IZABEL CRISTINA ALVES BATISTA

POPULATION STRUCTURE OF *Fusarium oxysporum* f. sp. *cubense* IN BRAZIL

Dissertação apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Fitopatologia, para obtenção do título de *Magister Scientiae*.

Orientador: Eduardo Seiti Gomide Mizubuti

Coorientadora: Camila Geovana Ferro

VIÇOSA – MINAS GERAIS

2019

Ficha catalográfica preparada pela Biblioteca Central da Universidade Federal de Viçosa - Câmpus Viçosa

T
B333p
2019

Batista, Izabel Cristina Alves, 1993-
Population structure of *Fusarium oxysporum* f. sp. *cubense* in Brazil / Izabel Cristina Alves Batista. – Viçosa, MG, 2019.
43 f. : il. (algumas color.) ; 29 cm.

Texto em inglês.
Orientador: Eduardo Seiti Gomide Mizubuti.
Dissertação (mestrado) - Universidade Federal de Viçosa.
Inclui bibliografia.

1. Fusariose. 2. Banana. I. Universidade Federal de Viçosa. Departamento de Fitopatologia. Programa de Pós-Graduação em Fitopatologia. II. Título.

CDD 22. ed. 632.4677

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APROVADA: 26 de julho de 2019.

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AGRADECIMENTOS

O presente trabalho foi realizado com o apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) – código de financiamento 001.

Agradeço à minha mãe, Francisca Alves Batista, pelo amor incondicional, apoio, dedicação e valores ensinados.

Ao Amarildo Junior, por estar sempre ao meu lado, pelo amor e companheirismo.

Ao professor Eduardo Seiti Gomide Mizubuti, pela orientação, palavras de incentivo, e ensinamentos valiosos.

À Drª Camila Geovana Ferro pela co-orientação e amizade.

A Universidade Federal de Viçosa, ao Departamento de Fitopatologia e à FAPEMIG, pela concessão da bolsa de estudos e oportunidade da realização deste curso.

Ao Dr. Randy Ploetz e Joshua Konkol pela contribuição na execução do trabalho.

Aos pesquisadores Miguel Angel Dita Rodriguez (EMBRAPA Mandioca e Fruticultura), Fernando Haddad (EMBRAPA Mandioca e Fruticultura), Sami Jorge Michereff (UFRPE), Christiana de Fatima Bruce da Silva (EMBRAPA Agroindústria Tropical), Kamilla Câmara Correia (UFCA), Paulo Eduardo Franca de Macedo (EMBRAPA Acre), Jânia Lília da Silva Bentes (UFAM), Daniel Augusto Schurt (EMBRAPA Roraima), Ludwig Pfenning (UFLA) e Gilson Soares da Silva (UEMA) pelo envio de amostras e contribuição na formação de uma coleção de Foc representativa do Brasil.

Aos amigos da pós-graduação em Fitopatologia, grupo Capesídeo e amigos paraenses pela amizade e todos os bons momentos.

Aos membros e ex-membros do Laboratório de Biologia de Populações de Fitopatógenos (BIOPOP) pelo convívio, amizade e ajuda. Muito obrigada!

BIOGRAFIA

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RESUMO

BATISTA, Izabel Cristina Alves Batista, M.Sc., Universidade Federal de Viçosa, julho de 2019. **Estrutura populacional de *Fusarium oxysporum* f. sp. *cubense* no Brasil**. Orientador: Eduardo Seiti Gomide Mizubuti. Coorientadora: Camila Geovana Ferro.

Murcha de Fusarium, causada pelo fungo *Fusarium oxysporum* f. sp. *cubense* (Foc), é considerada uma das doenças mais destrutivas da cultura da bananeira. Estudos de genética populacional são necessários para a implementação de uma abordagem de epidemiologia molecular visando ações efetivas de manejo ou mitigação. Neste estudo, uma coleção de 200 isolados monospóricos de várias regiões produtoras de banana com diferentes condições climáticas ao longo de um transecto Norte – Sul no Brasil foi formada para avaliar a estrutura genética da população de Foc. O grupo de compatibilidade vegetativa (VCG) de 140 isolados foi determinado por meio do pareamento com 17 testadores de VCG. Um grupo de 158 isolados foi selecionado para genotipagem por microssatélites. Houve diversidade moderada de Foc no Brasil. Foram identificados oito VCGs: 0120, 0122, 0124, 0125, 0128, 01215, 01220 e 01222. A distribuição de VCGs é desigual, independente do genótipo da banana e variada de acordo com as regiões geográficas. Quatro loci SSR foram polimórficos e, em média, 7,5 alelos foram detectados por locus. Trinta e cinco MGL foram identificados. Não houve associação entre VCG e SSR e nenhuma estrutura genética da população de Foc no Brasil foi detectada.

Palavras-chave: Panamá. Murcha. Banana.

ABSTRACT

BATISTA, Izabel Cristina Alves Batista, M.Sc., Universidade Federal de Viçosa, July, 2019. **Population structure of *Fusarium oxysporum* f. sp. *cubense* in Brazil**. Adviser: Eduardo Seiti Gomide Mizubuti. Co-adviser: Camila Geovana Ferro.

Fusarium wilt, caused by the soil-borne fungus *Fusarium oxysporum* f. sp. *cubense* (Foc), is considered one of the most destructive diseases of bananas. Population genetics studies are required for implementing a molecular epidemiology approach, which in turn is fundamental for effective management or mitigation actions. In this study, a collection of 200 monosporic isolates from several banana producing regions with different climate conditions along a South to North transect in Brazil was formed to assess the genetic structure of the population of Foc. The VCG of 140 isolates was determined by pairing against 17 VCG testers. A group of 158 isolates was selected for microsatellite genotyping. There was moderate diversity of Foc in Brazil. Eight VCGs were identified: 0120, 0122, 0124, 0125, 0128, 01215, 01220, and 01222. The distribution of VCGs is uneven, independent of the banana genotype and varied according to geographic regions. Four SSR loci were polymorphic and on average 7.5 alleles were detected per locus. Thirty-five MGL were identified. There was no association between VCG and SSR and no genetic structure of the population of Foc in Brazil was detected.

Keywords: Panama. Wilt. Banana.

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GENERAL INTRODUCTION

Fusarium oxysporum sensu lato is a species complex of soilborne fungi that comprises a range of plant pathogenic individuals. The complexity of this group can be further illustrated by the infraspecific divisions that allow the classification of strains into groups defined according to the ability to cause vascular wilt to a limited range of hosts, the *forma specialis* (f.sp.). The individuals of a given *forma specialis* can be classified into races according to the capacity to cause wilt in different cultivars or banana genotypes (Gordon and Okamoto 1992; Fourie et al. 2011). Furthermore, individuals that belong to a given race can cluster into vegetative compatibility groups (VCGs), which are groups based on the formation of stable vegetative heterokaryon between compatible mutant isolates, implying identity of alleles at vegetative incompatibility (*vic*) loci (Leslie 1993).

Fusarium oxysporum f.sp. *cubense* (E.F. Smith) Snyder and Hansen (Foc) causes Fusarium wilt (FW) in bananas and plantains, one of the most destructive plant diseases in history (Dita et al. 2018). The disease is also known as Panama disease and is characterized by a vascular disorder that seriously impairs water transport. The pathogen most likely originated in Southeast Asia but the disease was first reported in Australia in 1876 (Siamak and Zheng 2018). In 1908, the pathogen was first isolated in Cuba, and in less than 20 years, the disease was already present in several Central American islands (Stover 1962). During this period, the main commercial banana plantations consisted of "Gros Michel" cultivars, highly susceptible to FW and 40,000 ha of banana plantations were wiped out because of the disease in Central and South America (Su et al. 1986; Stover 1962).

Foc is a soil-borne fungus that infects the banana plant through the roots. After penetration, the pathogen moves through the xylem tissue and colonizes the rhizome of the banana plant, causing an internal discolouration of the vascular bundles (Li et al. 2011). Vascular bundle colonization occurs rapidly by the abundant formation of microconidia in the root system and in the banana rhizome. Microconidia can be carried by sap flow (Bishop and Cooper 1983). Eventually, the germinated spores prevent the translocation of water and nutrients (Jeger et al. 1995). The leaves in infected plants turn yellowish and wilt, and the plant usually dies after a few months (OGTR 2008).

Three races of Foc are recognized (Ploetz 2006): Race 1 (R1) is pathogenic to "Gros Michel" (AAA), “Prata” (Pome) and “Maçã” (Silk) (AAB) varieties. Race 2 (R2) affects Bluggoe bananas (ABB) and some tetraploids (AAAA) (Stover and Waite 1960; Ploetz 1990).

Race 4 is subdivided into Tropical race 4 (TR4) and Subtropical race 4 (STR4), both can infect cultivars belonging to the Cavendish subgroup (AAA), and all banana varieties susceptible to R1 and R2. STR4 can affect Cavendish in areas subject to low temperatures and other predisposing factors, while TR4 affects Cavendish in both tropical and subtropical conditions (Buddenhagen 2009).

The VCGs in Foc are determined based on the procedures adapted from a technique previously applied to *Aspergillus* (Puhalla 1985). This technique is based on the use of potassium chlorate ($KClO_3$) in the culture medium, a highly toxic analog of nitrate (NO_3). Thus, media supplemented with $KClO_3$ allow the microorganism to develop mutant sectors resistant to NO_3 due to loss of nitrate reductase activity and are recognized as nitrate non-utilising (*Nit*) mutants (Correll et al, 1987; Kistler 1997; Leslie and Summerell, 2006). These mutations that lead to loss of nitrate reductase activity may occur in different loci and should be phenotyped for complementarity tests. To identify the phenotype of *Nit* mutants it is necessary to relate the ability of mutant strain, assimilate different nitrogen sources based on the NO_3 metabolism pathway. Mutations in the loci involved in the production of the active enzyme nitrate reductase, inhibit mutant strains to use nitrate (NO_3) as a nitrogen source and are classified as *Nit*1. A molybdenum-containing cofactor is also part of the enzyme nitrate reductase and purine dehydrogenase and strains with mutations in the loci responsible for the synthesis of the molybdenum cofactor inhibit mutant strains to use both nitrate and hypoxanthine as a source of nitrogen, and are classified as *Nit*M. The mutation in the *Nit*3 locus affects a regulatory protein that interferes with NO_3 assimilation only. Thus, Nit3 mutants cannot use NO_3 and NO_2 as nitrogen source (Leslie and Summerell, 2006). *Nit* mutants, with mutations at different loci, can form a heterokaryon if they are compatible, i.e. when they are of the same group of vegetative compatibility (Puhalla 1985; Leslie 1993; Kistler 1997).

Knowledge of vegetative compatibility can be helpful to understand the population structure of the fungus. Because of the vegetative compatibility, genetic exchange is more likely to occur among individuals that share common alleles at putative *vic* loci. Due to mycelial incompatibility, groups of individuals may evolve independently. A population of *F. oxysporum* consisting of distinct clonal lineages corresponding to distinct VCGs suggests the absence of genetic recombination between members of those VCGs/lineages (Kistler 1997).

Currently, 24 Foc VCGs have been reported: VCG0120 through VCG0126 and VCG0128 through VCG01224 (Moore et al. 1993; Bentley and Dale 1995; Ploetz 2006;

Fourie et al. 2011; Ordonez et al. 2015). Some VCGs are compatible with each other and can form complexes, such as VCGs 0120/15, 0124/5/8/20, 0129/11 and 01213/16 (Ploetz 2006; Mostert et al. 2017; Czislowski et al. 2017).

