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VCG and AFLP analyses identify the same groups in the causal agents of mango malformation in Brazil

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Abstract The causal agents of mango malformation disease in Brazil are a new *Fusarium* lineage in the *Gibberella fujikuroi* species complex and *Fusarium sterilihyphosum*; however information on the genetic and geographical diversity of these pathogens in Brazil is missing. Vegetative compatibility group (VCG) and amplified fragment length polymorphism (AFLP) analyses were used to measure the genetic diversity within these populations. Both techniques identified the same genetic groups. Six VCG and AFLP groups were identified amongst isolates of the new lineage from Brazil. FB-VCG 1/AFLP I was the most widespread group, found in seven of the 13 sites sampled. The second most frequent group was recovered from three sites. The remaining four groups were recovered from single-sites. We think that this lineage represents a genetically and geographically diverse indigenous population that reproduces clonally. In *F. sterilihyphosum*, group FS-VCG 1/AFLP VII was found at three sites in the southeast region of Brazil. FS-VCG 2/AFLP VIII contained isolates from South Africa but not from Brazil. *Fusarium mangiferae* isolates from India and South Africa formed one group, while isolates from Egypt and the USA formed a second group. *F. sterilihyphosum* at present is represented by a small population that might have been introduced only once into a restricted area. The clonal nature of the observed populations suggests that these fungi either occur naturally on indigenous hosts and have jumped to the introduced mango host (introduced in Brazil) or that they originated with mango and went through a severe population bottleneck when they were introduced to Brazil from India or Southeast Asia.

Keywords *Gibberella fujikuroi* species complex · Plant disease · Tropical fruit crops · DNA fingerprint · Characterization

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Introduction

The mango tree, *Mangifera indica*, is cultivated commercially in many tropical and subtropical regions of the world, and mango malformation is one of the most important diseases affecting mango production in Brazil and elsewhere (Cunha et al. 2000; Ploetz 2001). The floral malformation occurs mainly on adult trees and severely reduces fruit set,

especially in semi-arid areas such as in the São Francisco River Valley, where the disease incidence is higher. In this region malformation occurs in ~30% of the commercial orchards, with up to 100% of the trees affected in some areas (Tavares and Lima 1997; São Jose et al. 2000). Vegetative malformation, which causes witch's broom, occurs primarily in nurseries, and is less important in mature trees (Cunha et al. 2000).

The cause of mango malformation has been the subject of discussion for >100 years, since its first observation in India in 1891 (Summanwar et al. 1966; Ploetz 2001). Fungal species in the genus *Fusarium* are now considered to be the causal agents of the disease (Freeman et al. 1999; Marasas et al. 2006; Lima 2006; Lima et al. 2008). Koch's postulates were completed for the first time for this disease in 1966, using isolates identified as *F. moniliforme* var. *subglutinans* (Summanwar et al. 1966).

Populations of *F. subglutinans sensu lato* from several plant species were elevated to the species category, based on phylogenetic studies (O'Donnell et al. 1998), and/or studies of sexual compatibility (Leslie et al. 2005). The population associated with maize was defined as *F. subglutinans sensu stricto* (teleomorph *Gibberella subglutinans*), that from sugar cane was *F. sacchari* (teleomorph *G. sacchari*), that from pineapple was *F. guttiforme*, and that from *Pinus* spp. was *F. circinatum* (teleomorph *G. circinata*) (Leslie and Summerell 2006).

Fusarium subglutinans sensu lato isolates associated with mango malformation have been divided into two phylogenetically distinct populations that were formally described as *Fusarium mangiferae* and *Fusarium sterilihyphosum* (Steenkamp et al. 2000; Britz et al. 2002). *F. mangiferae* has been reported in mango-producing countries (Marasas et al. 2006). *F. sterilihyphosum* has been reported in South Africa, from which it was first described, and from Brazil (Britz et al. 2002; Zheng and Ploetz 2002; Lima 2006). Recently, a new *Fusarium* lineage, referred in this study as *Fusarium* '*subglutinans*' was reported as the main causal agent of mango malformation in Brazil. *F. 'subglutinans'* belongs to the *Gibberella fujikuroi* species complex and is closely related to *F. sterilihyphosum* from which it cannot be differentiated based solely on morphological markers (Lima 2006; Lima et al. 2008).

Vegetative compatibility groups (VCGs) can be used to evaluate genotypic diversity in fungal populations (Zheng and Ploetz 2002; Heilmann et al. 2006; Ramirez et al. 2006). The relative number of VCGs is expected to be higher in a sexual population than in an asexual population. In asexual populations, VCG and pathogenicity may be correlated, which allows VCG to be used as a surrogate for pathogenicity whenever the population is primarily asexual (Leslie 1993). Vegetative compatibility data for populations of *F. oxysporum* have been used to identify sub-specific groups that can sometimes be correlated with *formae speciales* and/or pathogenicity (Baayen et al. 2000; Di Primo et al. 2001; Wang et al. 2006).

Isolates of *F. sterilihyphosum* from South Africa all belong to a single VCG (Steenkamp et al. 2000), while populations of *F. mangiferae* from India and South Africa each contain isolates belonging to a single, unique VCG. Populations from Israel, the United States and Egypt, contain two, three and four VCGs, respectively. *F. sterilihyphosum* isolates from Brazil analyzed thus far all belong to a single, unique VCG (Zheng and Ploetz 2002).

