphaerella fijiensis* Morelet [anamorphic stage: *Pseudocercospora fijiensis* (Morelet) Deighton], is the most destructive disease affecting banana plants (*Musa* spp) in all producing regions. The pathogen causes leaf necrosis and drying, significantly reducing photosynthesis and consequently, production, leading to a reduction in the size and quality of fruits, which renders them commercially unviable (Stover and Simmonds, 1987). Disease control is difficult, especially in the susceptible banana cultivars of the Cavendish (Churchill, 2011), Prata, and Terra (Burt et al., 1997) subgroups, for which the disease is more severe. Multiple fungicides are commonly applied at high frequencies given the susceptibility of these cultivars. However, fungicides are highly detrimental to the environment, the health of workers who handle them, the inhabitants of areas in which they are applied, and consumers. *M. fijiensis* is a serious problem for small producers who do not have the financial resources to perform chemical control in their banana plantations (Marín et al., 2003).

Losses caused by Black Sigatoka in banana and plantain crops may reach up to approximately 80% of production (Churchill, 2011) and 100% from the second cycle in the Maçã, Prata Comum, Terra, and D'Angola cultivars in the northern region of Brazil (Gasparotto et al., 2001). The continuous use of a single product or products of the same chemical group promotes the onset of pathogen strains resistant to the fungicides used. The use of doses below the recommended dose also generates selection pressure favoring resistant pathogen strains, resulting in reduction of the effectiveness of fungicides, as found in Central America in the case of strobilurin (Marín et al., 2003) and propiconazole (Castro et al., 1995; Guzmán and Romero, 1997; Romero and Sutton, 1997).

Studies using experimental hybrids are currently underway to create plants that are genetically resistant to *M. fijiensis* (Ferreira et al., 2004). However, the high genetic diversity found in *M. fijiensis* may represent an obstacle to the development of resistant plants because strains able to infect the resistant plants could be rapidly selected.

Understanding the genetic structure of fungal populations and how pathogens evolve is a key aspect for developing disease control strategies and breeding programs aimed at plant resistance to disease (Churchill, 2011). Studies in populations of *M. fijiensis* showed that this fungus exhibits high genetic variability (Rivas et al., 2004; Halkett et al., 2010). Recently, Robert et al. (2012) showed that Southeast Asia was the center of origin of *M. fijiensis* by analyzing the introduction routes of *M. fijiensis* in four continents using isolates from 37 countries. The authors also noted the occurrence of bottlenecks (decreased genetic diversity) in other continents and suggested that *M. fijiensis* originally migrated from southeastern Asia to Oceania and Africa, and simultaneously from Southeast Asia and Oceania to America. In Brazil, Black Sigatoka was first identified in 1998 in the municipalities of Tabatinga and Benjamin Constant in the state of Amazonas (Pereira et al., 1998). However, little is known about the current genetic diversity of this fungus in Brazil.

Various molecular marker systems have been used to study the genetic variability of populations of *M. fijiensis*, including random amplified polymorphic DNA (RAPD) (Johanson et al., 1994), restriction fragment length polymorphism (RFLP) (Carlier et al., 1996; Hayden et al., 2003), single nucleotide polymorphism (SNP) (Zandjanakou-Tachin et al., 2009), and microsatellite (Halkett et al., 2010; Robert et al., 2012) markers, which have demonstrated

high genetic variability in most populations studied.

Enterobacterial repetitive intergenic consensus (ERIC) elements were first reported by Sharples and Lloyd (1990), and originally termed intergenic repeat units (IRUs), and were subsequently renamed as ERIC (Hulton et al., 1991). These elements are similar to the miniature inverted repeat transposable elements (MITEs) of Archaea and eukaryotes (Delihas, 2008), and are 124-127 bp in size with highly conserved, repetitive, and reversed sequences in their central regions, which are located in extragenic regions (Sharples and Lloyd, 1990). Molecular marker systems based on ERIC elements have become a powerful alternative tool for microbiological diagnostics owing to their simplicity, speed, reproducibility, and accuracy (He et al., 2012). Thus, ERIC-based systems have been widely used in studies on genetic variability and genotyping in bacteria and fungi, as reported for *Acetobacter pasteurianus* (Wu et al., 2010), *Fusarium solani* (Godoy et al., 2004), *Candida* spp (Chong et al., 2007), and *Aureobasidium pullulans* (Loncaric et al., 2009).