Except for the unique and constant association between VCGs 01213 and 01216 with TR4, no other relationship between races and VCGs was observed or reported in Foc. Thus, the same VCG may be related to different races. The relationship between the VCGs and races was investigated using a multigenic phylogenetic approach and there was no clear association between these phenotypes (Fourie et al. 2009). However, using IGS PCR-RFLP, Foc isolates from different VCGs were grouped into eight distinct phylogenetic lineages distributed in two clades, A and B (Fourie et al. 2009). These findings were validated and extended when Foc isolates from 24 VCGs were genotyped-by-sequencing using Diversity Array Technology sequencing (DArTseq). Again, the analyses of the SNPs allowed the separation of the individuals into two clades (Ordonez et al. 2015). Clade A included lineages I (VCG 01219), II (VCGs 0126 and 01210), III (VCGs 0129, 0129/11, and 01211), IV (VCGs 0120, 01215, 0120/15, and 0122), and V (VCGs 0121, 01216, 01213, and 01213/16). Clade B included lineages VI (VCGs 0123, 01217, and 01218), VII (VCGs 0124, 0125, 0128, 01220, and 01212), and VIII (VCG 01214) (Fourie et al. 2009).

Diverse phenotypic and genetic characters have been used to measure variation in *F. oxysporum* f. sp. *cubense* population (Ploetz 2006; Fourie et al. 2011). These techniques include vegetative compatibility (Leslie 1993), restriction fragment length polymorphisms (RFLPs) (Koenig et al. 1997), and microsatellites or simple sequence repeats (SSRs) (Bogale et al. 2005; Fourie et al. 2011).

Previous study, using RFLPs and cDNA probes to characterize a worldwide collection of Foc, suggested that some *formae speciales* of *F. oxysporum* were not monophyletic (Koenig et al. 1997). Afterwards, O'Donnell et al (1998) showed that *Fusarium oxysporum* f. sp. *cubense* beside being polyphyletic still composed by several clonal lineages. Recently, a diagnostic method based on PCR-RFLPs of IGS was developed to assign Foc isolates to its multiple evolutionary lineages and could also allow separation of isolates of *F. oxysporum* f. sp. *cubense* from non pathogenic isolates of *F. oxysporum* (Fourie et al. 2009).

Microsatellite or single sequence repeats (SSRs) markers are widely used for genetic studies of pathogen populations because of the high resolution that they provide (Saeed et al. 2015; Bogale et al. 2006). SSRs are stretches of highly polymorphic repeats of di-, tri-, tetra,

penta- or hexa- nucleotide that are scattered throughout the genome (Groppe et al. 1995; Fourie, et al. 2011). Based on information from *Fusarium oxysporum* isolates genome, nine microsatellite markers were developed to genetic diversity analyses and a total of 71 alleles among the isolates were found (Bogale et al. 2005).

The genetic variability in the Foc population from Brazil was studied using 214 isolates from different geographic regions (Costa et al. 2015). Genetic variation was assessed using SSRs markers (Bogale et al. 2005) and 52 different haplotypes were identified. No evidence of sexual recombination was observed indicating that the reproductive mode of this population is predominantly asexual. Also, differences in virulence between haplotypes were identified. However, it was not possible to detect any population structure (Costa et al. 2015). Therefore, further studies are needed using a higher number of isolates, a broader sampling area and more accurate methods for SSR genotyping, such as fluorescence-based SSR markers. Additionally, it would be interesting to complement these analyses with PCR-RFLP to determine the lineages present in the different banana producing areas and the occurrence and distribution of the VCGs. The combination of several markers will provide a more robust basis for better understanding of the diversity of Foc in Brazil.

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CHAPTER 1

GENETIC DIVERSITY AND POPULATION STRUCTURE OF *Fusarium oxysporum* f. sp. *cubense* IN BRAZIL

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Abstract

Fusarium wilt, caused by the soil-borne fungus *Fusarium oxysporum* f. sp. *cubense* (Foc), is considered one of the most destructive diseases of bananas. Population genetics studies are required for implementing a molecular epidemiology approach and seek for effective management or mitigation actions. In this study, a collection of 200 monosporic isolates from several banana producing regions with different climate conditions along a South to North transect in Brazil was formed to assess the genetic structure of the population of Foc. The VCG of 140 isolates was determined by pairing against 17 VCG testers. A group of 158 isolates was selected for microsatellite genotyping. There was moderate diversity of Foc in Brazil. Eight VCGs were identified: 0120, 0122, 0124, 0125, 0128, 01215, 01220, and 01222. The distribution of VCGs is uneven, independent of the banana genotype and varied according to geographic regions. Four SSR loci were polymorphic and on average 7.5 alleles were detected per locus. Thirty-five MGL were identified. There was no association between VCG and SSR and no genetic structure of the population of Foc in Brazil was detected.

Introduction

Fusarium wilt (FW) of bananas (*Musa acuminata*, *M. balbisiana* and *Musa* x *paradisiaca*) is currently one of the most mentioned plant diseases due to the recent changes in the distribution of a particular race of its causal agent, the fungus *Fusarium oxysporum* f. sp. *cubense* (E.F. Smith) Snyder and Hansen (Foc). However, regardless of the race of the pathogen, FW is a destructive disease worldwide. The pathogen infects the banana plant through the roots, colonises and blocks vascular tissues, and causes a reddish-brown discolouration of the rhizome and pseudostem. As a consequence, leaves and whole plants normally collapse after FW development (Stover 1962; Fan et al. 2017).

Variants of the pathogen have different capabilities to infect banana genotypes. To date, three races of Foc are recognized (Ploetz 2006): Race 1 (R1) is pathogenic to "Gros Michel" (AAA), 'Prata' ('Pome') and 'Maçã' ('Silk') (AAB) varieties. Race 2 (R2) affects Bluggoe, a variety of the baking banana group (ABB) and some tetraploids (AAAA) (Stover and Waite 1960; Ploetz 1990). Race 4 is subdivided into Tropical race 4 (TR4) and Subtropical race 4 (SR4), both of which can cause wilt in cultivars belonging to the Cavendish subgroup (AAA), and all banana varieties susceptible to R1 and R2. SR4 can affect Cavendish in areas subjected to low temperatures and other predisposing stress factors, while TR4 can affect Cavendish in both tropical and subtropical conditions (Buddenhagen 2009). In addition to variability and the pathogenicity level, there is variation within the *formae speciales* which are detected based on vegetative compatibility groups and also based on molecular markers.

At least 24 vegetative compatibility groups (VCGs) are known to separate Foc strains (Fourie et al. 2009; O'Donnell et al. 2009; Mostert et al. 2017). VCG reflects the allelic homogeneity in *vic* (vegetative incompatibility) or heterokaryon (het) loci, that is, compatible individuals must have identical alleles in each of the *vic* loci (Correll 1991). When fungal isolates are vegetatively compatible, having the same *vic* or het loci, their hyphae may fuse and form heterokaryon, therefore, genetically distinct nuclei can be found in a hypha or in the thallus (Leslie 1993). To determine VCGs, nitrate non-utilizing (*nit*) mutants that are unable to assimilate nitrate (NO_3) from the media are paired with mutants of known tester strains.

VCGs play an important role in structuring the pathogen population, since nuclear exchange is possible only among individuals of the same compatibility group. The recognition of VCGs within a morphologically homogeneous population allows the identification of genetically isolated subpopulations with possible distinct characteristics, such as

pathogenicity or geographical origin (Leslie 1993). In Brazil, there is only one study carried out to characterize populations of Foc based on VCGs (Matos et al. 2009). Forty-four isolates were obtained from banana fields and characterized. Isolates were grouped into eight distinct VCGs: 0120, 0123, 0124, 0125, 0128, 0129, 01210, and 01215 (Matos et al. 2009). However, the small number of isolates with relatively limited representativeness of the banana production regions was not sufficient to draw safe conclusions about the occurrence and distribution of VCG's in Brazil.

Microsatellite or simple sequence repeats (SSRs) are widely used in population genetics studies, enabling the acquisition of large quantities of genetic information for gene and genotype identification (Bogale et al. 2006). In a previous study, Costa et al. (2015) used the SSR markers to investigate the diversity of Foc in the states of Bahia, Ceará, Rio Grande do Norte, Minas Gerais and Rio Grande do Sul. Although it was not possible to observe any population structure among isolates, this was the most detailed study on the genetic structure of Foc in Brazil to date. Since populations may change over time, it would be interesting to quantify the amount of genetic variability present in the current Brazilian population and to try to identify if there is any pattern of its distribution in the banana producing regions.

The characterization of VCGs currently present in Brazil combined with fluorescence-based SSR markers to genotype individuals from different areas can add useful information about the basic biology of Foc. This will contribute to a better understanding of the evolutionary processes that may be acting upon the pathogen populations and help to establish effective practices for management and control of FW.

The aim of this work was to study the genetic structure of the population of *F. oxysporum* f. sp. *cubense* in Brazil. The specific objectives were: a) to assess the variability of Foc in Brazil based on lineages and vegetative compatibility groups and b) to estimate the amount and distribution of genetic variation in the Foc population in Brazil using microsatellite markers (SSR).

Material and methods

Obtaining isolates

Fusarium oxysporum f. sp. *cubense* isolates were obtained after sampling different regions and banana cultivars. Infected plant samples or non-pure isolates were sent to the

Departamento de Fitopatologia (DFP) of the Universidade Federal de Viçosa (UFV), in order to form a collection of Foc representative of Brazil (Supplementary Table 1). Monosporic cultures were obtained and mycelial discs of the monosporic isolates were maintained in microtubes at 10 °C in the culture collection of the Laboratório de Biologia de Populações de Fitopatógenos. A total of 700 *Fusarium* isolates from 15 Brazilian states were received and screened for identification of *forma specialis cubense* and TR4 using a PCR-based approach with a set of primers developed by Lin et al. (2009) and Dita et al. (2010). In addition to rapid PCR diagnosis, pathogenicity tests were performed confirming that the isolates were *Fusarium oxysporum* f. sp. *cubense*. Population analyses of Foc were done with isolates selected based on geographic distribution, such as to include the widest range of locations, and cultivar of origin. Isolates collected from fields planted with different cultivars and banana groups such as Pome, Silk and Cavendish were selected to represent as better as possible different conditions along a South to North transect in eastern Brazil.

Vegetative compatibility groups

Vegetative compatibility tests were conducted to determine the VCG of Foc isolates. Wild type strains were first grown in minimal medium (MM: Sucrose 30g/L, $NaNO_3$ 2g/L, L-asparagine 1.6g/L, $KClO_3$ 15g/L, Agar 20g/L and trace element solution 200µl/L) to check for dense mycelial growth. After 3-5 days, when the colonies had grown vigorously, three mycelial discs from each isolate were placed in petri dishes (90 x 15 mm) containing chlorate medium (MM + 1.5-4.0% $KClO_3$) in a triangular arrangement, and incubated at 25º C for 7-21 days (Puhalla 1985).

Mycelial discs from spontaneous $KClO_3$-resistant sectors were transferred to MM. Plates presenting colonies with aerial mycelium growth on MM were discarded. Isolates that did not form aerial mycelium were classified as *nit* mutant and were phenotyped as *nit*1, *nit*3 or *nit*M mutants based on their inability to assimilate nitrogenous compounds as the sole source of nitrogen.