Amplified fragment length polymorphism (AFLP) is a powerful tool for evaluating genetic variation in *Fusarium* populations (Leslie and Summerell 2006). In populations of both *Fusarium* (Baayen et al. 2000; Wang et al. 2006) and *Colletotrichum* (Heilmann et al. 2006), the groups resulting from AFLP and VCG analyses are correlated, if not identical, especially if sexual reproduction is limited. If sexual recombination is relatively common, however, then members of the same VCG need not be clones and may have quite distinct AFLP banding patterns (Chulze et al. 2000).

There have been no studies of genetic variation in isolates of the main causal agent of mango malformation in Brazil. Our goal in this study was to identify VCGs in *F.* '*subglutinans*', *F. sterilihyphosum* and *F. mangiferae* and to determine if there is a correlation between the VCG and AFLP groupings for these species. Our working hypothesis is that VCG and AFLP analyses will group the isolates in a similar manner, suggesting that the mango pathogens reproduce primarily asexually.

Materials and methods

Fungal material

Thirty-five *Fusarium* isolates were obtained from mango tissue with either floral or vegetative malfor-

mation symptoms from several locations in Brazil and other countries (Table 1). Isolates were purified as single conidial subcultures and preserved in distilled water (Castellani 1939) at 10°C in Coleção Micológica de Lavras – CML, Universidade Federal de Lavras, Lavras, Minas Gerais, Brazil. Each Brazilian isolate used in this study was obtained from a different mango tree. Duplicates of isolates are maintained as spore suspensions in 15% glycerol at −70°C at the Plant Pathology Department, Throckmorton Plant Sciences Center, Kansas State University, Manhattan, KS, USA.

Induction of *nit* mutants

Fusarium isolates were grown on minimal medium (MM) for 7 days at 23°C. Agar plugs (1–2 mm^3) from the margin of the colonies were transferred to plates containing MM + $KClO_3$ (MMC). The concentration of $KClO_3$ ranged from 1.5 to 2.5% (Correll et al. 1987; Leslie and Summerell 2006). Initially, 1.6 g l^{-1} of L-asparagine was added as an alternative nitrogen source, but in later experiments the alternative nitrogen source was changed to 1.4 g l^{-1} of L-threonine. The change from L-asparagine to L-threonine increases the percentage of NitM mutants recovered (Klittich and Leslie 1988a). For each isolate, ten Petri dishes (90×15 mm) containing MMC were inoculated with three agar plugs per plate (30 mycelial plugs per isolate) and incubated at 25°C for 14 to 21 days. Rapidly growing sectors (one sector by mycelial plug) were transferred to MM. Sectors that grew well on MM were discarded as putative CRN mutants (Klittich and Leslie 1989), while those that had thin mycelial growth were retained as putative *nit* mutants (Leslie and Summerell 2006).

nit mutant phenotypes

Basal medium (BM) supplemented with either $NaNO_2$ (0.4 g l^{-1}) or hypoxanthine (0.5 g l^{-1}) as the sole nitrogen source was used to classify the mutants as *nit1*, *nit3* or NitM (Correll et al. 1987; Leslie and Summerell 2006). Nine unclassified *nit* mutants were cultured per Petri dish (90×15 mm), with an isolate representative of each phenotypic class included on each plate as a control. Plates were incubated at 25°C for 3 to 4 days (Leslie and Summerell 2006). Isolates with wild-type growth on BM + $NaNO_2$ and on BM + hypoxanthine were classified as *nit1*, those with wild-type growth only on BM + hypoxanthine were classified as *nit3*, and those with wild-type growth only on BM + $NaNO_2$ were classified as NitM (Correll et al. 1987; Leslie and Summerell 2006).

Complementation tests using 24-well plates

Complementation tests were made as previously described (Klittich and Leslie 1988b; Leslie and Summerell 2006), by placing drops of spore suspensions from mutants in complementary phenotypic classes, *e.g.*, *nit1*/NitM or *nit3*/NitM, in 24-well tissue-culture plates containing 1.7 ml of MM + $NaNO_3$ per well and incubated at 25°C for 7–14 days. As negative controls, NitM mutants were inoculated singly in the first horizontal row, and either a *nit1* or a *nit3* mutant was inoculated in the first vertical row. The 24-well plates were incubated at 25°C under fluorescent bulbs in a 12 h light/12 h dark photoperiod. Heterokaryon formation was evaluated after 3 days.

Complementation results were confirmed by pairing mycelial plugs from different phenotypic classes, *nit1*/NitM or *nit3*/NitM in Petri dishes (90×15 mm), incubated as described above. In both the 24-well plates and the Petri dishes, heterokaryon formation was considered positive only if robust mycelial growth occurred where the mutant colonies met.

Heterokaryon stability

Ten consecutive mycelial mass transfers were done to test the stability of the prototrophic heterokaryons formed in the complementation tests. After the formation of the robust mycelial growth, a mycelial plug was transferred to Petri dishes containing MM + $NaNO_3$ and incubated at 25°C for 7 days. Heterokaryons that maintained the prototrophic (robust) growth after ten subcultures were considered stable (Di Primo et al. 2001; Leslie and Summerell 2006).

