Comparative analysis of pathogenic and nonpathogenic *Fusarium oxysporum* populations associated with banana on a farm in Minas Gerais, Brazil

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Fusarium wilt is one of the most devastating diseases on banana. The causal agent, *Fusarium oxysporum* f. sp. *cubense* (Foc) is genetically diverse and its origin and virulence are poorly understood. In this study, pathogenic Foc isolates and nonpathogenic *F. oxysporum* isolates from Minas Gerais in Brazil were compared using *EF-1α* and IGS sequences. This allowed the examination of the origin and evolutionary potential of Foc in a country outside the region of origin of the banana plant. Two different sequence types were found among Foc isolates. One appeared to be of local origin because it was identical to the sequence type of the largest group of nonpathogenic isolates. To explore if the 'local' Foc isolates had acquired pathogenicity either independently through coevolution with the host, or through horizontal gene transfer (HGT) of pathogenicity genes from other, probably introduced, Foc isolates, the presence and sequence of putative *SIX* effector genes were analysed. Homologues of *SIX1*, *SIX3* and *SIX8* were found. *SIX1* sequences were identical and exclusively found in all pathogenic isolates, while variable ratios of sequences of multicopy gene *SIX8* were found among nonpathogenic and different pathogenic isolates. This observation supports the HGT hypothesis. Horizontal transfer of genes between isolates of *F. oxysporum* has important implications for the development of reliable diagnostic tools and effective control measures. Full genome sequencing is required to confirm HGT and to further unravel the virulence mechanisms of forma specialis *cubense*.

Keywords: evolution, Panama disease, phylogenetics, virulence

Introduction

Fusarium oxysporum is a ubiquitous fungus found in soils and plants all over the world (Lori *et al.*, 2004). The *Fusarium oxysporum* species complex (FOSC) is best known for its wilt-causing members. Pathogenic *F. oxysporum* often display narrow host specificity and are subdivided into formae speciales according to the affected host. However, the species complex also harbours large populations of putative nonpathogenic *F. oxysporum*, which seem to surpass pathogenic strains in genetic diversity (Lori *et al.*, 2004).

Fusarium oxysporum f. sp. *cubense* (Foc) is the causal agent of fusarium wilt on banana. Based on pathogenicity tests, Foc is divided into different races that attack different groups of banana cultivars. Race 1 affects, among others, bananas of the Silk (AAB) and Gros

Michel (AAA) cultivars, while race 2 affects cultivar Silver Bluggoe (ABB) and other cooking bananas. Bananas susceptible to race 1 and 2, together with bananas from the Cavendish (AAA) subgroup, are affected by race 4. Race 4 is subdivided into a subtropical and tropical race 4, depending on the climatic conditions in which they can affect bananas of the subgroup Cavendish. While race 1 is found in almost all banana-growing regions in the world, race 4 is largely restricted to Southeast Asia and Australia, but the race is spreading and has already been reported in a few other Asian countries and Mozambique (Ordonez *et al.*, 2015). Many different clonal lineages of Foc are known, which do not form a monophyletic clade (O'Donnell *et al.*, 1998).

Both race 1 and race 2 are reported in Brazil (Ploetz, 1990; Pereira *et al.*, 2005), but the diversity of Foc seems to differ greatly between regions. In Santa Catarina, all Foc isolates collected by Cunha *et al.* (2015) had an identical elongation factor 1α sequence type (*EF-1α*). Another study, comprising isolates from several states in Brazil, showed a higher diversity but with dominance of two microsatellite profiles, indicating a clonal structure of the pathogen (Costa *et al.*, 2015).