The ERIC-based molecular marker was chosen in this study because approximately 50% of the *M. fijiensis* genome consists of repetitive elements, 11.7% of which correspond to transposable elements (TEs) (Santana et al., 2012). Therefore, this study aimed to analyze the genetic diversity in populations of *M. fijiensis* from various regions of Brazil using ERIC-polymerase chain reaction (PCR).

MATERIAL AND METHODS

Preparation of *M. fijiensis* isolates

The isolates were prepared from leaves of banana plants with symptoms typically induced by *M. fijiensis* collected at 15 locations in Brazil between the years 2008 and 2009 (Table 1), which belonged to the culture collection of Empresa Brasileira de Pesquisa Agropecuária (Embrapa) Amazônia Ocidental/Centro de Pesquisa Agroflorestal da Amazônia Ocidental. Conidia were isolated from young lesions using the direct isolation method. All the 123 isolates were prepared from monosporic cultures.

DNA extraction

Mycelia were grown for 15 days in 50 mL enriched potato dextrose broth (PDB; 250 g boiled potato extract in 500 mL water, 10 g dextrose, 2 g peptone, 1.5 g hydrolyzed casein, and 2 g yeast extract per L water), and maintained with constant stirring at 120 rpm. The mycelia were recovered, washed, filtered, and stored at -80°C for subsequent extraction. The DNA was prepared by grinding the mycelium using the cetyltrimethyl ammonium bromide (CTAB) method as described by Doyle and Doyle (1987). The quantity and quality of DNA was assessed by 0.8% agarose gel electrophoresis and using the NanoDrop spectrophotometer.

PCR parameters for ERIC

Each PCR was performed in a total volume of 20 µL with 0.5 mM each primer, ERIC1R (5'-ATGTAAGCTCCTGGGGTTCAC-3') and ERIC2 (5'-AAGTAAGTGACTGGG GTGAGCG-3'), 0.8 mM each dNTP, 2 mM $MgCl_2$, 50 ng DNA, 1X buffer, and 1 unit *Taq* DNA

Table 1. *Mycosphaerella fijiensis* isolates used in this study.

Isolates	Origin*	Cultivars
1	Presidente Figueiredo-AM	Maçã
2	Presidente Figueiredo-AM	Thap Maeo
3	Presidente Figueiredo-AM	FHIA 18
5	Presidente Figueiredo-AM	D'Angola
105	Presidente Figueiredo-AM	D'Angola
113	Presidente Figueiredo-AM	Prata Comum
115	Presidente Figueiredo-AM	Unidentified
123	Presidente Figueiredo-AM	D'Angola
127	Presidente Figueiredo-AM	Caru Roxa
129	Presidente Figueiredo-AM	Maçã
4	Manaus-AM	Prata Anã
44	Manaus-AM	Prata Comum
46	Manaus-AM	Maçã
47	Manaus-AM	Maçã
49	Manaus-AM	D'Angola
50	Manaus-AM	Maçã
52	Manaus-AM	Maçã
54	Manaus-AM	Prata Comum
58	Manaus-AM	Maçã
59	Manaus-AM	D'Angola
6	Manacapuru-AM	Prata Comum
7	Manacapuru-AM	D'Angola
8	Manacapuru-AM	D'Angola
9	Manacapuru-AM	D'Angola
10	Manacapuru-AM	Maçã
13	Manacapuru-AM	D'Angola
16	Manacapuru-AM	Unidentified
62	Manacapuru-AM	Maçã
63	Manacapuru-AM	Prata Comum
68	Manacapuru-AM	D'Angola
72	Manacapuru-AM	D'Angola
76	Manacapuru-AM	D'Angola
77	Manacapuru-AM	Maçã
17	Rio Preto da Eva-AM	Prata Comum
18	Rio Preto da Eva-AM	Caru Verde
19	Rio Preto da Eva-AM	Prata Comum
20	Rio Preto da Eva-AM	Maçã
24	Rio Preto da Eva-AM	Prata Comum
25	Rio Preto da Eva-AM	D'Angola
29	Rio Preto da Eva-AM	D'Angola
30	Rio Preto da Eva-AM	Prata Comum
31	Rio Preto da Eva-AM	Prata Comum
32	Rio Preto da Eva-AM	Prata Comum
33	Rio Preto da Eva-AM	Prata Comum
37	Rio Preto da Eva-AM	Maçã
38	Rio Preto da Eva-AM	Prata Comum
40	Rio Preto da Eva-AM	D'Angola
41	Rio Preto da Eva-AM	Maçã
42	Rio Preto da Eva-AM	Maçã
22	Itacoatiara-AM	Caru Verde
43	Itacoatiara-AM	Caru Roxa
150	Itacoatiara-AM	Prata Comum
151	Itacoatiara-AM	D'Angola
182	Itacoatiara-AM	FHIA 18
188	Itacoatiara-AM	Maçã
189	Itacoatiara-AM	Caru Verde
190	Itacoatiara-AM	Prata Comum
191	Itacoatiara-AM	Caru Roxa
192	Itacoatiara-AM	Nanica
195	Itacoatiara-AM	D'Angola
144	Careiro Castanho-AM	D'Angola