DNA extraction and AFLP analysis

Isolates were cultivated in complete medium broth (Correll et al. 1987; Leslie and Summerell 2006) and incubated on a shaker (150 rpm) for two days at 25–28°C. The mycelia were harvested, frozen with liquid

Table 1 Vegetative compatibility groups (VCGs) in isolates of *Fusarium* spp. obtained from malformed panicles and shoots of mango

Species	Origin[a]	Isolates[b]	AFLP	VCG[c]
F. mangiferae	India	CML 406 (BBA 69662, KSU 16242)	IX	FM-VCG 1
F. mangiferae	India	CML 407 (BBA 70826, KSU 16243)	IX	FM-VCG 1
F. mangiferae	South Africa (ex-paratype)	CML 412 (MRC 2730, KSU 3873)	IX	nd (VCG 3[d]; VCG 5[e])
F. mangiferae	Egypt	CML 409 (BBA 70896, KSU 16245)	X	FM-VCG 2
F. mangiferae	Egypt	KSU 4700 (MRC 8091)	X	nd (VCG 1[e])
F. mangiferae	USA, Florida	KSU 4079 (MRC 8088)	X	nd (VCG 5[e])
F. sterilihyphosum	Brazil, IJaci, MG	CML 280 (KSU 16213)	VII	FS-VCG 1
F. sterilihyphosum	Brazil, Itumirim, MG	CML 282 (KSU 16215)	VII	FS-VCG 1
F. sterilihyphosum	Brazil, Piracicaba, SP	CML 283 (KSU 16216)	VII	FS-VCG 1
F. sterilihyphosum	Brazil, Lavras, MG	CML 401 (KSU 16240)	VII	FS-VCG 1
F. sterilihyphosum	South Africa (*ex*-holotype)	CML 414 (MRC 2802, NRRL 25623, KSU 16250)	VIII	FS-VCG 2 (VCG 4[d])
F. sterilihyphosum	South Africa	KSU 11783 (MRC 8095)	VIII	FS-VCG 2
F. '*subglutinans*'	Brazil, Almofala, CE	CML 386 (KSU 16230)	I	FB-VCG 1
F. '*subglutinans*'	Brazil, Eloi Mendes, MG	CML 278 (KSU 16211)	I	FB-VCG 1
F. '*subglutinans*'	Brazil, Juazeiro, BA	CML 388 (KSU 16232)	I	FB-VCG 1
F. '*subglutinans*'	Brazil, Lavras, MG	CML 258 (KSU 16191)	I	FB-VCG 1
F. '*subglutinans*'	Brazil, Lavras, MG	CML 275 (KSU 16208)	I	FB-VCG 1
F. '*subglutinans*'	Brazil, Lavras, MG	CML 262 (KSU 16195)	I	nd
F. '*subglutinans*'	Brazil, Livramento, BA	CML 346 (KSU 16218)	I	FB-VCG 1
F. '*subglutinans*'	Brazil, Livramento, BA	CML 349 (KSU 16221)	I	FB-VCG 1
F. '*subglutinans*'	Brazil, Passos, MG	CML 269 (KSU 16202)	I	FB-VCG 1
F. '*subglutinans*'	Brazil, Rondonópolis, MT	CML 345 (KSU 16217)	I	FB-VCG 1
F. '*subglutinans*'	Brazil, São João Del Rei, MG	CML 279 (KSU 16212)	I	nd
F. '*subglutinans*'	Brazil, Cruz, CE	CML 381 (KSU 16225)	II	nd
F. '*subglutinans*'	Brazil, Petrolina, PE	CML 387 (KSU 16231)	II	FB-VCG 2
F. '*subglutinans*'	Brazil, Várzea Grande, MT	CML 350 (KSU 16222)	II	FB-VCG 2
F. '*subglutinans*'	Brazil, Nova Porteirinha, MG	CML 382 (KSU 16226)	III	FB-VCG 3
F. '*subglutinans*'	Brazil, Nova Porteirinha, MG	CML 383 (KSU 16227)	III	FB-VCG 3
F. '*subglutinans*'	Brazil, Nova Porteirinha, MG	CML 384 (KSU 16228)	IV	FB-VCG 4
F. '*subglutinans*'	Brazil, Nova Porteirinha, MG	CML 385 (KSU 16229)	IV	FB-VCG 4
F. '*subglutinans*'	Brazil, Juazeiro, BA	CML 389 (KSU 16233)	V	FB-VCG 5
F. '*subglutinans*'	Brazil, Petrolina, PE	CML 390 (KSU 16234)	V	FB-VCG 5
F. '*subglutinans*'	Brazil, Uberlândia, MG	CML 259 (KSU 16192)	VI	FB-VCG 6
F. '*subglutinans*'	Brazil, Uberlândia, MG	CML 260 (KSU 16193)	VI	FB-VCG 6
F. '*subglutinans*'	Brazil, Uberlândia, MG	CML 261 (KSU 16194)	VI	FB-VCG 6
F. '*subglutinans*'	Brazil, Uberlândia, MG	CML 264 (KSU 16197)	VI	FB-VCG 6
F. '*subglutinans*'	Brazil, Uberlândia, MG	CML 266 (KSU 16199)	VI	FB-VCG 6

F. '*subglutinans*' is a new phylogenetic lineage in the *Gibberella fujikuroi* species complex and is the main causal agent of mango malformation disease in Brazil (Lima 2006; Lima et al. 2008).

[a] Brazilian states: *BA* Bahia, *CE* Ceará, *MG* Minas Gerais, *MT* Mato Grosso, *PE* Pernambuco, *SP* São Paulo

[b] Culture collection abbreviations: *BBA* Biologische Bundesanstalt für Land- und Forstwirschaft, Berlin, Germany; *CML* Coleção Micológica de Lavras, Plant Pathology Department, Universidade Federal de Lavras, Brazil; *KSU* Kansas State University, Manhattan, Kansas, USA; *MRC* Medical Research Council, Programme on Mycotoxins and Experimental Carcinogenesis (PROMEC), Tygerberg, South Africa; *NRRL* National Center for Agricultural Utilization Research, Peoria, Illinois, USA

[c] VCGs FM, FS and FB were determined in this study, while groups inside brackets were determined as indicated. *nd* used for isolates where a VCG could not be assigned.