The genome of *F. oxysporum* can be divided into a core and a lineage-specific region. The core genomic

regions reside on conserved chromosomes and are present in all *F. oxysporum* strains, regardless of their hosts (Ma, 2014). The lineage-specific genomic regions are variable between strains, contain no housekeeping genes and are generally rich in transposons. In the tomato pathogen, *Fusarium oxysporum* f. sp. *lycopersici* (Fol) isolate Fol4287, the lineage-specific region consists of four chromosomes and two smaller regions at the end of core chromosomes. One of the lineage-specific chromosomes contains a high fraction of effector genes, and is largely responsible for its pathogenicity, as horizontal transfer of this pathogenicity chromosome to a nonpathogenic strain, Fo47, turned it virulent to tomato (Ma *et al.*, 2010). One major group of effector genes on this pathogenicity chromosome is formed by the *SIX* (secreted in xylem) genes. They code for small, commonly cysteine-rich proteins that lack homology to other proteins, contain a signal peptide for secretion and are probably involved in the interaction with the plant during infection (de Sain & Rep, 2015). To date, 14 *SIX* genes have been described in Fol (Lievens *et al.*, 2009; Schmidt *et al.*, 2013), four of which are needed for full virulence on tomato, namely *SIX1*, *SIX3*, *SIX5* and *SIX6* (Gawehns *et al.*, 2015). Some *SIX* genes are involved in gene-for-gene interactions with the tomato plant (de Sain & Rep, 2015). Homologues of *SIX* genes have been detected in *F. oxysporum* pathogenic on several hosts such as cabbage, cotton, onion, *Arabidopsis* and banana (Chakrabarti *et al.*, 2011; Meldrum *et al.*, 2012; Thatcher *et al.*, 2012; Kashiwa *et al.*, 2013; Fraser-Smith *et al.*, 2014; Sasaki *et al.*, 2015), but their role in virulence on other hosts has not yet been revealed. In Foc, homologues of *SIX1*, *SIX2*, *SIX4*, *SIX6*, *SIX7*, *SIX8*, *SIX9* and *SIX13* have been reported (Fraser-Smith *et al.*, 2014). The *SIX* genes have been used to predict host specificity of *F. oxysporum* (van Dam *et al.*, 2016). Primers have been developed based on *SIX1*, *SIX2* and *SIX3* to distinguish Fol from other formae speciales. In addition, different races of Fol can be identified based on *SIX3* and *SIX4* (Lievens *et al.*, 2009). Fraser-Smith *et al.* (2014) developed primers based on *SIX8* to discriminate the different races of Foc.

Fusarium oxysporum is a cosmopolitan soil inhabitant, suggesting that it is also indigenous to South American soils. On the contrary, banana, with a natural distribution covering Southeast Asia, was probably only introduced to the Americas in the post-Columbian period (De Langhe *et al.*, 2009). Being an introduced crop, the coevolution of banana with indigenous *F. oxysporum* has probably been too short to result in pathogenic isolates, and it could be suggested that all Foc are introduced in Brazil. By studying the relation of the local nonpathogenic population and Foc, outside the region of origin of the host plant, this study aims to answer questions about the origin and evolutionary potential of Foc.

A population of *F. oxysporum* isolates from a farm in Pedra Dourada, Minas Gerais, Brazil were characterized. Isolates were collected from soil, graminoids, and banana cultivars Maçã, Prata and Ouro, to explore the occurrence of Foc inside and outside its host. This collection of isolates, which comprised both Foc and strains nonpathogenic to banana, was molecularly characterized to study the local population structure and resemblance between pathogenic and nonpathogenic strains. Two marker regions of the core genome, *EF-1α* and IGS, were used because these have been shown to contain the highest nucleotide variation of marker region used in the *F. oxysporum* species complex (O'Donnell *et al.*, 2009). The population comprised Foc with both divergent and identical sequence types as the nonpathogenic isolates, suggesting the occurrence of a local pathogenic population. To test if the 'local' Foc isolates attained pathogenicity through coevolution with banana or via horizontal gene transfer (HGT) between an introduced pathogen and a native nonpathogenic strain, the presence and sequence of putative *SIX* effectors was analysed. Throughout the text, the terms pathogenic and nonpathogenic always refer to the virulence on banana subgroup Silk, unless clearly mentioned.