Four phenotyping media were used to characterize the mutants: MM supplemented with $NaNO_3$ (2 g/L), $NaNO_2$ (0.5 g/L, Sigma Chemical Co., St. Louis), hypoxanthine (0.2 g/L, Sigma Chemical Co.), and ammonium tartrate (1.6 g/L). The pH of the culture media was adjusted to 6.5 using 1 M NaOH.

Each phenotyping medium was dispensed into Petri dishes (90 mm diameter x 15 mm height). The reverse side of each plate was marked with 14 evenly spaced dots to guide the

location of mycelial discs of *nit* mutants of a given phenotype. Strains were incubated at 25 ºC for 3 to 4 days before phenotyping assessments. Growth on phenotyping medium was interpreted according to the assimilation capacity of the nitrogen source (Table 1). MM+Ammonium tartrate medium was used as control. Strains that did not grow normally on this medium have another nutritional requirement and were discarded. MM+Hypoxanthine medium was used to differentiate *nit*M mutants. MM+$NaNO_2$ medium differentiated *nit*3 mutants. Isolates that did not show dense mycelial growth only in MM+$NaNO_3$ medium were classified as *nit*1 mutants (Correll et al. 1987). Each *nit* mutant was maintained in a 2 mL tube at 10 °C. The recovered mutants were used in the vegetative compatibility tests.

Table 1. Nitrogen sources utilized in the phenotype screening of *nit* mutants.

Strain type	**NH_4**	**NO_3**	**NO_2**	**Hypoxanthine**	**ClO_3**
Wild type	+	+	+	+	-
*Nit*1	+	-	+	+	+
*Nit*3	+	-	-	+	+
*Nit*M	+	-	+	-	+
crn	+	+	+	+	+

Leslie and Summerell, 2006.

Strains of 22 VCGs testers were provided by Prof. Randy Ploetz of the University of Florida, Homestead FL in the USA. Due to quarantine restrictions tester 01213/16, associated with TR4, was not available for the assays. Only testers of seventeen VCGs were viable for use in this essay: 0120, 0121, 0122, 0123, 0124, 0125, 0126, 0128, 0129, 01210, 01211, 01212, 01214, 01215, 01219, 01220, and 01222 (Supplementary Table 2). The pairings were done on MM supplemented with $NaNO_3$ as the sole nitrogen source. For each isolate, *nit*1 mutants were paired with its *nit*M mutant cognate to confirm heterokaryon self-compatibility, and then with the *nit*M or *nit*1 of the tester to determine VCG identity (Leslie 1993; Leslie and Summerell 2006). Different sets were formed, each set with six mutants from different isolates of the same phenotype and one mutant tester with a different phenotype. Mycelial

discs were distributed in a Petri dish (90 x 15 mm): the mutants tester (*nit*1 or *nit*M) were set at the center of the Petri dish and the mutants from different isolates (*nit*M or *nit*1) surrounding the tester.

Pairing tests on Petri dishes were kept at 25 °C in complete darkness and scored for heterokaryon formation after 14 days. Complementation tests were done in duplicates and repeated at least twice in time. About 2000 Petri dishes were used for the pairing tests. Positive reactions were considered those that presented aerial mycelium growth in the line of intersection between paired mutants, confirming the classification of these strains as belonging to the same VCG. Negative reactions were those that had incipient growth in the line of intersection between the paired mutants.

A Fisher's exact test was conducted to test for the independence of VCGs and geographic location using package stats in R.

DNA extraction

Mycelial discs of Foc isolates were transferred to Erlenmeyer containing potato dextrose (PD) medium (Leslie and Summerell, 2006) and grown for 7 days at 25 ºC, in the dark. After this period, the mycelial mass on PD was transferred over to sterilized filter paper and the mycelium retained on the filter was allowed to dry at room temperature for 24 h. The dried fungal mass was stored at -20 °C.

DNA extractions were carried out using the Wizard® Genomic DNA Purification Kit (Promega Corporation) following the manufacturer's instructions. The extracted DNA was quantified by spectrophotometry (Nanodrop 2000), and its integrity was assessed after electrophoresis on 1% agarose gel. The DNA was diluted to the final working concentration of 10 ng /μL^{-1}.

IGS PCR-RFLP

A fragment of the IGS region of rDNA was amplified using primers PNFo (5'- CCC GCC TGG CTG CGT CCG ACT C - 3') and PN22 (5'- CAA GCA TAT GAC TAC TGG C - 3') (Edel et al. 1995). PCRs were performed with 10 ng of template DNA in 50 µl reaction volume using 0.5 µl of each primer; 0.5 µl of dNTP; 0.3 µl of Taq DNA polymerase; and T5x Buffer. PCR conditions were 30 cycles of 90s at 95 ºC, 60 s at 50 ºC, and 90 s at 72 ºC. The amplification products were visualized by electrophoresis on a 0.8 % agarose gel stained with Gel Red (Biotium, Hayward, USA) at 80 V.

Five restriction enzymes were used for PCR-RFLP analysis: *Ava*I, *Bbv*I, *BceA*I, *BsrD*I, and *CviQ*I (New England BioLabs). All enzymes were used separately in PCR-RFLP digestion reactions, which was performed in a total volume of 20 µl and consisted of 5 µl IGS PCR product, 2U of restriction enzyme and 2 µl of the provided restriction buffer. After incubation at 37 °C for 1 h or 3 h, depending on manufacturer recommendation, the restricted fragments were visualized on a 3% agarose gel electrophoresis at 60 V.

The pattern of digestion with *Ava*I enzyme allowed to differentiate Foc isolates into clades A and B (Fourie et al. 2009). Clade A isolates were then digested with the following enzymes: *BceA*I to separate the Foc V lineages from lineages I, II, III and IV; *CviQ*I to separate lineages I and II from lineages III and IV; and *BsrD*I to separate lineage III and lineage IV. Isolates of clade B were digested with the enzyme *Bbv*I to separate lineage VII from lineages VI and VIII.

Microsatellites genotyping

The genetic variability of the Foc population was analyzed with nine microsatellite markers described by Bogale et al. (2005). Forward primers were labeled with the fluorophores 6-FAM, VIC, NED or PET (Supplementary Table 3). The primers were first tested in an individual PCR for optimization. Only primers producing good quality PCR products for all samples were retained for further analysis. The reactions were performed using the Multiplex 5X Master Mix (New England Biolabs, Inc.) in a final volume of 12.5µl following the protocol described by the manufacturer. Multiplex conditions were 35 cycles of 90s at 95 ºC, 60 s at 52 ºC, and 90 s at 72 ºC. The final concentration of the primers was 0.2 µM. The multiplex products were lyophilized and fragment analysis was done using the GeneScan-500 LIZ size standard (Applied Biosystems) on an ABI 3730xl analyzer. The fragments were analyzed with the software GENEMARKER v.1.191 (SoftGenetics). The size of DNA fragments was manually binned into alleles according to the number of repeat units at each locus.

Data analyses

Population genetic analysis was conducted in R. The *poppr* R package (Kamvar et al. 2014) was used to calculate the number of multilocus genotypes (MLGs) combining the alleles identified at each SSR locus, excluding monomorphic loci. The clonal fraction in the whole population was calculated as 1-[(number of different genotypes)/(total number of isolates)]. Based on the frequency of MLGs in the whole population, true diversity indices were

estimated by Hill numbers (N) of orders 0, 1, and 2. The diversity analyses were conducted using the iNEXT package (Hsieh et al. 2016). A principal components analysis (PCA) was performed on the data set without any population assignment to identify clusters of individuals. The *adegenet* R package (Jombart 2008; Jombart and Ahmed 2011) was used to create minimum spanning networks based on Bruvo's genetic distance (Kamvar et al. 2015; Kamvar et al. 2014).

Results

A total of 200 monosporic Foc isolates were obtained in this study. No isolate was classified as TR4 based on the PCR test. A subset of 140 isolates were selected for the VCG test. A group of 158 isolates was selected for microsatellite genotyping. A total of 98 isolates were subjected to both the SSR genotyping and VCG test (Supplementary Table 1).

VCG pairing

Out of 140 isolates, *Nit* mutants were generated for 117 isolates (84%). Twenty-three isolates were chlorate resistant (*crn* mutants) and could not be used in VCG testing. Most isolates (N = 88) had both *nit* 1 and *nit* M or *nit* 1 and *nit* 3 mutants, and no self-incompatible mutant was noticed. About 5.945 possible pairing combinations were performed (205 *nit*1/*nit*3/*nit*M x 29 *nit*1/*nit*M testers), isolates were arranged in different combinations on each plate.

When the 117 Foc mutants were paired with the testers of 17 known VCGs available, 73 isolates formed heterokaryon. In total, 8 of the 24 known VCGs were identified in Brazil: 0120, 0122, 0124, 0125, 0128, 01215, 01220 and 01222 (Fig. 1A).

The predominant VCG was 0120 and its complex VCG 0120/15 (48% of the isolates). The other VCGs found, 0122, 0124, 01222 and the VCGs complexes 0124/22, 0124/5/22, 0124/5/8/22 and 0125/8/20, accounted for 12% of the isolates. However, for 50 isolates (40%) there was no heterokaryon formation with any of the testers. These isolates may belong to other VCGs for which no testers were available, or they may belong to novel VCGs.

From the isolates sampled from 15 Brazilian states, we identified VCGs for isolates collected in 9 states: Bahia, Ceará, Maranhão, Minas Gerais, Pernambuco, Paraná, Rio Grande do Sul, Santa Catarina and São Paulo (Fig 1B). The state of Ceará, located in the Northeast region, had the highest VCG richness in this study. Six VCGs were detected among the eight isolates analyzed: 0122, 0124, 0125, 0128, 01220 and 01222. Some of these VCGs formed

specific VCG complexes: 0124/22, 0124/5/22, 0124/5/8/22 and 0125/8/20. All Foc strains from Ceará were isolated from the Prata cultivar (AAB).

Four VCGs were found in São Paulo state: 0120, 0120/15 and 0124/22; three in Minas Gerais: 0120, 01215 and 0124; and three in Maranhão state: only complex 0124/5/22. The isolates from São Paulo and Minas Gerais were sampled mainly from Prata cultivar. There was no clear association between VCGs and cultivar or genotype of the crop. In the states of Santa Catarina (n=2), Paraná (n=1), Bahia (n=1) and Rio Grande do Sul (n=1), only VCG 0120 and/or its complex 0120/15 was found. In the state of Pernambuco only VCG 0124 was found. The two most prevailing VCGs occurring in Brazil 0120 and 0124 exhibited a significantly ($p < 0.001$) uneven distribution among North-Northeast and South-Southeast.

PCR-RFLP diagnostic

The IGS primers PNFo and PN22 amplified a 1700bp band for all 140 Foc isolates. Based on the restriction pattern with AvaI, 96 isolates belong to Clade A (68.5%) and 19 isolates to Clade B (13.5%). The PCR-RFLP profile for 25 isolates (18%) was not clear and it was not possible to classify the isolates in clades. The cleavage with the enzymes *BceA*I and *Bbv*I, generated illegible patterns, preventing classification of Foc isolates into lineages.