[d] VCG determined by Steenkamp et al. (2000).

[e] VCG determined by Zheng and Ploetz (2002).

N_2, and ground to a powder in a mortar with a pestle. Approximately 150 mg of ground mycelium was added to a 1.5 ml microcentrifuge tube and kept at −70°C until DNA was extracted. The DNA was purified by the CTAB-based protocol of Leslie and Summerell (2006).

AFLP patterns (Vos et al. 1995) were generated as previously described by Leslie and Summerell (2006). The *Eco*RI + GG/*Mse*I + CT, *Eco*RI + TT/*Mse*I + AC and *Eco*RI + AA/*Mse*I + TT primer combinations were used to generate the AFLP band patterns. *Eco*RI primers in the selective amplification were labelled with ATP γ-^{33}P. Following PCR amplification, AFLP fragments were separated in denaturing 6% polyacrylamide gels (Long Ranger; FMC Scientific, Rockland, ME, USA) in ×1 TBE buffer [100 mM Tris Base, 100 mM boric acid, and 2 mM EDTA (pH 8.0)]. The gel was blotted on a 3MW membrane (Midwest Scientific, Valley Park, MO, USA) and dried prior to exposure to X-ray film (Classic Blue Sensitive; Midwest Scientific) for 3 to 7 days. Bands between 200 and 800 bp in size were scored manually and a binary matrix was generated where '1' corresponded to the presence of the allele and '0' to its absence.

AFLP bands of the same molecular size were considered homologous in the analyses. The unweighted pair-group method with arithmetic means (UPGMA) as implemented by PAUP (PAUP 4.0 version beta 10; Swofford 2000) was used to generate the network presented. Dendrogram topologies were assessed through bootstrap analysis in PAUP with 1,000 replicates.

Results

Generation of *nit* mutants and phenotypes

At 1.5% $KClO_3$ 25% of the colonies formed *nit* mutant sectors, while at 2.5% $KClO_3$ the frequency of sectoring colonies increased to 40%. When L-asparagine was used as the primary nitrogen source, then 20% of the *nit* mutants recovered were NitM. However when L-threonine was used as the primary nitrogen source, then 80% of the *nit* mutants recovered belonged to the NitM class. *Nit1, nit3* and NitM mutants were obtained from all of the wild-type isolates except CML 262, 279, 381 and 412, and KSU 4700 and 4079.

Complementation tests

A line of robust mycelial growth was observed when *nit1*/NitM or *nit3*/NitM mutants from the same wild-type isolate were paired, *i.e.* all of the isolates with *nit* mutants in at least two phenotypic classes were heterokaryon self-compatible (Correll et al. 1989; Leslie and Summerell 2006). Isolates CML 262, 279, 381 and 412, and KSU 4700 and 4079 could not have a VCG assigned due to the lack of at least two phenotypic classes to test for self-compatibility when they fail to complement with the testers or the absence of sectors when cultivated on MM + chlorate. Based on complementation patterns, six VCGs (FB-VCG 1 to 6) were identified amongst the *F.* '*subglutinans*' isolates, two VCGs (FM-VCG 1 and 2) were identified for *F. mangiferae*, and two more (FS-VCG 1 and 2) for *F. sterilihyphosum* (Table 1). None of the VCGs identified in this study contained isolates from more than one species. Isolate BBA 70896 of *F. mangiferae* from Egypt and other isolates of this species from India were in different VCGs. The Brazilian isolates of *F. sterilihyphosum* collected in the southeast region formed a unique VCG, FS-VCG 1, and isolates from South Africa formed a second group, FS-VCG 2. For *F.* '*subglutinans*': FB-VCG 1 was widespread across the mango-producing regions and dominated in southern Minas Gerais. FB-VCG 2 was restricted to the states of Pernambuco and Mato Grosso. FB-VCG 3, FB-VCG 4 and FB-VCG 6 were found only in Minas Gerais. FB-VCG 6 was restricted to a single site in the western part of Minas Gerais, while FB-VCG 3 and FB-VCG 4 each were recovered only from the northern part of the state. FB-VCG 5 was present in the states of Pernambuco and Bahia, but at geographically close sites (Fig. 1). All of the heterokaryons formed after pairing of complementary auxotrophic *nit* mutants retained prototrophic growth after 10 consecutive mycelial mass transfers on MM. It was possible to obtain isolates belonging to different species or to different VCGs of the same species from the same site (Fig. 1).