Materials and methods

Fusarium oxysporum isolates

Samples were collected from a farm in Pedra Dourada, MG, Brazil (20°50′S, 42°07′W). The farm is managed as an agroforestry system with banana and coffee as main crops, which are intercropped with various fruit and native tree species. On the farm, banana cv. Maçã (subgroup: Silk) was affected by fusarium wilt, but disease was variable and variation could be attributed to differences in disease suppressiveness of the soil (Deltour *et al.*, 2017). Soil samples, different graminoids, pseudostem and roots of banana cultivars Ouro (AA, Sucrier subgroup, resistant to Foc race 1) and Prata (AAB, Pome subgroup, moderately susceptible to Foc race 1) and pseudostem of cv. Maçã (AAB, Silk subgroup, very susceptible to Foc race 1) were collected from the farm for isolation of *F. oxysporum* (Table 1). Isolates were also recovered from roots, rhizome and pseudostem of tissue culture-derived plantlets of cv. Maçã (Multiplanta Tecnologia Vegetal) grown in soil sampled from the farm. In addition, diseased bananas of cv. Maçã from two farms in Araponga (approximately 42 km from Pedra Dourada, straight distance) were sampled. All plants sampled were evaluated for internal discolouration.

For fungal isolation, plant samples were surface sterilized in NaOCl, washed in sterile water and plated on potato dextrose agar (PDA; 200 g L^{-1} potatoes, 20 g L^{-1} dextrose, 15 g L^{-1} agar, complemented with distilled water), supplemented with 10 mg L^{-1} rifampicin. Soil samples were serially diluted in sterile water and plated on rose bengal agar. Isolates morphologically identified as *F. oxysporum* were kept as single spore culture on dried filter paper and slants with PDA.

Pathogenicity test on banana

The isolates collected from the pseudostem of cv. Maçã in Pedra Dourada were tested for pathogenicity at the Federal University of Viçosa. Tissue culture-derived plantlets of cv. Maçã (AAB, subgroup Silk, susceptible to Foc race 1) and cv. Grande Naine (AAA, subgroup Cavendish, susceptible to Foc race 4) (Multiplanta Tecnologia Vegetal) were dipped for 2 h in a spore suspension (10^6 spores mL^{-1}) prepared from 10-day-old cultures

Table 1 Isolates of the *Fusarium oxysporum* species complex used in this study.