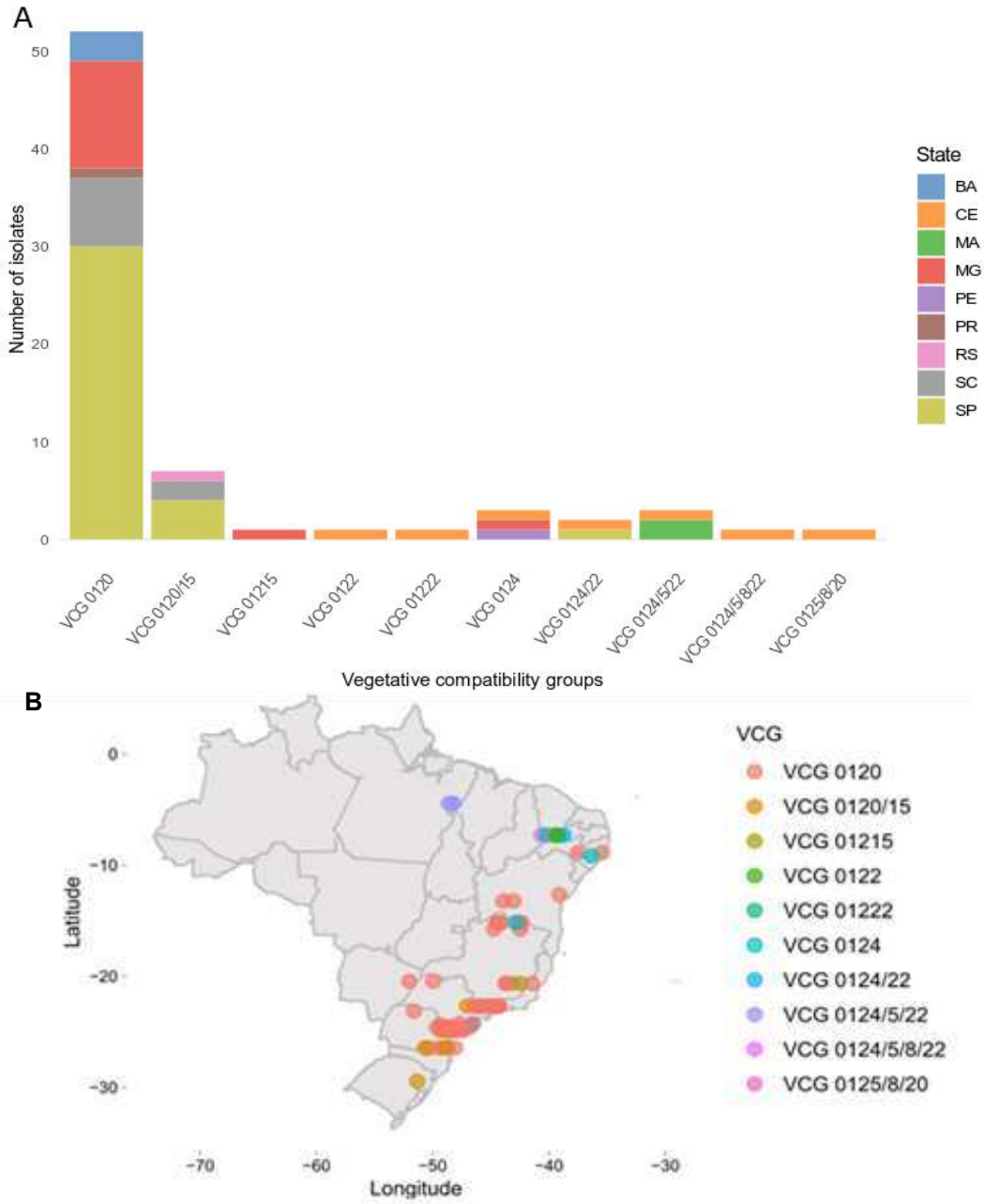

Figure 1 A. Vegetative compatibility groups (VCGs) of *Fusarium oxysporum* f. sp. *cubense* (Foc) occurring in the Brazilian states. BA = Bahia, CE = Ceará, MA = Maranhão, MG = Minas Gerais, PE = Pernambuco, PR = Paraná, RS = Rio Grande do Sul, SC = Santa Catarina, SP = São Paulo state. B. Distribution of the Foc VCGs found in Brazil.

Microsatellite markers

There was no amplification for four SSR loci: FO5, FO9, FO10, and FO13. An *in silico* analysis was performed to diagnose this issue and it was observed that there were no hits or similarities between the regions of the microsatellite FO5, FO9 and FO10 loci (Genbank: AY931030, AY931029, and AY931028 respectively) against the database of four genomes of Foc assembled at the scaffold level (Genbank: AGND01, AMGP01, AMGQ01 and MBFV01). The only observed hits were with the genome of Fol (*Fusarium oxysporum* f. sp. *lycopersici*) assembled at the chromosome level (Genbank: AAXH01). For the FO13 locus, although there were hits between the flanking sequence and the Foc genome, the software Primer3web (version 4.1.0) pointed out a problem in the reverse sequence design of MB13 primer (5'-3': CTAAGCCTGCTACACCCTCG). Thus, these four loci were not used for genotyping. All analyses were conducted based on the polymorphism found in five loci: FO2, FO11, FO14, FO17, and FO18.

A total of 35 haplotypes were identified among the 158 isolates analyzed. The number of alleles at each locus varied from 2 (FO14) to 10 (FO2 and FO17) (Supplementary Table 3). Two alleles were revealed at FO14 locus, but one occurred at low frequency (0.01), thus it was considered as uninformative and removed from the analyses. The four remaining loci had an average of 7.5 alleles per locus. The average gene diversity over all loci was 0.59 but estimates varied from 0.42 (FO11) to 0.67 (FO2).

Table 2 Summary of observed and estimated diversities and standard errors (SE) for the SSR genotyping data

Diversity[1]	Observed	Estimated	SE
Genotype richness (q = 0)[2]	35.0	47.7	8.3
Shannon diversity (q = 1)[3]	10.3	12.2	1.7
Simpson diversity (q = 2)[4]	3.9	4.0	0.6

[1] Calculation of diversity indices based for the whole population (sample size n = 158)
[2] Hill number (q = 0) number of individuals regardless of their relative abundances
[3] Hill number (q = 1) effective number of common individual in the population
[4] Hill number (q = 2) effective number of dominant individuals in the population

The sample size for the whole population was 158 individuals, and the genotypic diversity based on Hill numbers for 0, 1, 2 were 35, 10.3, and 3.9 respectively (Table 2).

Both I_A and r_d calculated for clone-corrected dataset were significantly different from zero ($P < 0.01$) in the overall population. Thus, the random mating hypothesis was rejected, indicating a clonal population.

Population Structure

The PCA for all 158 isolates of the dataset showed that the first two components were not sufficient to explain the total variation observed. The first component explained 43.7% of the total variation, and the second component only 16.3% (Fig. 2).

The isolates were not grouped in clear clusters (Fig. 2). The formation of putative groups within quadrants was exploited through a discriminant analysis of principal components (data not shown) and no biological or environmental pattern was noticed in these analyses. Thus, no evidence of population structure among Foc isolates was observed.

The minimum spanning network also revealed no distinct pattern of genotype by VCGs or by location, with different VCGs within the same genotypes (Figure 3A), and genotypes from the Northeast heavily contributing to the main groups of MLGs found in Foc (Figure 3B).

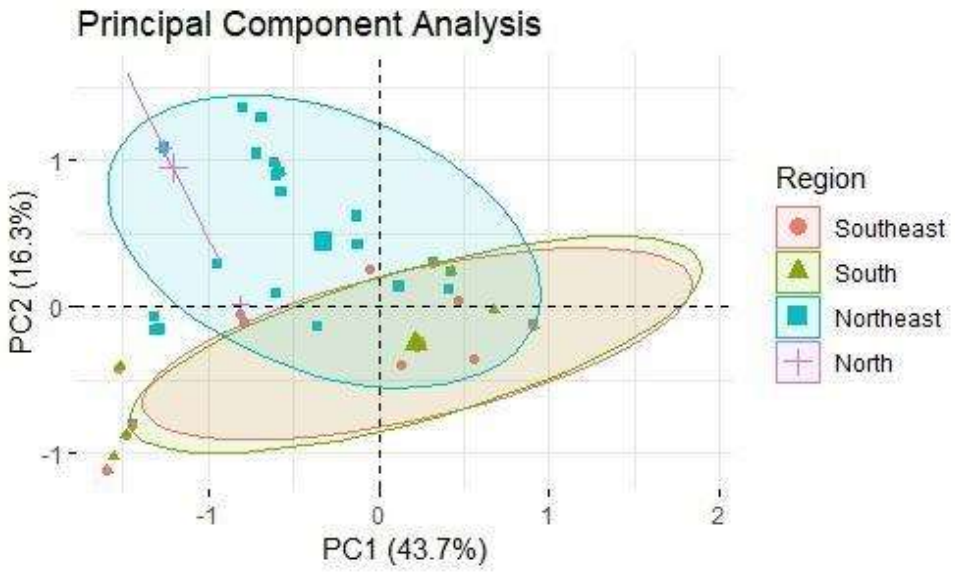

Figure 2. Scatter plot of principal component analysis (PCA) of 35 haplotypes from this study.

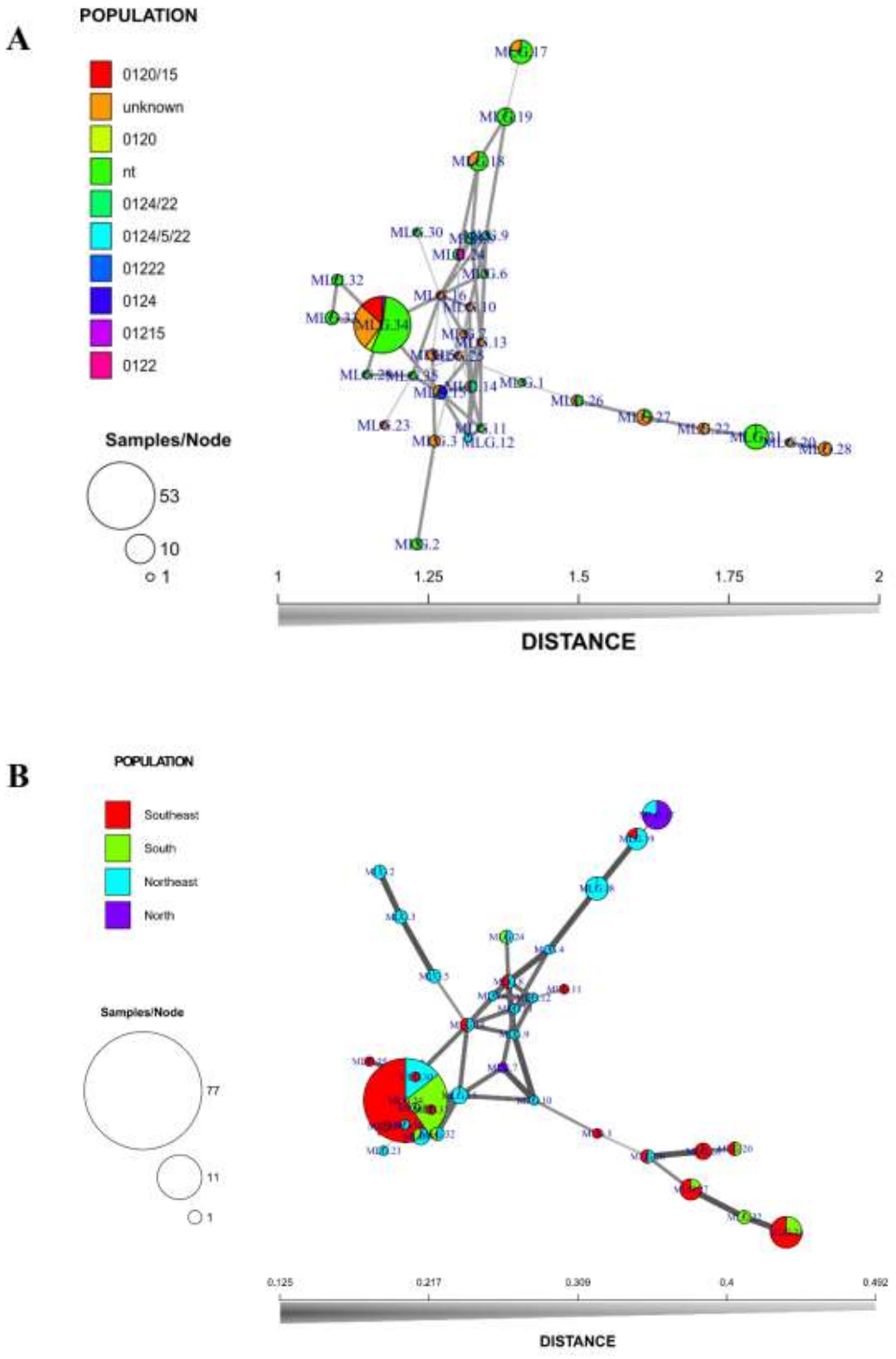

Figure 3 Minimum spanning tree of 35 multilocus genotypes detected in 158 isolates of *Fusarium* oxysporum f.sp. cubense. A. Colors refers to the vegetative compatibility group of isolates. B. Colors refers to isolates geographic region of origin.