AFLP analysis

Two hundred and eighty-six bands were scored from the gels for the three primer sets used. In the UPGMA network generated with *Eco*RI + GG/*Mse*I + CT, *Eco*RI + TT/*Mse*I + AC and *Eco*RI + AA/*Mse*I + TT primer combinations, three groups were identified that

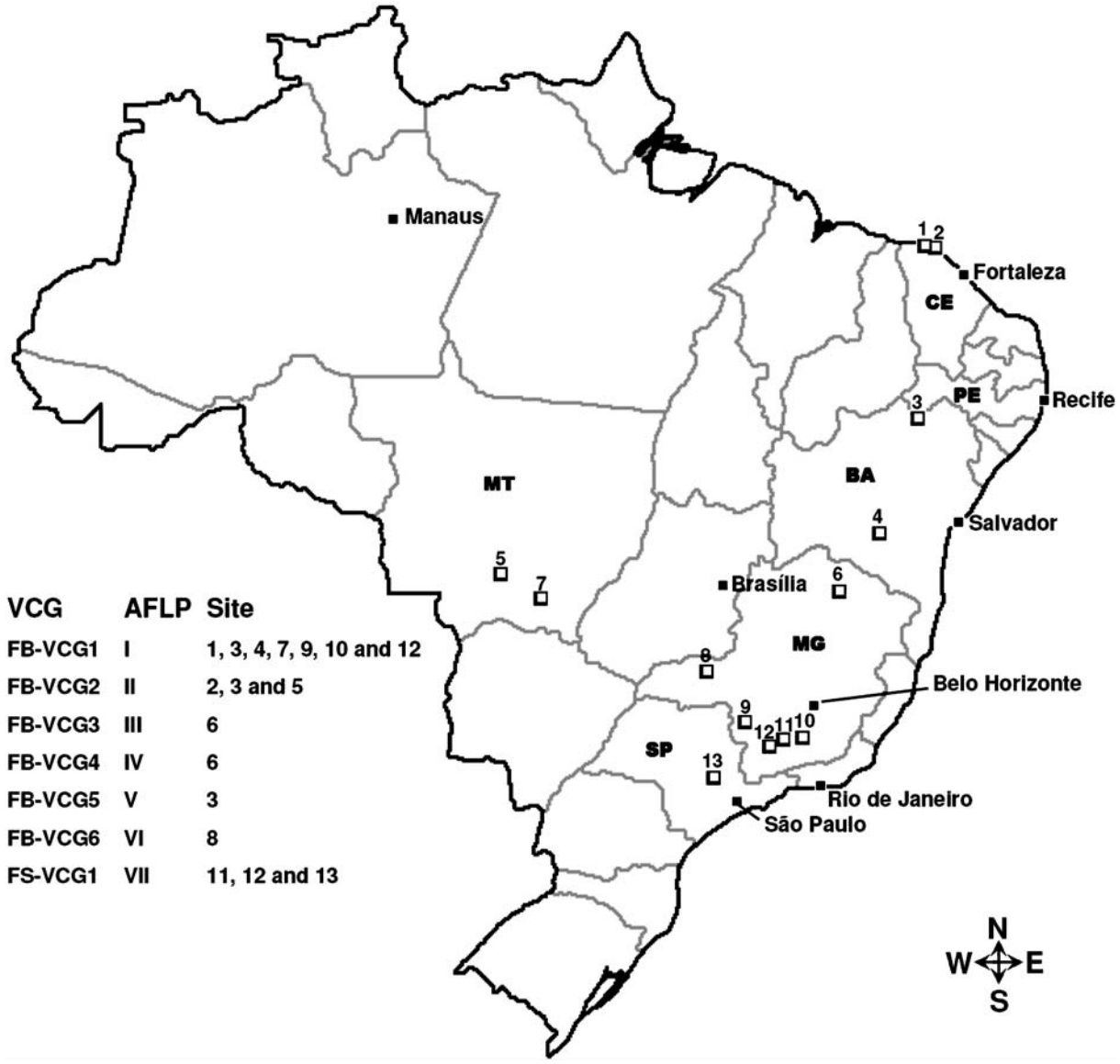

Fig. 1 Geographic distribution of vegetative compatibility-amplified fragment length polymorphism groups in *F.* '*subglutinans*' and *F. sterilihyphosum* isolates analyzed from Brazil. The *open boxes numbered from 1 to 13 in the map* represent the collection sites in Brazil: *1* Almofala, *2* Cruz, *3* Juazeiro and Petrolina (São Francisco River Valley), *4* Livramento, *5* Várzea Grande, *6* Nova Porteirinha, *7* Rondonópolis, *8* Uberlândia, *9* Passos, *10* São João Del Rei, *11* Ijaci and Itumirim, *12* Lavras and Eloi Mendes, and *13* Piracicaba. Brazilian States: *BA* Bahia, *CE* Ceará, *MG* Minas Gerais, *MT* Mato Grosso, *PE* Pernambuco, and *SP* São Paulo

had 100% bootstrap support and correspond to *F.* '*subglutinans*', *F. sterilihyphosum* and *F. mangiferae* (Fig. 2). The group representing *F.* '*subglutinans*' could be subdivided into six subgroups (I to VI), each with 100% bootstrap support. The *F. sterilihyphosum* group had two subgroups, VII—containing four isolates obtained from mango malformation in Brazil with 82% bootstrap support, and VIII—containing two isolates from South Africa with 100% bootstrap support. *Fusarium mangiferae* isolates also were divided into two groups, IX—with 56% bootstrap support containing isolates from India and South Africa, and X with 54% bootstrap support containing isolates from Egypt and the United States. Each AFLP subgroup corresponded to a VCG group (Table 1).