Name	Host	Plant part	Location[a]	Sequence type		Pathogenicity			Accession no.	
				EF-1α	IGS	Banana cvs of Silk subgroup	Banana cv. Grande Naine	Tomato cv. Moneymaker	*EF-1α*	IGS
Fusarium oxysporum f. sp. *cubense*										
FoxPDa1	Banana cv. Maça	Pseudostem	PD	9	Q	+	−	−	KU577989	KY296389
FoxPDa9	Banana cv. Maça	Pseudostem	PD	9	Q	+	−	nt	KU577989	KY296389
FoxPDa10	Banana cv. Maça	Pseudostem	PD	9	Q	+	−	nt	KU577989	KY296389
FoxPDa13	Banana cv. Maça	Pseudostem	PD	9	Q	+	−	nt	KU577989	KY296389
FoxPDa14	Banana cv. Maça	Pseudostem	PD	9	Q	+	−	nt	KU577989	KY296389
FoxPDa15	Banana cv. Maça	Pseudostem	PD	9	Q	+	−	−	KU577989	KY296389
FoxPDb1	Banana cv. Maça	Pseudostem	PD	9	Q	+	−	−	KU577989	KY296389
FoxPDb2	Banana cv. Maça	Pseudostem	PD	9	Q	+	−	nt	KU577989	KY296389
MrPDa9a	Banana cv. Maça	Root	ePD	9	Q	+	nt	nt	KU577989	KY296389
MsPDa9a	Banana cv. Maça	Rhizome	ePD	9	Q	+	nt	nt	KU577989	KY296389
MsPDa13	Banana cv. Maça	Rhizome	ePD	9	Q	+	nt	nt	KU577989	KY296389
MsPDb2b	Banana cv. Maça	Rhizome	ePD	9	Q	+	nt	nt	KU577989	KY296389
FoxPDa3	Banana cv. Maça	Pseudostem	PD	1	A	+	+	−	KU577991	KY296373
Ara1	Banana cv. Maça	Pseudostem	Araponga	1	A	+	nt	−	KU577991	KY296373
Ara2	Banana cv. Maça	Pseudostem	Araponga	1	A	+	nt	−	KU577991	KY296373
F. oxysporum nonpathogenic on banana cv. Silk										
SPDa1	Soil	n/a	PD	1	A	−	−	nt	KU577991	KY296373
SPDa5	Soil	n/a	PD	11	V	−	−	nt	KY274447	KY296394
SPDa7	Soil	n/a	PD	13	R	−	−	nt	KY274449	KY296390
SPDa14a	Soil	n/a	PD	1	B	−	−	nt	KU577991	KY296374
SPDa14b	Soil	n/a	PD	3	H	−	−	nt	KY274441	KY296380
SPDa9	Soil	n/a	PD	4	G	−	−	nt	KY274442	KY296379
FoxPDa2	Banana cv. Maça	Pseudostem	PD	1	A	−	−	−	KU577991	KY296373
FoxPDa6a	Banana cv. Maça	Pseudostem	PD	10	N	−	−	nt	KU577992	KY296386
FoxPDa6b	Banana cv. Maça	Pseudostem	PD	1	A	−	−	−	KU577991	KY296373
FoxPDa9b	Banana cv. Maça	Pseudostem	PD	1	C	−	−	−	KU577991	KY296375
FoxPDa9c	Banana cv. Maça	Pseudostem	PD	1	I	−	nt	nt	KU577991	KY296381
PpPDa9	Banana cv. Prata	Pseudostem	PD	1	A	−	nt	nt	KU577991	KY296373
PrPDa9	Banana cv. Prata	Root	PD	1	F	−	nt	nt	KU577991	KY296378
PrPDa10	Banana cv. Prata	Root	PD	1	C	−	nt	−	KU577991	KY296375
PrPDa11	Banana cv. Prata	Root	PD	1	B	−	nt	−	KU577991	KY296374
PpPDa13	Banana cv. Prata	Pseudostem	PD	1	C	−	nt	nt	KU577991	KY296375
OrPDa14	Banana cv. Ouro	Root	PD	3	H	−	nt	nt	KY274441	KY296380
OrPDa16	Banana cv. Ouro	Root	PD	2	A	−	nt	nt	KY274440	KY296373
OpPDa16	Banana cv. Ouro	Pseudostem	PD	1	J	−	nt	nt	KU577991	KY296382
OrPDa17	Banana cv. Ouro	Root	PD	5	A	−	nt	nt	KY274443	KY296373
GrPDb1a	*Melinis minutiflora*	Root	PD	7	E	−	−	nt	KY274445	KY296377
GrPDb1b	*Hyparhernia sufa*	Root	PD	14	K	−	nt	nt	KY274450	KY296383
GsPDb1c	*Digitaria insularis*	Shoot	PD	12	O	−	−	nt	KY274448	KY296387
GrPDa1a	*Digitaria insularis*	Root	PD	1	C	−	−	nt	KU577991	KY296375
GrPDa1b	*Cyperus iria*	Root	PD	1	B	−	−	nt	KU577991	KY296374
GrPDb2	*Brachiaria brizantha*	Root	PD	6	B	−	−	nt	KY274444	KY296374
GrPDa12a	*Hypolytrum schradarianum*	Root	PD	7	S	−	nt	nt	KY274445	KY296391
GsPDa12b	*Digitaria (horizontalis)*	Shoot	PD	1	B	−	nt	nt	KU577991	KY296374
GrPDa12b	*Digitaria (horizontalis)*	Root	PD	1	A	−	nt	nt	KU577991	KY296373
GrPDa5	*Rhynchospora aurea*	Root	PD	1	A	−	nt	nt	KU577991	KY296373
GrPDa15	*Paspalum* sp.	Root	PD	2	A	−	nt	nt	KY274440	KY296373
GrPDa17a	*Panicum repens*	Root	PD	2	F	−	nt	nt	KY274440	KY296378
GsPDa17b	*Cyperus* sp.	Shoot	PD	6	B	−	nt	nt	KY274444	KY296374
GrPDa17b	*Cyperus* sp.	Root	PD	13	X	−	nt	nt	KY274449	KY296396
MrPDa1a	Banana cv. Maça	Root	ePD	3	H	−	nt	nt	KY274441	KY296380
MrPDa1b	Banana cv. Maça	Root	ePD	1	A	−	nt	nt	KU577991	KY296373
MrPDa5	Banana cv. Maça	Root	ePD	1	H	−	nt	nt	KU577991	KY296380