Discussion

In this study phenotypic and genotypic markers were combined to investigate the genetic structure of the population of Foc in Brazil. It was hypothesised that the population of Foc was highly variable regarding compatibility groups since 8 VCGs were found in a previous study that phenotyped 44 isolates (Matos et al. 2009). In the present study, using approximately three times as many isolates, the same number of VCGs was identified, but there were differences regarding them. Among the VCGs reported by Matos et al. (2009) the VCG 0123, 0129 and 01210 were not observed in this study. In contrast, we found the VCGs 0122, 01220 and 01222. To our knowledge, this is the first report of the occurrence of these VCGs in the Americas.

Overall, VCG diversity in Brazil can be interpreted as high. Mostert et al. (2017), found 17 Foc VCGs from a total of 615 *Fusarium* isolates distributed in Asian countries, the putative center of origin of both banana and pathogen. Eleven of these VCGs occur in Indonesia (N= 47 isolates) and Malaysia (N=67 isolates) . The VCG richness in Brazil was greater than in some Asian countries: Thailand (7 VCGs from 117 isolates), Bangladesh (6 VCGs from 61 isolates), India, Vietnam and Taiwan (5 VCGs from 66, 35 and 102 isolates) and Sri Lanka (4 VCGs from 27 isolates) (Mostert et al. 2017).

Highest richness was identified in Ceará state. Our results suggest that the high diversity of VCGs and the occurrence of different multilocus genotypes in Ceará state, may be related to the local evolution of the pathogen. Similar observations have been made in previous studies, in which the diversity of *Fusarium oxysporum* would be explained by the relation with local non-pathogenic isolates of *F. oxysporum* from nearby uncultivated areas and other *formae speciales* (Skovgaard et al. 2002; Fourie et al. 2009). Local pathogenic variants can be affected by different evolutionary mechanisms and as a consequence, a variable population is originated in a region (Summerell et al. 2010). Apparently, local variants present in Ceará state can infect a specific banana cultivar, such as 'Prata' in Northeast Brazil, and contribute to high levels of haplotypic diversity in the region .

The distribution of VCGs in Brazil depends on Brazilian regions, and seems more related to the climatic conditions of the producing area than to the banana variety. The most prevalent VCG 0120 is of low occurrence in the North and Northeast, the hottest regions in Brazil where the average annual temperature varies between 25 °C to 36 °C (INMET, 2019). VCG 0120 was mainly found in the Southeast region, where the average annual temperature

varies from 22 ºC to 24 ºC (INMET, 2019); Other VCGs and complexes that belong to Clade B were limited to a very specific region, such as 0122, 0125/8/20 and 0124/5/22 found only in the Northeast of Brazil. In all cases aforementioned, the isolates were sampled mainly from cultivar Prata (AAB - 'Pome'). Contrary to what seems to happen in Brazil, in Asian countries the occurrence and distribution of VCGs seem to be influenced by banana varieties (Mostert et al. 2017).

Another important note about the VCG 0120 is that many authors refer to it as the STR4 - subtropical race 4 (Buddenhagen, 2009; Czilowski et al. 2017). The lack of accurate markers for the detection of Foc races precludes this association. However, some strains of VCG 0120 characterized in this study show the STR4 phenotype, since they were collected from symptomatic Nanica and Nanicão plants (Cavendish subgroup - AAA) sampled from São Paulo and Santa Catarina states, located in the Southeast and South regions of Brazil, respectively. Both states can experience low temperatures during the winter, varying from 16 to 22 ºC in Santa Catarina, and from 18 to 24 ºC in São Paulo (INMET, 2019). The occurrence or predominance of VCGs in different geographic locations in Brazil, independent of the banana genotype, may be evidence of a fitness advantage of individuals in the Foc population.

The VCG tests could have been less laborious and more efficient had the diagnosis by PCR-RFLP for lineage classification worked. The separation of the Foc strains into lineages correlates well with the vegetative compatibility group (Fourie et al. 2009). The PCR-RFLP analyses were successful in classifying the isolates into Clade A and Clade B as proposed by Fourie et al (2009). However, the digestion that would classify into lineages generated atypical cleavage profiles. Karangwa et al. (2018) reported a similar result, when digested some Clade A isolates with *BceA*I restriction enzyme. Although this method was consistent for the characterization of Foc isolates from Asia and Africa (Fourie et al. 2009; Mostert et al. 2017; Karangwa et al. 2018), it was not suitable in this study either because it lacked reproducibility or because of the lack of a control reaction. Consequently, we cannot safely state that strains that do not form heterokaryon with any of the VCG testers correlate to a lineage or belong to a VCG not reported. Previous studies have also identified strains that do not pair with testers, and the authors raised the possibility of these "un-matched" isolates to belong to novel VCGs (Mostert et al. 2017; Karangwa et al. 2018).

All previous studies that aimed to describe the structure population of Foc in Brazil had certain limitations in relation to the few markers used and to the sampling, that could

underestimate the population dynamics (Costa et al. 2015; Cunha et al. 2015). In this study, we attempted to minimize the effects of these limitations by expanding sampling to other regions, including isolates from different banana producing areas such as the Ribeira Valley in São Paulo, the Cariri Region of Ceará, and other northern and southern regions that had not yet been explored. We performed an exploratory analysis based on two markers (VCGs and SSR) that could elucidate how the population of the pathogen causing FW in banana is occurring in Brazil. Based on SSR genotyping, the Foc population appears to have low to moderate genetic variability. Clonal fraction in this study was high (77.8%), similar to that reported in the previous study with Brazilian isolates (74.7%) (Costa et al, 2015). MLG richness estimate for the previous study was 52 among 214 isolates and 35 among 158 isolates used in the present study. Similarly, the genotypic diversity estimated by the Stoddart & Taylor index in the studies was 5.96 and 3.96, for the study conducted by Costa et al (2015) and the present one, respectively. Even though sample sizes differ, estimates are anticipated to be similar if a rarefaction analysis is conducted. A limitation of the current study was the low number of polymorphic microsatellite loci.

The set of SSR markers developed by Bogale et al (2005) is not ideal for studies of Foc population. These markers were not developed for Foc. Most likely, the two strains of *Fusarium oxysporum* chosen for the development of these SSR markers, *F. oxysporum* f. sp. *lycopersici* (Fol) and probably a non-pathogenic strain isolated from the soil where banana plants were cultivated did not allow for the selection of a good set of polymorphic loci in Foc (Bogale et al. 2005). Thus, we did not have enough resolution to infer about the genetic structure of Foc in Brazil. The availability of four genomes of Foc opens the opportunity for the development of a more specific and suitable set of SSR markers. Future studies using these markers may reveal a different genetic reality.

Conclusions

Eight vegetative compatibility groups were identified in the Brazilian population of *Fusarium oxysporum* f. sp. *cubense*: 0120, 0122, 0124, 0125, 0128, 01215, 01220 and 01222.

The distribution of VCGs in Brazil is uneven, independent of the banana genotype and is influenced by geographic region.

There is no evidence of genetic structure of the population of Foc in Brazil.

Acknowledgments

This research was supported by the Fundação de Amparo à Pesquisa do Estado de Minas Gerais - FAPEMIG. We thank Dr. Randy Ploetz and Joshua Konkol for supplying strains of known VCG testers of *Fusarium oxysporum* f. sp. *cubense*.

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Supplementary Table 1 Information of isolates of *Fusarium oxysporum* f. sp. *cubense*

Isolate code	Year	Collector/ Sponsor	Municipality	Region	State	Longitude	Latitude	Altitude	Host	Cultivar	Genotype	Clado	VCG	SSR Genotyping
UFV-Foc001	2015	Miguel Dita	Jacupiranga	Southeast	SP	-48.0957780	-24.8858060	33	Banana	Gran Naine	AAA	A	0120	+
UFV-Foc003	2016	Miguel Dita	Cajati	Southeast	SP	-48.2214720	-24.7013780	168	Banana	Prata anã	AAA	A	0120	+
UFV-Foc016	2016	Miguel Dita	Jacupiranga	Southeast	SP	-48.0551330	-24.8860330	148	Banana	Prata comum	AAB	A	0120/15	+
UFV-Foc024	2016	Miguel Dita	Pariquera-Açú	Southeast	SP	-47.8853080	-24.6181440	47	Banana	Prata Catarina	AAB	A	0120	+
UFV-Foc032	2016	Miguel Dita	Eldorado	Southeast	SP	-48.2023830	-24.6296140	92	Banana	Prata Anã	AAB	A	0120	+
UFV-Foc053	2016	Miguel Dita	Marinópolis	Southeast	SP	-50.8332250	-20.4611890	418	Banana	Maçã	AAB	A	Unknown	+
UFV-Foc059	2016	Miguel Dita	Marinópolis	Southeast	SP	-50.8333110	-20.4611830	418	Banana	Maçã	AAB	A	Unknown	+
UFV-Foc061	2016	Miguel Dita	Marinópolis	Southeast	SP	-50.8167080	-20.4366500	389	Banana	Maçã	AAB	A	Unknown	+
UFV-Foc063	2016	Miguel Dita	Marinópolis	Southeast	SP	-50.8166030	-20.4365940	389	Banana	Maçã	AAB	A	Unknown	+
UFV-Foc069	2016	Miguel Dita	Marinópolis	Southeast	SP	-50.8258610	-20.4348440	406	Banana	Maçã	AAB	A	Unknown	+
UFV-Foc072	2016	Miguel Dita	Marinópolis	Southeast	SP	-50.8258080	-20.4348830	406	Banana	Maçã	AAB	A	Unknown	+
UFV-Foc078	2016	Miguel Dita	Marinópolis	Southeast	SP	-50.8042920	-20.4398170	400	Banana	Maçã	AAB	A	Unknown	+
UFV-Foc081	2016	Miguel Dita	Marinópolis	Southeast	SP	-50.8040420	-20.4397170	400	Banana	Maçã	AAB	A	*crn* mutant	+
UFV-Foc086	2016	Miguel Dita	Aparecida D´Oeste	Southeast	SP	-50.8767420	-20.4798470	370	Banana	Maçã	AAB	A	NT	+
UFV-Foc087	2016	Miguel Dita	Corupá	South	SC	-49.2433330	-26.4261110	52	Banana	Prata	AAB	A	Unknown	+
UFV-Foc088	2016	Miguel Dita	Corupá	South	SC	-49.2433330	-26.4261110	52	Banana	Nanica	AAA	A	0120	+
UFV-Foc101	2016	Miguel Dita	São Bento do Sapucaí	Southeast	SP	-45,7007690	-22.6884440	1045	Banana	Prata Mineira	AAB	A	0120/15	+
UFV-Foc111	2016	Miguel Dita	São Bento do Sapucaí	Southeast	SP	-45,6968170	-22.6873970	1111	Banana	Prata Mineira	AAB	A	0120	+
UFV-Foc113	2016	Miguel Dita	São Bento do Sapucaí	Southeast	SP	-45,6967140	-22.6874170	1114	Banana	Prata Mineira	AAB	A	0120/15	+
UFV-Foc136	2016	Miguel Dita	Santa Mariana	South	PR	-50.5186360	-23,1838650	507	Banana	Maçã	AAB	A	Unknown	+
UFV-Foc142	2016	Miguel Dita	Santa Mariana	South	PR	-50.5186360	-23,1838650	507	Banana	Maçã	AAB	A	Unknown	+
UFV-Foc144	2016	Miguel Dita	Santa Mariana	South	PR	-50.5465310	-22.9533090	411	Banana	Maçã	AAB	A	Unknown	+
UFV-Foc145	2016	Miguel Dita	Santa Mariana	South	PR	-50.5465310	-22.9533090	411	Banana	Maçã	AAB	-	NT	+
UFV-Foc146	2016	Miguel Dita	Santa Mariana	South	PR	-50.5465310	-22.9533090	411	Banana	Maçã	AAB	-	NT	+
UFV-Foc151	2015	Miguel Dita	Cândido Mota	Southeast	SP	-50.4834570	-22.7365170	465	Banana	Maçã	AAB	A	NT	+
UFV-Foc152	-	Miguel Dita	Penápolis	Southeast	SP	-49.6717810	-21.2433780	383	Banana	Maçã	AAB	A	Unknown	+
UFV-Foc154	-	Miguel Dita	Penápolis	Southeast	SP	-50.1089860	-21.2272530	383	Banana	Maçã	AAB	A	Unknown	+
UFV-Foc161	2016	Miguel Dita	Penápolis	Southeast	SP	-50.1089860	-21.2272530	383	Banana	Maçã	AAB	A	Unknown	+
UFV-Foc166	2016	Miguel Dita	Penápolis	Southeast	SP	-50.1087810	-21.2272860	381	Banana	Maçã	AAB	A	Unknown	+