Continued on next page

Table 1. Continued.

Isolates	Origin*	Cultivars
154	Careiro Castanho-AM	Prata Comum
164	Careiro Castanho-AM	Caru Verde
167	Careiro Castanho-AM	Prata Comum
173	Careiro Castanho-AM	D'Angola
174	Careiro Castanho-AM	Maçã
223	Iranduba-AM	Unidentified
224	Iranduba-AM	Nanica
225	Iranduba-AM	Nanica
226	Iranduba-AM	Prata Comum
227	Iranduba-AM	Prata Comum
228	Iranduba-AM	Caru Verde
229	Iranduba-AM	Caru roxa
230	Iranduba-AM	Prata Comum
231	Iranduba-AM	Prata Comum
232	Iranduba-AM	Prata Comum
233	Iranduba-AM	D'Angola
234	Iranduba-AM	D'Angola
198	Miracatu-SP	Prata Comum
199	Miracatu-SP	Prata Comum
204	Miracatu-SP	Prata Comum
205	Miracatu-SP	Prata Comum
201	Pedro de Toledo-SP	Nanica
202	Pedro de Toledo-SP	Prata Comum
203	Pedro de Toledo-SP	Terra
240	Pedro de Toledo-SP	Prata Comum
209	Eldorado-SP	Prata Comum
210	Eldorado-SP	Prata Comum
212	Eldorado-SP	Nanica
213	Eldorado-SP	Prata Comum
219	Pariquera-Açu-SP	Maçã
220	Pariquera-Açu-SP	Nanica
221	Pariquera-Açu-SP	Nanica
222	Pariquera-Açu-SP	Figo
67	Cáceres-MT	Figo (Cinza)
81	Cáceres-MT	Calypso
82	Cáceres-MT	IAC 2001
86	Cáceres-MT	Maçã
87	Cáceres-MT	Grand naine
88	Cáceres-MT	Terrinha
109	Cáceres-MT	D'Angola
106	Caroebe-RR	Maçã
111	Caroebe-RR	Prata Comum
114	Caroebe-RR	Prata Comum
118	Caroebe-RR	Maçã
119	Caroebe-RR	Prata Comum
120	Caroebe-RR	Maçã
121	Caroebe-RR	Prata Comum
124	Caroebe-RR	Prata Comum
126	Caroebe-RR	Maçã
130	Caroebe-RR	Pacovan
131	Caroebe-RR	Pacovan
132	Caroebe-RR	Prata Comum
157	Rio Branco-AC	Grand naine
163	Rio Branco-AC	Prata Comum
171	Rio Branco-AC	D'Angola
172	Rio Branco-AC	SH 3640
177	Rio Branco-AC	Figo Cinza
161	Porto Velho-RO	Maçã
162	Porto Velho-RO	Pacovan
169	Porto Velho-RO	Prata Comum
170	Porto Velho-RO	Caru Roxa
175	Porto Velho-RO	Caru Roxa

*AM = Amazonas; AC = Acre; RO = Rondônia; RR = Roraima; MT = Mato Grosso; and SP = São Paulo.

polymerase. The PCR program consisted of: initial denaturation at 94°C for 1 min, followed by 30 cycles at 94°C for 1 min, 52°C for 1 min, and 65°C for 5 min, with a final extension of 16 min at 65°C. The amplification products were separated by electrophoresis on 1.5% agarose gel stained with 0.3 µg/mL ethidium bromide in 0.5X Tris/borate/EDTA (TBE) buffer (2 mM ethylenediaminetetraacetic acid (EDTA), 0.1 M Tris-HCl, 0.1 M boric acid, pH 8.0). The detection of amplification products was performed using the L-PIX Image software (for acquisition and treatment of images from Loccus Biotecnologia). The 1-kb Plus DNA Ladder (Invitrogen) was used to estimate the size of amplicons.