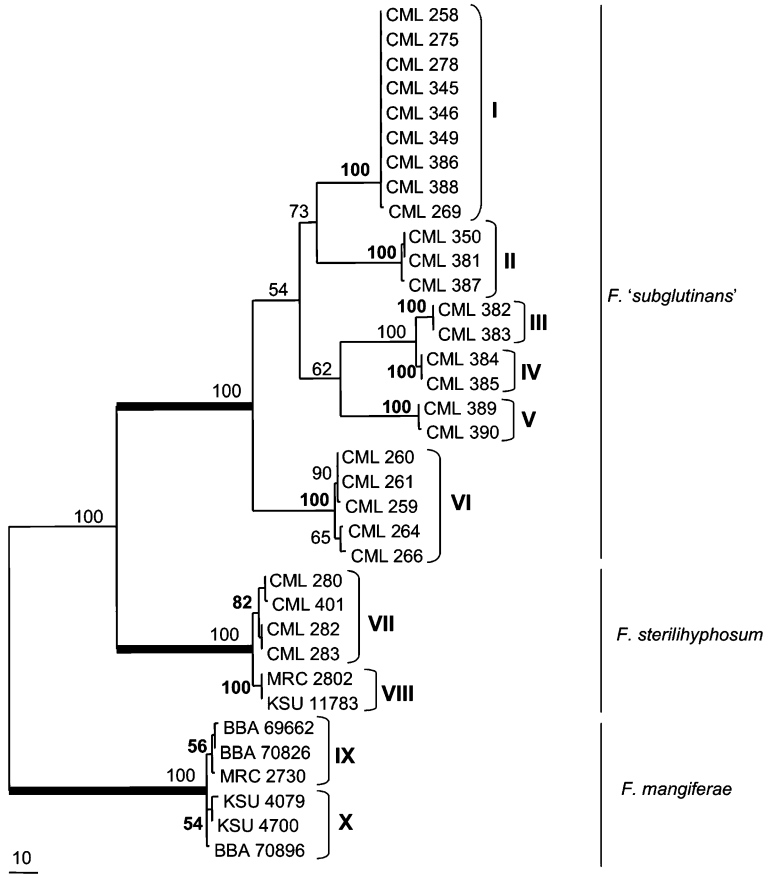

Fig. 2 UPGMA network for *F. mangiferae*, *F. sterilihyphosum*, and *F.* '*subglutinans*' generated after combination of AFLP patterns produced with EGG-MCT, ETT-MAC and EAA-MTT primers. Bands scored between 200 and 800 bp interval (286 total markers). Bootstrap values are shown above internodes (1,000 replicates). *Thick horizontal lines* represent the groups formed by *F. mangiferae*, *F. sterilihyphosum*, and *F.* '*subglutinans*' isolates. AFLP sub-groups are indicated by *roman numerals* and the bootstrap support is indicated in *bold*. The number of band changes is indicated by *scale at the bottom of dendrogram*. For culture collection abbreviations see Table 1

Discussion

The presence of six subgroups within isolates of the main causal agent of mango malformation in Brazil was observed in both the VCG and the AFLP analyses. This result is consistent with the hypothesis that there is significant genetic variation present in this population, and that this variation is present as a series of effectively clonal genotypes that reproduce primarily, if not exclusively, asexually. Mango is cultivated in all regions of Brazil and the transit of propagation material amongst the different producing areas within the country is not rigorously regulated. The exchange of propagation material could explain the widespread distribution and apparent clonality of *F.* '*subglutinans*' populations as well as the presence of multiple fungal VCGs at a single location.

Fusarium sterilihyphosum isolates recovered in Brazil formed only a single group (FS-VCG 1). This limited variation could be due to (1) the evaluation of only four isolates, (2) genetic uniformity within the species, or (3) the recent introduction of the species either into the country or to the host. Isolates from South Africa were closely related to those from Brazil, but were distinct in terms of AFLP banding patterns and VCG compatibility. The origin of either the South African nor the Brazilian populations of this species are clear.

If AFLP banding patterns were used to assign VCGs, then *F. mangiferae* isolates from South Africa

and India would belong to the same VCG, and a common origin for these isolates could be inferred. A similar argument could be made for the isolates from Egypt and the isolate from the United States (Table 1). On the other hand Zheng and Ploetz (2002) placed these isolates in different VCGs. In the same study other isolates from Egypt and the United States were placed in the same VCG. Isolates from India and Egypt also belonged to the same VCG, while isolates from South Africa and the United States were in a second VCG. These differences are probably due to differences in the isolates examined, which highlights the need for larger, more diverse collections of isolates associated with mango malformation.

Isolates not assigned to a VCG on the basis of a complementation test could tentatively be assigned to a VCG based on the AFLP analysis by comparing the banding pattern of the unassigned isolate with those of reference tester isolates from each VCG. A correlation between AFLP, VCG and gene phylogeny groupings is known for several *formae speciales* of *F. oxysporum* (Baayen et al. 2000) and *Colletotrichum* (Heilmann et al. 2006). *Fusarium* populations responsible for *Fusarium* wilt of cotton, *F. oxysporum* f. sp. *vasinfectum*, in Australia were represented by just two VCGs in that country (VCG 01111 and VCG 01112), and these VCGs also formed two distinct AFLP groups (Wang et al. 2006).

Isolates of *F. subglutinans sensu lato* obtained from mango malformation in Israel, the United States and South Africa were reported to group into four VCGs, with isolates from Israel forming VCG 1, those from the United States forming VCG 2, and those from South Africa forming VCG 3 and VCG 4 (Steenkamp et al. 2000). The isolates in VCGs 1, 2 and 3 were identified as *F. mangiferae*, and the isolates in VCG 4 were identified as *F. sterilihyphosum* (Britz et al. 2002).