Supplementary Table 1 (Continued)

Isolate code	Year	Collector/ Sponsor	Municipality	Region	State	Longitude	Latitude	Altitude	Host	Cultivar	Genotype	Clado	VCG	SSR Genotyping
UFV-Foc171	2016	Miguel Dita	Penápolis	Southeast	SP	-50.1085220	-21.2270000	380	Banana	Maçã	AAB	A	*crn* mutant	+
UFV-Foc182	2016	Miguel Dita	Penápolis	Southeast	SP	-49.6717810	-21.2433780	420	Banana	Maçã	AAB	A	Unknown	+
UFV-Foc191	2016	Miguel Dita	Penápolis	Southeast	SP	-49.6717810	-21.2444890	413	Banana	Maçã	AAB	A	Unknown	+
UFV-Foc201	2016	Miguel Dita	Penápolis	Southeast	SP	-49.4732920	-21.4149860	421	Banana	Maçã	AAB	A	Unknown	+
UFV-Foc210	2016	Daniel Heck/ Eduardo Mizubuti	São Bento do Sapucaí	Southeast	SP	-45,7363890	-22.6883330	874	Banana	Prata	AAB	A	0120	+
UFV-Foc212	2016	Daniel Heck/ Eduardo Mizubuti	Penápolis	Southeast	SP	-50.0780560	-21.4208330	407	Banana	Maçã	AAB	A	Unknown	+
UFV-Foc213	2016	Daniel Heck/ Eduardo Mizubuti	Santa Mariana	South	PR	-50.4880560	-23,1413890	421	Banana	Maça	AAB	A	0120	+
UFV-Foc216	2016	Daniel Heck/ Eduardo Mizubuti	Jacupiranga	Southeast	SP	-48.0988770	-24.8902540	102	Banana	Nanica	AAA	A	0120/15	+
UFV-Foc217	-	Fernando Haddad	Cruz das Almas	Northeast	BA	-39.1219440	-12.6530560	212	Banana	Maçã	AAB	B	Unknown	+
UFV-Foc218	-	Fernando Haddad	Itajuípi	Northeast	BA	-39.3770000	-14.6747000	108	Banana	Prata-Anã	AAB	-	NT	+
UFV-Foc223	2011	Fernando Haddad	Porteirinha	Southeast	MG	-43.3733300	-15.9233330	556	Banana	Prata anã	AAB	-	NT	+
UFV-Foc227	2011	Fernando Haddad	Guanambi	Northeast	BA	-42.8736100	-14.4652780	515	Banana	Prata anã	AAB	-	NT	+
UFV-Foc242	2011	Fernando Haddad	Tancredo Neves	Northeast	BA	-39.5191700	-13.5822220	253	Banana	Maçã	AAB	-	NT	+
UFV-Foc243	2011	Fernando Haddad	Tancredo Neves	Northeast	BA	-39.5230600	-13.5722220	253	Banana	Maçã	AAB	-	NT	+
UFV-Foc244	2011	Fernando Haddad	Porteirinha	Southeast	MG	-43.3733300	-15.9233330	556	Banana	Prata anã	AAB	-	NT	+
UFV-Foc245	2011	Fernando Haddad	Juazeiro	Northeast	BA	-40.3991700	-9.4694440	369	Banana	Maçã	AAB	-	NT	+
UFV-Foc248	2011	Fernando Haddad	Juazeiro	Northeast	BA	-40.3411100	-9.5447220	369	Banana	Maçã	AAB	-	NT	+
UFV-Foc249	2011	Fernando Haddad	Juazeiro	Northeast	BA	-40.4261100	-9.5144440	369	Banana	Maçã	AAB	-	NT	+
UFV-Foc250	2011	Fernando Haddad	Ponto Novo	Northeast	BA	-40.3663900	-10.9502780	368	Banana	Prata anã	AAB	-	NT	+
UFV-Foc251	2011	Fernando Haddad	Lavras	Southeast	MG	-44.9913900	-21.2313890	919	Banana	Maçã	AAB	-	NT	+
UFV-Foc252	2011	Fernando Haddad	Lavras	Southeast	MG	-44.9913900	-21.2313890	919	Banana	Maçã	AAB	-	NT	+
UFV-Foc256	2011	Fernando Haddad	Guanambi	Northeast	BA	-42.8663900	-14.8647220	515	Banana	Prata Anã	AAB	-	NT	+
UFV-Foc262	2011	Fernando Haddad	Itajuípe	Northeast	BA	-39.3834100	-14.9663890	108	Banana	Maçã	AAB	-	NT	+
UFV-Foc264	2011	Fernando Haddad	Itajuípe	Northeast	BA	-39.4269400	-14.7630560	108	Banana	Prata	AAB	-	NT	+
UFV-Foc265	2011	Fernando Haddad	Itajuípe	Northeast	BA	-39.4241700	-14.7566670	108	Banana	Prata	AAB	-	NT	+
UFV-Foc266	2011	Fernando Haddad	Itajuípe	Northeast	BA	-39.6180600	-14.7708330	108	Banana	Prata	AAB	-	NT	+
UFV-Foc267	2011	Fernando Haddad	Gandu	Northeast	BA	-39.7100000	-13.9477780	155	Banana	Prata	AAB	-	NT	+
UFV-Foc268	2011	Fernando Haddad	Wenceslau	Northeast	BA	-39.9086100	-13.7922220	146	Banana	Prata	AAB	-	NT	+

Supplementary Table 1 (Continued)

Isolate code	Year	Collector/ Sponsor	Municipality	Region	State	Longitude	Latitude	Altitude	Host	Cultivar	Genotype	Clado	VCG	SSR Genotyping
UFV-Foc269	2011	Fernando Haddad	Teolândia	Northeast	BA	-39.5841700	-13.7911110	209	Banana	Prata	AAB	-	NT	+
UFV-Foc270	2011	Fernando Haddad	Pedra Branca	Northeast	CE	-39.7166700	-5.4508330	500	Banana	Maçã	AAB	A	Unknown	+
UFV-Foc271	2011	Fernando Haddad	Mossoró	Northeast	RN	-37.3438900	-5.1877780	500	Banana	Tropical	AAAB	-	Unknown	+
UFV-Foc272	2011	Fernando Haddad	Dom Pedro de Alcântara	South	RS	-49.8716400	-29,3524440	7	Banana	Prata	AAB	-	Unknown	+
UFV-Foc273	2011	Fernando Haddad	Mâmpituba	South	RS	-49.9354400	-29,3960560	37	Banana	Prata	AAB	-	NT	+
UFV-Foc274	2011	Fernando Haddad	Três Cachoeiras	South	RS	-49.9244400	-29,4555560	9	Banana	Prata	AAB	-	NT	+
UFV-Foc275	2011	Fernando Haddad	Três Cachoeiras	South	RS	-49.9244400	-29,4555560	39	Banana	Prata	AAB	A	0120/15	+
UFV-Foc276	2011	Fernando Haddad	Ponto Novo	Northeast	BA	-40.3663900	-10.9502780	368	Banana	Prata Gorutuba	AAB	-	NT	+
UFV-Foc277	2011	Fernando Haddad	Ponto Novo	Northeast	BA	-40.3663900	-10.9502780	368	Banana	Thap Maeo	AAB	-	NT	+
UFV-Foc278	2011	Fernando Haddad	Ponto Novo	Northeast	BA	-40.3663900	-10.9502780	368	Banana	Prata Gorutuba	AAB	-	NT	+
UFV-Foc297	2014	Fernando Haddad	Eldorado	Southeast	SP	-48.2023830	-24.6296140	92	Banana	Nanica	AAA	A	0120	+
UFV-Foc298	2014	Fernando Haddad	Pedro de Toledo	Southeast	SP	-47.2336000	-24.2768000	44	Banana	Nanica	AAA	B	0124/22	+
UFV-Foc303	2014	Fernando Haddad	Miracatu	Southeast	SP	-47.4585000	-24.2860000	27	Banana	Prata-Anã	AAB	A	0120	+
UFV-Foc334	2014	Fernando Haddad	Palmitos	South	SC	-53.0929000	-27.0402000	406	Banana	Nanicão	AAA	-	NT	+
UFV-Foc342	2014	Fernando Haddad	Registro	Southeast	SP	-47.8494000	-24.5083000	25	Banana	Prata Comum	AAB	A	0120	+
UFV-Foc354	2014	Fernando Haddad	Corupá	South	SC	-49.2430560	-26.4252780	75	Banana	Nanica	AAA	A	0120	+
UFV-Foc355	2014	Fernando Haddad	Corupá	South	SC	-49.2826270	-26.4567230	52	Banana	Prata-Anã	AAB	A	*crn* mutant	+
UFV-Foc356	2014	Fernando Haddad	Corupá	South	SC	-49.2826270	-26.4567230	52	Banana	Prata-Anã	AAB	A	Unknown	+
UFV-Foc358	2014	Fernando Haddad	Corupá	South	SC	-49.2826270	-26.4567230	52	Banana	Nanica	AAA	A	0120	+
UFV-Foc387	-	Fernando Haddad	Bom Jesus da Lapa	Northeast	BA	-43.2505000	-13.1518000	436	Banana	Prata Gorotuba	AAB	-	NT	+
UFV-Foc412	2008	Sami Michereff	Bom Conselho	Northeast	PE	-36.6833330	-9.1641670	630	Banana	-	-	B	0124	+
UFV-Foc413	2006	Sami Michereff	Vicencia	Northeast	PE	-35.3275000	-7.6683330	157	Banana	-	-	A	Unknown	+
UFV-Foc438	2006	Sami Michereff	Cruz das Almas	Northeast	BA	-39.1219440	-12.6530560	212	Banana	-	-	A	0120	+
UFV-Foc440	2004	Sami Michereff	Campina Grande	Northeast	PB	-35.8816670	-7.2308330	512	Algodão	-	-	-	NT	+
UFV-Foc470	2007	Sami Michereff	Cruz das Almas	Northeast	BA	-39.1219440	-12.6530560	212	Banana	-	-	-	Unknown	+
UFV-Foc471	2007	Sami Michereff	Cruz das Almas	Northeast	BA	-39.1219440	-12.6530560	212	Banana	-	-	-	Unknown	+
UFV-Foc473	2006	Sami Michereff	Rio Largo	Northeast	AL	-35.8580560	-9.4802780	44	Banana	-	-	-	Unknown	+
UFV-Foc478	2006	Sami Michereff	Rio Largo	Northeast	AL	-35.8580560	-9.4802780	44	Banana	-	-	-	Unknown	+
UFV-Foc480	2006	Sami Michereff	Rio Largo	Northeast	AL	-35.8580560	-9.4802780	44	Banana	-	-	-	Unknown	+