Data analysis

The bands were identified with the numbers 1 (presence) and 0 (absence). The isolates were grouped into 15 and 7 populations according to collection site and original host, respectively. Genetic diversity in the two groups of populations was measured based on Nei's gene diversity index (Nei, 1978). The genetic distance between isolates was calculated using Nei's genetic distance (Nei, 1973; Nei and Li, 1979). Based on this coefficient, a dendrogram was drawn using the unweighted pair group method with arithmetic mean (UPGMA) and with the POPGENE population genetic analysis software (Yeh et al., 2000). Genotypic diversity (D_G) was estimated with the Shannon-Wiener *H'* index (Lewontin, 1972). The Arlequin software (Excoffier et al., 2005) was used to conduct the analysis of molecular variance (AMOVA).

RESULTS

The subpopulations were analyzed based on their geographical origins and were subdivided by collection site and host cultivar. The primers used exhibited high reproducibility and enabled the generation of amplicons in all specimens analyzed. Nine loci were amplified, 77.8% of which were polymorphic; 19 haplotypes were identified, including 6 singletons. Some haplotypes were widely distributed across various subpopulations.

A similar level of genetic diversity (H_E) and genotypic diversity (D_G) was detected in both the populations subdivided by collection site and in the populations subdivided by host cultivar (Table 2). The AMOVA results indicated that most of the genetic variability occurs within populations: 73.4% exists within the populations subdivided by collection site, and 100% exists within the populations subdivided by host cultivar (Table 3).

Table 2. Indices of genetic diversity in populations of *Mycosphaerella fijiensis*.

Population group	N	H_E (SE)	D_G (SE)
Collection site	123	0.20 (0.18)	0.32 (0.26)
Host cultivar	103	0.21 (0.19)	0.33 (0.26)

N = number of specimens; H_E= gene diversity (Nei, 1978); D_G = genotypic diversity; SE = standard error.

Table 3. Analysis of molecular variance (AMOVA) of ERIC-PCR in *Mycosphaerella fijiensis* isolates divided into 15 and 7 subpopulations according to geographical origin (collection sites) and host cultivar.

Source of variation	Degrees of freedom	Sum of squares	Variance components	Variation (%)
Between populations of cultivars	6	5.58	0.00	0.0
Within populations of cultivars	96	94.02	0.98	100.0
Between populations of collection sites	14	38.60	0.25	26.54
Within populations of collection sites	108	76.05	0.70	73.46

Cluster analysis based on collection site grouped the populations into three main groups (I, II, and II). The populations from Presidente Figueiredo-AM, Caroebe-RR, Cáceres-MT, Manaus-AM, Miracatu-SP, and Pedro de Toledo-SP, with a Nei's genetic distance of 2.0, were included in group I. The populations from Manacapuru-AM, Rio Preto da Eva-AM, Iranduba-AM, Eldorado-SP, and Pariquera-Açu-SP were included in group II, and the populations from Itacoatiara-AM, Careiro Castanho-AM, Porto Velho-RO, and Rio Branco-AC were included in group III (Figure 1).