Zheng and Ploetz (2002) used a larger set of isolates associated with mango malformation from several countries, including three isolates from Brazil. They found that the genetic diversity in *F. mangiferae* based on RAPD markers was relatively low. Each of the seven VCGs could be correlated with a geographic origin, with one of the seven (VCG 7) containing only three isolates from Brazil. These three isolates have been confirmed as *F. sterilihyphosum* (Marasas et al. 2006). Based on the results obtained, Zheng and Ploetz (2002) proposed that geographic origin and international dissemination of isolates through germplasm transport could explain the observed genetic diversity. They suggested that *F. mangiferae* originated in India, the centre of origin of cultivated mango, and that it was disseminated from that country to other locations as mango was introduced.

Fusarium mangiferae cannot survive for more than 18 weeks on the soil surface or when buried (Youssef et al. 2007), which strengthens the hypothesis that the fungus is disseminated through wind, water and propagation material. Dissemination across large distances is most likely to occur via propagative material. This hypothesis also could describe spread of the mango pathogens that occur in Brazil since none of these *Fusarium* spp. produce chlamydospores (Lima 2006).

Curiously, we have not recovered *F. mangiferae* from Brazil. Mango germplasm was introduced to Brazil in the 16th century, initially as cultivars from the Philippines. In 1931 and 1970 mangoes from the Indian cvs Haden and Tommy Atkins were introduced from the United States (Ferreira et al. 2002). The Philippine mango germplasm introduced over 400 years ago could have brought with it the *F.* '*subglutinans*' pathogen population. Alternatively, the *F.* '*subglutinans*' population is native to Brazil and jumped from a native host to mango, as has been suggested for *F. oxysporum* f. sp. *vasinfectum* on cotton in Australia (Wang et al. 2006). Both hypotheses are consistent with the relatively clonal nature of the population. Presumably each VCG represents a separate 'jump' to mango from the native host or a separate introduction into the country. The second hypothesis also is supported by the putative origin of a number of closely related species from the Americas, e.g., *Fusarium circinatum*, *Fusarium guttiforme* and *Fusarium konzum* (O'Donnell et al. 1998; Zeller et al. 2003).

Identification of VCGs in *Fusarium* is an efficient tool for assessing genetic diversity and monitoring the spread of specific isolates within the country. Nevertheless, the difficulty in recovering NitM mutants and the consistent correlation between AFLPs and VCGs, suggests that AFLPs could be used to screen large numbers of isolates and to assign isolates to a VCG whenever NitM mutants cannot be obtained. In conclusion, the two *Fusarium* populations that cause mango malformation disease in Brazil differ in terms

of their genetic variation but not necessarily their reproductive strategy. The predominant population, *F.* '*subglutinans*', is genetically and geographically diverse probably reproduces clonally and may represent an indigenous species. *F. sterilihyphosum* also appears to reproduce clonally, but this conclusion is based on a small population which could have been introduced only once to a limited geographic region. Testing the hypothesis that either or both of these populations originated from indigenous Brazilian *Fusarium* species will require, surveys in Brazil of *F. subglutinans sensu lato* isolates in native hosts that may, or may not, be closely related to mango. The genetic diversity observed in *F.* '*subglutinans*' from mango in Brazil should be considered in disease management programs and in studies of the epidemiology of this important tropical plant disease.

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References

Baayen, R. P., O'Donnell, K., Bonants, P. J. M., Cigelnik, E., Kroon, L. P. N. M., Roebroeck, E. J. A., et al. (2000). Gene genealogies and AFLP analyses in the *Fusarium oxysporum* complex identify monophyletic and nonmonophyletic *formae speciales* causing wilt and rot disease. *Phytopathology*, *90*, 891–900.

Britz, H., Steenkamp, E. T., Coutinho, T. A., Wingfield, B. D., Marasas, W. F. O., & Wingfield, M. J. (2002). Two new species of *Fusarium* section *Liseola* associated with mango malformation. *Mycologia*, *94*, 722–730.

Castellani, A. (1939). Viability of some pathogenic fungi in distilled water. *Journal of Tropical Medicine and Hygiene*, *42*, 225–226.

Chulze, S. N., Torres, A., Ramirez, M. L., & Leslie, J. F. (2000). Genetic variation in *Fusarium* section *Liseola* from no-till maize in Argentina. *Applied and Environmental Microbiology*, *66*, 5312–5315.

Correll, J. C., Klittich, C. J. R., & Leslie, J. F. (1987). Nitrate nonutilizing mutants of *Fusarium oxysporum* and their use in vegetative compatibility tests. *Phytopathology*, *77*, 1640–1646.

Correll, J. C., Klittich, C. J. R., & Leslie, J. F. (1989). Heterokaryon self-incompatibility in *Gibberella fujikuroi* (*Fusarium moniliforme*). *Mycological Research*, *93*, 21–27.

Cunha, M. M., Santos Filho, H. P., & Nascimento, A. S. (2000). *Manga Fitossanidade*. Brasília DF: Embrapa.

Di Primo, P., Cartia, G., & Katan, T. (2001). Vegetative compatibility and heterokaryon stability in *Fusarium oxysporum* f. sp. *radicis-lycopersici* from Italy. *Plant Pathology*, *50*, 371–382.

Ferreira, F. R., Possidio, E. L., Soares, N. B., Castro Neto, M. T., Pinto, A. C. Q., Donadioa, L. C., et al. (2002). Germoplasma de manga no Brasil. In A. R. São Jose (Ed.), *O agronegócio manga: produção e mercado* (pp. 111–118). Vitória da Conquista BA: Universidade Estadual do Sudoeste da Bahia.

Freeman, S., Maimon, M., & Pinkas, Y. (1999). Use of GUS transformants of *Fusarium subglutinans* for determining etiology of mango malformation disease. *Phytopathology*, *89*, 456–461.