Supplementary Table 1 (Continued)

Isolate code	Year	Collector/ Sponsor	Municipality	Region	State	Longitude	Latitude	Altitude	Host	Cultivar	Genotype	Clado	VCG	SSR Genotyping
UFV-Foc511	2017	Daniel Heck/ Eduardo Mizubuti	Janaúba	Southeast	MG	-43.3521700	-15.7644800	524	Banana	Prata Anã	AAB	A	0120	+
UFV-Foc512	2017	Daniel Heck/ Eduardo Mizubuti	Jaíba	Southeast	MG	-43.7885320	-15.1791840	475	Banana	Prata Catarina	AAB	A	0120	+
UFV-Foc513	2017	Daniel Heck/ Eduardo Mizubuti	Jaíba	Southeast	MG	-43.7885320	-15.1791840	475	Banana	Prata Catarina	AAB	A	0120	+
UFV-Foc514	2017	Daniel Heck/ Eduardo Mizubuti	Jaíba	Southeast	MG	-43.7880210	-15.1766520	474	Banana	Prata Gorutuba	AAB	A	0120	+
UFV-Foc515	2017	Daniel Heck/ Eduardo Mizubuti	Jaíba	Southeast	MG	-43.7873160	-15.1748270	473	Banana	Prata Catarina	AAB	A	0120	+
UFV-Foc516	2017	Daniel Heck/ Eduardo Mizubuti	Serra do Ramalho	Northeast	BA	-43.7021950	-13.2359800	449	Banana	Prata Gorutuba	AAB	A	*crn* mutant	+
UFV-Foc517	2017	Daniel Heck/ Eduardo Mizubuti	Serra do Ramalho	Northeast	BA	-43.7021950	-13.2359800	449	Banana	Prata Gorutuba	AAB	A	0120	+
UFV-Foc518	2017	Daniel Heck/ Eduardo Mizubuti	Serra do Ramalho	Northeast	BA	-43.7021950	-13.2359800	449	Banana	Prata Gorutuba	AAB	A	0120	+
UFV-Foc519	2017	Daniel Heck/ Eduardo Mizubuti	Janaúba	Southeast	MG	-43.3503930	-15.7668810	528	Banana	Prata Catarina	AAB	A	0120	+
UFV-Foc520	2017	Daniel Heck/ Eduardo Mizubuti	Jaíba	Southeast	MG	-43.7880210	-15.1766520	474	Banana	Prata Gorutuba	AAB	-	NT	+
UFV-Foc521	2017	Daniel Heck/ Eduardo Mizubuti	Janaúba	Southeast	MG	-43.3521700	-15.7644800	524	Banana	Prata Anã	AAB	-	NT	+
UFV-Foc522	2017	Daniel Heck/ Eduardo Mizubuti	Jaíba	Southeast	MG	-43.7885320	-15.1791840	475	Banana	Prata Catarina	AAB	-	NT	+
UFV-Foc523	2017	Daniel Heck/ Eduardo Mizubuti	Jaíba	Southeast	MG	-43.7880210	-15.1766520	474	Banana	Prata Gorutuba	AAB	-	NT	+
UFV-Foc526	2017	Daniel Heck/ Eduardo Mizubuti	Corupá	South	SC	-49.2927850	-26.4567230	140	Banana	Caturra	AAA	A	0120	+
UFV-Foc529	2017	Daniel Heck/ Eduardo Mizubuti	Corupá	South	SC	-49.2927850	-26.4567230	140	Banana	Caturra	AAA	A	0120	+
UFV-Foc530	2017	Daniel Heck/ Eduardo Mizubuti	Corupá	South	SC	-49.2826270	-26.4413000	113	Banana	Nanicão	AAA	A	0120	+
UFV-Foc531	2017	Daniel Heck/ Eduardo Mizubuti	Corupá	South	SC	-49.2826270	-26.4413000	113	Banana	Nanicão	AAA	-	NT	+
UFV-Foc533	2017	Daniel Heck/ Eduardo Mizubuti	Corupá	South	SC	-49.2826270	-26.4413000	113	Banana	Nanicão	AAA	-	NT	+
UFV-Foc534	2017	Daniel Heck/ Eduardo Mizubuti	Corupá	South	SC	-49.2985920	-26.4772360	166	Banana	Prata Anã	AAB	A	0120	+
UFV-Foc535	2017	Daniel Heck/ Eduardo Mizubuti	Corupá	South	SC	-49.2985920	-26.4413000	166	Banana	Prata Anã	AAB	-	NT	+
UFV-Foc536	2017	Daniel Heck/ Eduardo Mizubuti	Jaraguá do Sul	South	SC	-49.1896930	-26.4378210	45	Banana	Prata Catarina	AAB	A	0120/15	+
UFV-Foc537	2017	Daniel Heck/ Eduardo Mizubuti	Jaraguá do Sul	South	SC	-49.1896930	-26.4378210	45	Banana	Prata Catarina	AAB	-	NT	+

Supplementary Table 1 (Continued)

Isolate code	Year	Collector/ Sponsor	Municipality	Region	State	Longitude	Latitude	Altitude	Host	Cultivar	Genotype	Clado	VCG	SSR Genotyping
UFV-Foc539	2017	Daniel Heck/ Eduardo Mizubuti	Corupá	South	SC	-49.2430560	-26.4252780	75	Banana	Figo	ABB	A	0120/15	+
UFV-Foc540	2017	Daniel Heck/ Eduardo Mizubuti	Serra do Ramalho	Northeast	BA	-43.7021950	-13.2359800	449	Banana	Prata Gorutuba	AAB	-	NT	+
UFV-Foc541	2017	Daniel Heck/ Eduardo Mizubuti	Serra do Ramalho	Northeast	BA	-43.7021950	-13.2359800	449	Banana	Prata Gorutuba	AAB	-	Unknown	+
UFV-Foc542	2017	Daniel Heck/ Eduardo Mizubuti	Janaúba	Southeast	MG	-43.3521700	-15.7644800	524	Banana	Prata Anã	AAB	B	*crn* mutant	+
UFV-Foc543	2017	Daniel Heck/ Eduardo Mizubuti	Jaíba	Southeast	MG	-43.7873160	-15.1748270	473	Banana	Prata Catarina	AAB	B	0124	+
UFV-Foc557	2017	Daniel Heck/ Eduardo Mizubuti	Janaúba	Southeast	MG	-43.3521700	-15.7644800	524	Banana	Prata Anã	AAB	-	NT	+
UFV-Foc559	2017	Daniel Heck/ Eduardo Mizubuti	Janaúba	Southeast	MG	-43.3503930	-15.7668810	528	Banana	Prata Catarina	AAB	-	NT	+
UFV-Foc560	2017	Daniel Heck/ Eduardo Mizubuti	Janaúba	Southeast	MG	-43.3503930	-15.7668810	528	Banana	Prata Catarina	AAB	-	NT	+
UFV-Foc561	2017	Daniel Heck/ Eduardo Mizubuti	Jaíba	Southeast	MG	-43.7880210	-15.1766520	474	Banana	Prata Gorutuba	AAB	-	NT	+
UFV-Foc562	2017	Daniel Heck/ Eduardo Mizubuti	Corupá	South	SC	-49.2985920	-26.4772360	166	Banana	Prata Anã	AAB	-	NT	+
UFV-Foc569	2017	Daniel Heck/ Eduardo Mizubuti	Corupá	South	SC	-49.2826270	-26.4413000	113	Banana	Nanicão	AAA	-	NT	+
UFV-Foc571	2017	Daniel Heck/ Eduardo Mizubuti	Corupá	South	SC	-49.1896930	-26.4378210	45	Banana	Prata Catarina	AAB	-	NT	+
UFV-Foc572	2017	Daniel Heck/ Eduardo Mizubuti	Janaúba	Southeast	MG	-43.3521700	-15.7644800	524	Banana	Prata Anã	AAB	-	NT	+
UFV-Foc575	2017	Daniel Heck/ Eduardo Mizubuti	Jaíba	Southeast	MG	-43.7880210	-15.1766520	474	Banana	Prata Gorutuba	AAB	-	NT	+
UFV-Foc576	2017	Daniel Heck/ Eduardo Mizubuti	Jaíba	Southeast	MG	-43.7873160	-15.1748270	473	Banana	Prata Catarina	AAB	A	0120	+
UFV-Foc577	2017	Daniel Heck/ Eduardo Mizubuti	Teixeiras	Southeast	MG	-42.8337730	-20.6384300	690	Banana	Prata	AAB	A	0120	+
UFV-Foc578	2017	Daniel Heck/ Eduardo Mizubuti	Teixeiras	Southeast	MG	-42.8337730	-20.6384300	690	Banana	Prata	AAB	A	0120	+
UFV-Foc579	2017	Daniel Heck/ Eduardo Mizubuti	Teixeiras	Southeast	MG	-42.8337730	-20.6384300	690	Banana	Prata	AAB	B	*crn* mutant	+
UFV-Foc580	2017	Daniel Heck/ Eduardo Mizubuti	Teixeiras	Southeast	MG	-42.8337730	-20.6384300	690	Banana	Prata	AAB	-	NT	+
UFV-Foc581	2017	Daniel Heck/ Eduardo Mizubuti	Teixeiras	Southeast	MG	-42.8337730	-20.6384300	690	Banana	Prata	AAB	A	01215	+
UFV-Foc582	2017	Daniel Heck/ Eduardo Mizubuti	Teixeiras	Southeast	MG	-42.8337730	-20.6384300	690	Banana	Prata	AAB	A	0120	+
UFV-Foc583	2017	Daniel Heck/ Eduardo Mizubuti	Teixeiras	Southeast	MG	-42.8337730	-20.6384300	690	Banana	Prata	AAB	A	0120	+
UFV-Foc584	2017	Daniel Heck/ Eduardo Mizubuti	Teixeiras	Southeast	MG	-42.8337730	-20.6384300	690	Banana	Prata	AAB	-	NT	+