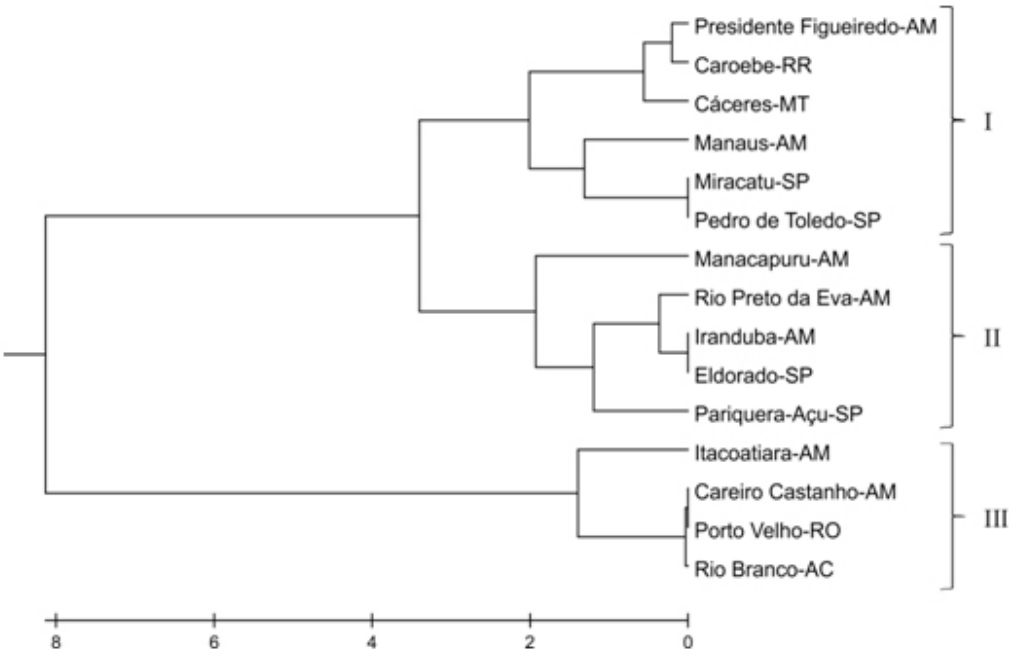

Figure 1. Genetic distance dendrogram including 15 populations of *Mycosphaerella fijiensis* according to the collection site in Brazil. The population of *M. fijiensis* was divided into subpopulations according to the collection site. The numbers above and below each node indicate the percentage of times each branch appears in the bootstrap analysis with 1000 replicates.

DISCUSSION

Recent data on the distribution of mating types in the *M. fijiensis* population of Brazil demonstrate the occurrence of sexual recombination, which should increase genetic diversity (Queiroz et al., 2013). However, the data obtained in our study indicate a decrease in genetic and genotypic diversity among subpopulations of *M. fijiensis* from different geographical origins in Brazil. This result may be due to the recent introduction of the fungus in the country, which was first reported in 1998 in the municipalities of Tabatinga and Benjamin Constant in the state of Amazonas (Pereira et al., 1998). Therefore, the results corroborate those found by Robert et al. (2012), who noted low genetic diversity in recently introduced populations and a trend toward decreased H_E with increased geographical distance in America, ranging from 0.44 in Honduras to 0.31 in Venezuela, when studying the introduction routes and global dispersion pattern of *M. fijiensis*.

The values found by AMOVA for populations subdivided by geographical origin demonstrated that the genetic variability of isolates was greater within collection sites than between collection sites; 73.4% of the total variability was found within each collection site. Similar results were found within isolates grouped by host, with 100% of the total variability observed in local populations. There was low genetic variability observed between populations subdivided by geographical origin and host cultivar because many alleles are shared between the different subpopulations. Conidia and ascospores are dispersed by wind from infected crops and may be spread over large distances from the source (inoculum), although ascospores play a key epidemiological role in the dispersal of *M. fijiensis* (Gauhl et al., 2000; Churchill, 2011). Therefore, according to Hayden et al. (2003), the dispersion of haplotypes may occur through the transport of material from the infected plant.

The dendrogram constructed based on the analysis of Nei's genetic distances revealed no correlation between geographic and genetic distance. Isolates from geographically distant regions exhibited high genetic similarity, as found in isolates from Iranduba-AM and Eldorado-SP, which are more than 2700 km apart in a straight line. Conversely, the isolates from Presidente Figueiredo-AM and Itacoatira-AM, separated by only 210 km in a straight line, exhibited high genetic distance. In the dendrogram of group I, the isolates from Presidente Figueiredo-AM and Caroebe-RR exhibited high genetic similarity. Presidente Figueiredo-AM is located at the edge of a highway linking the state of Roraima to Manaus, the capital of Amazonas. In Amazonas, the transport of bananas destined for Manaus is not conducted properly. The banana bunches are stacked directly on the boats and on the bodies of the trucks, and are bundled together using banana leaves, which often show symptoms of Black Sigatoka (Fancelli, 2003). Therefore, the high genetic similarity between the isolates from Presidente Figueiredo-AM and Caroebe-RR apparently results from anthropogenic activity, which is responsible for pathogen dispersion through the transport of infected plant material (Robert et al., 2012) because the same trucks transport bananas within the state of Amazonas and between the state of Roraima (city of Caroebe) and Amazonas (city of Manaus). Thus, the dispersion of ascospores may lead to exchange of genetic material between the fungal populations (Churchill, 2011). Indeed, *M. fijiensis* was originally found in Amazonas, and the results from this study indicate that specimens from Amazonas migrated to other regions, thereby enabling the formation of new populations.