Heilmann, L. J., Nitzan, N., Johnson, D. A., Pasche, J. S., Doetkott, C., & Gudmestad, N. C. (2006). Genetic variability in the potato pathogen *Colletotrichum coccodes* as determined by amplified fragment length polymorphism and vegetative compatibility group analyses. *Phytopathology*, *96*, 1097–1107.

Klittich, C. J. R., & Leslie, J. F. (1988a). Nitrate reduction mutants of *Fusarium moniliforme* (*Gibberella fujikuroi*). *Genetics*, *118*, 417–423.

Klittich, C. J. R., & Leslie, J. F. (1988b). Multi-well plates for complementation tests of *Fusarium*. *Fungal Genetics Newsletter*, *35*, 21–22.

Klittich, C. J. R., & Leslie, J. F. (1989). Chlorate-resistant, nitrate-utilizing mutants of *Fusarium moniliforme* (*Gibberella fujikuroi*). *Journal of General Microbiology*, *135*, 721–727.

Leslie, J. F. (1993). Fungal vegetative compatibility. *Annual Review of Phytopathology*, *31*, 127–150.

Leslie, J. F., Summerell, B. A., Bullock, S., & Doe, F. J. (2005). Description of *Gibberella sacchari* and neotypification of its anamorph *Fusarium sacchari*. *Mycologia*, *97*, 718–724.

Leslie, J. F., & Summerell, B. A. (2006). *The Fusarium Laboratory Manual*. Ames: Blackwell.

Lima, C. S. (2006). Biological and molecular characterization of *Fusarium* species involved in the etiology of mango malformation. PhD thesis, Universidade Federal de Lavras.

Lima, C. S., Pfenning, L. H., Costa, S. S., Campos, M. A., & Leslie, J. F. (2008). A new *Fusarium* lineage within the *Gibberella fujikuroi* species complex is the main causal agent of mango malformation disease in Brazil. *Plant Pathology* (in press).

Marasas, W. F. O., Ploetz, R. C., Wingfield, M. J., Wingfield, B. D., & Steenkamp, E. T. (2006). Mango malformation disease and the associated *Fusarium* species. *Phytopathology*, *96*, 667–672.

O'Donnell, K., Cigelnik, E., & Nirenberg, H. I. (1998). Molecular systematics and phylogeography of the *Gibberella fujikuroi* species complex. *Mycologia*, *90*, 465–493.

Ploetz, R. C. (2001). Malformation: a unique and important disease of mango, *Mangifera indica* L. In B. A. Summerell,

J. F. Leslie, D. Backhouse, W. L. Bryden, & L. W. Burgess (Eds.), *Fusarium: Paul E. Nelson Memorial Symposium* (pp. 233–247). St. Paul: APS Press.

Ramirez, M. L., Reynoso, M. M., Farnochi, M. C., & Chulze, S. (2006). Vegetative compatibility and mycotoxin chemotypes among *Fusarium graminearum* (*Gibberella zeae*) isolates from wheat in Argentina. *European Journal of Plant Pathology*, *115*, 139–148.

São Jose, A. R., Souza, S. E., Pina, A. V., & Ataide, E. M. (2000). Incidence and severity of mango flower malformation in Bahia State, Brazil. *Acta Horticulturae*, *509*, 765–797.

Steenkamp, E., Britz, H., Coutinho, T., Wingfield, B., Marasas, W., & Wingfield, M. (2000). Molecular characterization of *Fusarium subglutinans* associated with mango malformation. *Molecular Plant Pathology*, *1*, 187–193.

Summanwar, A. S., Raychaudhuri, S. P., & Phatak, S. C. (1966). Association of the fungus *Fusarium moniliforme* Sheld. with the malformation in mango (*Mangifera indica* L.). *Indian Phytopathology*, *19*, 227–228.

Swofford, D. L. (2000). *PAUP*: Phylogenetic Analysis Using Parsimony (*and other methods). Version 4.0b10*. Sunderland: Sinauer Associates.

Tavares, S. C. C. H., & Lima, J. A. S. (1997). Isolamento do agente causal da malformação vegetativa em mangueira no Submédio São Francisco. *Fitopatologia Brasileira*, *22*, 322 (Supplement).

Vos, P., Hogers, R., Bleeker, M., Reijans, M., Van der Lee, T., Hornes, M., et al. (1995). AFLP: A new technique for DNA fingerprinting. *Nucleic Acids Research*, *23*, 4407–4414.

Wang, B., Brubaker, C. L., Tate, W., Woods, M. J., Matheson, B. A., & Burdon, J. J. (2006). Genetic variation and population structure of *Fusarium oxysporum* f. sp. *vasinfectum* in Australia. *Plant Pathology*, *55*, 746–755.

Youssef, S. A., Maymon, M., Zveibil, A., Klein-Gueta, D., Sztejnberg, A., Shalaby, A. A., et al. (2007). Epidemiological aspects of mango malformation disease caused by *Fusarium mangiferae* and source of infection in seedlings cultivated in orchards in Egypt. *Plant Pathology*, *56*, 257–263.

Zeller, K. A., Summerell, B. A., Bullock, S., & Leslie, J. F. (2003). *Gibberella konza* (*Fusarium konzum*) sp. nov., a new biological species within the *Gibberella fujikuroi* species complex from prairie grass. *Mycologia*, *95*, 943–954.

Zheng, Q., & Ploetz, R. (2002). Genetic diversity in the mango malformation pathogen and development of a PCR assay. *Plant Pathology*, *51*, 208–216.