Supplementary Table 1 (Continued)

Isolate code	Year	Collector/ Sponsor	Municipality	Region	State	Longitude	Latitude	Altitude	Host	Cultivar	Genotype	Clado	VCG	SSR Genotyping
UFV-Foc585	2017	Daniel Heck/ Eduardo Mizubuti	Teixeiras	Southeast	MG	-42.8337730	-20.6384300	690	Banana	Prata	AAB	-	NT	+
UFV-Foc586	2017	Daniel Heck/ Eduardo Mizubuti	Teixeiras	Southeast	MG	-42.8337730	-20.6384300	690	Banana	Prata	AAB	-	NT	+
UFV-Foc590	2018	Daniel Heck/ Eduardo Mizubuti	Teixeiras	Southeast	MG	-42.8337730	-20.6384300	690	Banana	Maca	AAB	-	NT	+
UFV-Foc592	2018	Daniel Heck/ Eduardo Mizubuti	Vicosa	Southeast	MG	-42.8786000	-20.7549000	690	Banana	Maca	AAB	-	NT	+
UFV-Foc705	-	-	Barbalha	Northeast	CE	-39.3025000	-7.3055100	415	Banana	Prata	AAB	B	0124/22	+
UFV-Foc706	-	-	Barbalha	Northeast	CE	-39.3025000	-7.3055100	415	Banana	Prata	AAB	B	0124/5/8/22	+
UFV-Foc707	-	-	Barbalha	Northeast	CE	-39.3025000	-7.3055100	415	Banana	Prata	AAB	B	0124	+
UFV-Foc708	-	-	Barbalha	Northeast	CE	-39.3025000	-7.3055100	415	Banana	Prata	AAB	B	0125/8/20	+
UFV-Foc709	-	-	Barbalha	Northeast	CE	-39.3025000	-7.3055100	415	Banana	Prata	AAB	B	0124/5/22	+
UFV-Foc711	-	-	Barbalha	Northeast	CE	-39.3025000	-7.3055100	415	Banana	Prata	AAB	B	*crn* mutant	+
UFV-Foc712	-	-	Barbalha	Northeast	CE	-39.3025000	-7.3055100	415	Banana	Prata	AAB	B	01222	+
UFV-Foc713	-	-	Barbalha	Northeast	CE	-39.3025000	-7.3055100	415	Banana	Prata	AAB	A	0122	+
UFV-Foc715	-	-	Itinga do Maranhão	Northeast	MA	-47.5300000	-4.4521500	144	Banana	Prata	AAB	B	0124/5/22	+
UFV-Foc716	-	-	Itinga do Maranhão	Northeast	MA	-47.5300000	-4.4521500	144	Banana	Prata	AAB	B	0124/5/22	+
UFV-Foc775	2017	Jânia Bentes	Manaus	North	AM	-60.0261000	-3.1071900	39	Banana	-	-	-	Unknown	+
UFV-Foc776	2017	Jânia Bentes	Manaus	North	AM	-60.0261000	-3.1071900	39	Banana	-	-	-	Unknown	+
UFV-Foc777	2018	Daniel Heck/ Eduardo Mizubuti	Teixeiras	Southeast	MG	-42.8262700	-20.6314960	695	Banana	Prata	AAB	-	NT	+
UFV-Foc785	2009	Daniel Schurt	Boa Vista	North	RR	-60.6714000	-2.8195400	76	Banana	-	-	-	Unknown	+
UFV-Foc793	-	Gilson Silva	São Luís	Northeast	MA	-44.3068000	-2.5300000	4	Banana	-	-	-	*crn* mutant	+
UFV-Foc795	2017	Jânia Bentes	Manaus	North	AM	-60.0261000	-3.1071900	39	Banana	-	-	-	NT	+
UFV-Foc796	2017	Jânia Bentes	Manaus	North	AM	-60.0261000	-3.1071900	39	Banana	-	-	-	Unknown	+
UFV-Foc797	2017	Jânia Bentes	Manaus	North	AM	-60.0261000	-3.1071900	39	Banana	-	-	-	Unknown	+
UFV-Foc798	2017	Jânia Bentes	Manaus	North	AM	-60.0261000	-3.1071900	39	Banana	-	-	-	Unknown	+
UFV-Foc799	2017	Jânia Bentes	Manaus	North	AM	-60.0261000	-3.1071900	39	Banana	-	-	-	Unknown	+

VCG: Vegetative compatibility group identified in this study as described by Puhalla 1985
NT: Not tested

Supplementary Table 2 Vegetative compatibility tester strains used in this study.

Code	Species	Formae speciales	Mutation	Cultivar	Location	Country	VCG	Provided by	Year	Race[1]
O-1219-2	*Fusarium oxysporum*	*cubense*	*nit*M	Mons	Queensland	Australia	0120	Randy Ploetz, UF	2017	4?
O-1219-10	*Fusarium oxysporum*	*cubense*	*nit*1	Mons	Queensland	Australia	0120	Randy Ploetz, UF	2017	4?
F9130-2	*Fusarium oxysporum*	*cubense*	*nit*1	Cavendish	-	Taiwan	0121	Randy Ploetz, UF	2017	4
F9130-7	*Fusarium oxysporum*	*cubense*	*nit*M	Cavendish	-	Taiwan	0121	Randy Ploetz, UF	2017	4
PH2-3	*Fusarium oxysporum*	*cubense*	*nit*1	Cavendish	-	Philippines	0122	Randy Ploetz, UF	2017	4?
PHL1-8	*Fusarium oxysporum*	*cubense*	*nit*M	Latundan	-	Philippines	0123	Randy Ploetz, UF	2017	?
PHL2-8	*Fusarium oxysporum*	*cubense*	*nit*1	Latundan	-	Philippines	0123	Randy Ploetz, UF	2017	?
BLUG15	*Fusarium oxysporum*	*cubense*	*nit*M	Bluggoe	-	Honduras	0124	Randy Ploetz, UF	2017	2
S?-1	*Fusarium oxysporum*	*cubense*	*nit*1	Tetraploid 1242	Bodles	Jamaica	0124	Randy Ploetz, UF	2017	2
8610-2	*Fusarium oxysporum*	*cubense*	*nit*1	Lady finger	Murwillumbah	Australia	0125	Randy Ploetz, UF	2017	1
8611-9	*Fusarium oxysporum*	*cubense*	*nit*M	Lady finger	Queensland	Australia	0125	Randy Ploetz, UF	2017	1
STM2-1	*Fusarium oxysporum*	*cubense*	*nit*M	Maqueno	-	Honduras	0126	Randy Ploetz, UF	2017	1
22994-5	*Fusarium oxysporum*	*cubense*	*nit*1	Bluggoe	South Johnstone	Australia	0128	Randy Ploetz, UF	2017	2
22994-6	*Fusarium oxysporum*	*cubense*	*nit*M	Bluggoe	South Johnstone	Australia	0128	Randy Ploetz, UF	2017	2
8622-9	*Fusarium oxysporum*	*cubense*	*nit*1	Cavendish	Queensland	Australia	0129	Randy Ploetz, UF	2017	4?
A1-1-7	*Fusarium oxysporum*	*cubense*	*nit*1	Apple	Florida	United State	01210	Randy Ploetz, UF	2017	1?
A1-1-9	*Fusarium oxysporum*	*cubense*	*nit*M	Apple	Florida	United State	01210	Randy Ploetz, UF	2017	1?
SH3142-3	*Fusarium oxysporum*	*cubense*	*nit*M	SH 3142	Queensland	Australia	01211	Randy Ploetz, UF	2017	?
SH3142-4	*Fusarium oxysporum*	*cubense*	*nit*1	SH 3142	Queensland	Australia	01211	Randy Ploetz, UF	2017	?
STNP2-3	*Fusarium oxysporum*	*cubense*	*nit*M	Ney Poovan	Tenguero Station	Tanzania	01212	Randy Ploetz, UF	2017	?

Supplementary Table 2 (Continued)

Code	Species	Formae speciales	Mutation	Cultivar	Location	Country	VCG	Provided by	Year	Race[1]
STNP4-8	*Fusarium oxysporum*	*cubense*	*nit*1	Ney Poovan	Bukaba Station	Tanzania	01212	Randy Ploetz, UF	2017	?
MA2-2	*Fusarium oxysporum*	*cubense*	*nit*M	Harare	Karonga	Malawi	01214	Randy Ploetz, UF	2017	?
MW40-1	*Fusarium oxysporum*	*cubense*	*nit*1	Harare	Karonga	Malawi	01214	Randy Ploetz, UF	2017	?
Cr2-3-1	*Fusarium oxysporum*	*cubense*	*nit*M	Gros Michel	Isolona	Costa Rica	01215	Randy Ploetz, UF	2017	?
Cr2-2-1	*Fusarium oxysporum*	*cubense*	*nit*1	Gros Michel	Hamburgo	Costa Rica	01215	Randy Ploetz, UF	2017	?
Indo25-9	*Fusarium oxysporum*	*cubense*	*nit*1	Pisang Ambon	Sumatra	Indonesia	01219	Randy Ploetz, UF	2017	?
24223	*Fusarium oxysporum*	*cubense*	*nit*M	Williams	Carnarvon	Australia	01220	Randy Ploetz, UF	2017	?
RPML9-1	*Fusarium oxysporum*	*cubense*	*nit*1	Pisang awak legor	Pantai Acheh	Malaysia	01222	Randy Ploetz, UF	2017	?
RPML4-6	*Fusarium oxysporum*	*cubense*	*nit*M	Pisang awak legor	Pantai Acheh	Malaysia	01222	Randy Ploetz, UF	2017	?

[1] Information provided by the collector.

Supplementary table 3 Primer sequences, SSR motifs, number of alleles and allele sizes, used in this study.

SSR Primer[2]	Locus	Motifs	Primers sequences 5′–3′	Fluorescent dye	Allele size range (bp)	No. of alleles[1]
MB2	FO2	(GT)11(GA)6	F: TGCTGTGTATGGATGGATGG R: CATGGTCGATAGCTTGTCTCAG	NED	240-272	10
MB5	FO5	(TG)9	F: ACTTGGAGGAAATGGGCTTC R: GGATGGCGTTTAATAAATCTGG	VIC	-	-
MB9	FO9	(CA)9	F: TGGCTGGGATACTGTGTAATTG R: TTAGCTTCAGAGCCCTTTGG	NED	-	-
MB10	FO10	(AAC)6	F: TATCGAGTCCGGCTTCCAGAAC R: TTGCAATTACCTCCGATACCAC	6FAM	-	-
MB11	FO11	(GGC)7	F: GTGGACGAACACCTGCATC R: AGATCCTCCACCTCCACCTC	VIC	173-182	4
MB13	FO13	(CTTGGAAGTGGTAGCGG)14	F: GGAGGATGAGCTCGATGAAG R: CTAAGCCTGCTACACCCTCG	PET	-	-
MB14	FO14	(CCA)5	F: CGTCTCTGAACCACCTTCATC R: TTCCTCCGTCCATCCTGAC	6FAM	181-184	2
MB17	FO17	(CA)21	F: ACTGATTCACCGATCCTTGG R: GCTGGCCTGACTTGTTATCG	6FAM	302-334	10
MB18	FO18	(CAACA)6	F: GGTAGGAAATGACGAAGCTGAC R: TGAGCACTCTAGCACTCCAAAC	PET	254-294	6

[1]Number of observed alleles.
[2]Simple sequence repeats primers (SSRs) described by Bogale et al. 2005.