This study found that ERIC-PCR is a low-cost and highly reproducible marker system in *M. fijiensis* that is useful in the study of genetic variability of the species. Our results further demonstrated that the Brazilian population of *M. fijiensis* exhibits high genetic variability, which is higher within each population than between populations. Furthermore, the results of our study are important to help disease control and to promote the success of genetic breeding programs aimed at developing cultivars of banana plants that are resistant to Black Sigatoka.

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REFERENCES

Burt PJA, Rutter J and Gonzales H (1997). Short-distance wind dispersal of the fungal pathogens causing Sigatoka diseases in banana and plantain. *Plant Pathol.* 46: 451-458.

Carlier J, Lebrun MH, Zapater MF and Dubois C (1996). Genetic structure of the global population of banana black leaf

streak fungus, *Mycosphaerella fijiensis*. *Mol. Ecol.* 5: 499-510.
Castro O, Wang A and Campos LF (1995). Análisis *in vitro* de la sensibilid de *Mycosphaerella fijiensis* a los fungicidas fenarimol, tridemorph y propiconazole. *Phytopathology* 85: 382.
Chong PP, Abdul Hadi SR, Lee YL, Phan CL, et al. (2007). Genotyping and drug resistance profile of *Candida* spp. in recurrent and one-off vaginitis, and high association of non-albicans species with non-pregnant status. *Infect. Genet. Evol.* 7: 449-456.
Churchill AC (2011). *Mycosphaerella fijiensis*, the black leaf streak pathogen of banana: progress towards understanding pathogen biology and detection, disease development, and the challenges of control. *Mol. Plant Pathol.* 12: 307-328.
Delihas N (2008). Small mobile sequences in bacteria display diverse structure/function motifs. *Mol. Microbiol.* 67: 475-481.
Doyle JJ and Doyle JL (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem. Bull.* 19: 11-15.
Excoffier L, Laval G and Schneider S (2005). Arlequin (version 3.0): an integrated software package for population genetics data analysis. *Evol. Bioinform. Online.* 1: 47-50.
Fancelli M (2003). Cultivo da banana para o estado do Amazonas. In: Embrapa Mandioca e Fruticultura. Sistema de Produção. In: Brazilian Agricultural Research Corporation (Embrapa) Cassava and Fruits. Production System. Available at [http://sistemasdeproducao.cnptia.embrapa.br/FontesHTML/Banana/BananaAmazonas/mercado.htm]. Accessed January 17, 2013.
Ferreira CF, Silva SO, Sobrinho NPD and Da Paz OP (2004). Molecular characterization of banana (AA) diploids with contrasting levels of black and yellow sigatoka resistance. *Am. J. Appl. Sci.* 1: 276-278.
Gasparotto L, Pereira JCR and Trindade DR (2001). Situação atual da Sigatoka negra da bananeira. *Fitopatol. Bras.* 26: 449.
Gauhl F, Pasberg-Gauhl C and Jones DR (2000). Black Leaf Streak. Disease Cycle and Epidemiology. In: Disease of Banana, Abaca and Enset (Jones DR, ed.). CAB International, Wallingford, 56-62.
Godoy P, Cano J, Gene J, Guarro J, et al. (2004). Genotyping of 44 isolates of *Fusarium solani*, the main agent of fungal keratitis in Brazil. *J. Clin. Microbiol.* 42: 4494-4497.
Guzmán M and Romero AA (1997). Comparison of azoxystrobin, propiconazole and difenoconazole fungicides on black Sigatoka control *Mycosphaerella fijiensis* Morelet in bananas Musa AAA. *Corbana* 22: 49-59.
Halkett F, Coste D, Platero GG, Zapater MF, et al. (2010). Genetic discontinuities and disequilibria in recently established populations of the plant pathogenic fungus *Mycosphaerella fijiensis*. *Mol. Ecol.* 19: 3909-3923.
Hayden HL, Carlier J and Aitken EAB (2003). Genetic structure of *Mycosphaerella fijiensis* populations from Australia, Papua New Guinea and the Pacific Islands. *Plant Pathol.* 52: 703-712.
He GZ, Deng SX and Qian N (2012). Intestinal microbial community diversity between healthy and orally infected rabbit with *Entamoeba histolytica* by ERIC-PCR. *Parasitol. Res.* 111: 1123-1126.
Hulton CS, Higgins CF and Sharp PM (1991). ERIC sequences: a novel family of repetitive elements in the genomes of *Escherichia coli*, *Salmonella typhimurium* and other enterobacteria. *Mol. Microbiol.* 5: 825-834.
Johanson A, Crowhurst RN, Rikkerink EHA and Fullerton RA (1994). The use of species-specific DNA probes for the identification of *Mycosphaerella fijiensis* and *M. musicola*, the causal agents of Sigatoka disease of banana. *Plant Pathol.* 43: 701-707.
Lewontin RC (1972). The apportionment of human diversity. *Evol. Biol.* 6: 381-398.
Loncaric I, Oberlerchner JT, Heissenberger B and Moosbeckhofer R (2009). Phenotypic and genotypic diversity among strains of *Aureobasidium pullulans* in comparison with related species. *Antonie Van Leeuwenhoek* 95: 165-178.
Marín DH, Romero RA, Guzmán M and Sutton TB (2003). Black Sigatoka: an increasing threat to banana cultivation. *Plant Dis.* 87: 208-222.
Nei M (1973). Analysis of gene diversity in subdivided populations. *Proc. Natl. Acad. Sci. U. S. A.* 70: 3321-3323.
Nei M (1978). Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* 89: 583-590.
Nei M and Li WH (1979). Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proc. Natl. Acad. Sci. U. S. A.* 76: 5269-5273.
Pereira JCR, Gasparotto L, Coelho AFS and Urben AF (1998). Sigatoka negra no Brasil. *Fitopatol. Bras.* 23: 295.
Queiroz CB, Miranda EC, Hanada RE, Sousa NR, et al. (2013). Distribution of mating-type alleles and M13 PCR markers in the black leaf spot fungus *Mycosphaerella fijiensis* of bananas in Brazil. *Genet. Mol. Res.* 12: 443-452.
Rivas GG, Zapater MF, Abadie C and Carlier J (2004). Founder effects and stochastic dispersal at the continental scale of the fungal pathogen of bananas *Mycosphaerella fijiensis*. *Mol. Ecol.* 13: 471-482.
Robert S, Ravigne V, Zapater MF, Abadie C, et al. (2012). Contrasting introduction scenarios among continents in the worldwide invasion of the banana fungal pathogen *Mycosphaerella fijiensis*. *Mol. Ecol.* 21: 1098-1114.

Romero RA and Sutton TB (1997). Sensitivity of *Mycosphaerella fijiensis*, causal agent of Black Sigatoka of banana, to Propiconazole. *Phytopathology* 87: 96-100.

Santana MF, Silva JC, Batista AD, Ribeiro LE, et al. (2012). Abundance, distribution and potential impact of transposable elements in the genome of *Mycosphaerella fijiensis*. *BMC Genomics* 13: 720.

Sharples GJ and Lloyd RG (1990). A novel repeated DNA sequence located in the intergenic regions of bacterial chromosomes. *Nucleic Acids Res.* 18: 6503-6508.

Stover RH and Simmonds NW (1987). Bananas. Longman Scientific and Technical, Essex., England.

Wu J, Gullo M, Chen F and Giudici P (2010). Diversity of *Acetobacter pasteurianus* strains isolated from solid-state fermentation of cereal vinegars. *Curr. Microbiol.* 60: 280-286.

Yeh FC, Yang R, Boyle TJ and Ye Z (2000). PopGene32, Microsoft Windows-Based Freeware for Population Genetic Analysis, Version 1.32. Molecular Biology and Biotechnology Centre, University of Alberta, Edmonton.

Zandjanakou-Tachin M, Vroh-Bi I, Ojiambo PS and Tenkouano A (2009). Identification and genetic diversity of *Mycosphaerella* species on banana and plantain in Nigeria. *Plant Pathol.* 58: 536-546.