(continued)

Table 1 (continued)

Name	Host	Plant part	Location[a]	Sequence type		Pathogenicity			Accession no.	
				EF-1α	IGS	Banana cvs of Silk subgroup	Banana cv. Grande Naine	Tomato cv. Moneymaker	EF-1α	IGS
MrPDa6	Banana cv. Maça	Root	ePD	14	K	–	nt	nt	KY274450	KY296383
MrPDa7	Banana cv. Maça	Root	ePD	7	T	–	nt	nt	KY274445	KY296392
MrPDa13	Banana cv. Maça	Root	ePD	1	A	–	nt	nt	KU577991	KY296373
MrPDa14a	Banana cv. Maça	Root	ePD	8	D	–	nt	nt	KY274446	KY296376
MrPDa14b	Banana cv. Maça	Root	ePD	14	M	–	nt	nt	KY274450	KY296385
MrPDa15	Banana cv. Maça	Root	ePD	1	C	–	nt	nt	KU577991	KY296375
MrPDb1	Banana cv. Maça	Root	ePD	11	W	–	nt	nt	KY274447	KY296395
MrPDb2	Banana cv. Maça	Root	ePD	7	E	–	nt	nt	KY274445	KY296377
MpPDa1	Banana cv. Maça	Pseudostem	ePD	1	G	–	nt	nt	KU577991	KY296379
MpPDa10	Banana cv. Maça	Pseudostem	ePD	1	B	–	nt	nt	KU577991	KY296374
MsPDa4	Banana cv. Maça	Rhizome	ePD	1	C	–	nt	nt	KU577991	KY296375
MsPDa7	Banana cv. Maça	Rhizome	ePD	1	A	–	nt	nt	KU577991	KY296373
MsPDa9b	Banana cv. Maça	Rhizome	ePD	1	F	–	nt	nt	KU577991	KY296378
MsPDa10	Banana cv. Maça	Rhizome	ePD	15	L	–	nt	nt	KY274451	KY296384
MsPDa14b	Banana cv. Maça	Rhizome	ePD	2	A	–	nt	nt	KY274440	KY296373
MsPDa15	Banana cv. Maça	Rhizome	ePD	2	C	–	nt	nt	KY274440	KY296375
MsPDb1a	Banana cv. Maça	Rhizome	ePD	8	Y	–	nt	nt	KY274446	KY296397
MsPDb1b	Banana cv. Maça	Rhizome	ePD	7	U	–	nt	nt	KY274445	KY296393
MsPDb2a	Banana cv. Maça	Rhizome	ePD	16	P	–	nt	nt	KY274452	KY296388
Fusarium oxysporum f. sp. *lycopersici*										
FolR1	Tomato	—	Brazil	—	—	nt	nt	+ (race 1)	—	—
BFOL-70	Tomato	—	USA	—	—	nt	nt	+ (race 2)	—	—
IPO3	Tomato	—	Netherlands	—	—	nt	nt	+ (race 3)	—	—
FOL-MM10	Tomato	—	USA	—	—	nt	nt	+ (race 3)	—	—

n/a, not applicable; nt, not tested.
[a]PD: Pedra Dourada, ePD: experiment with soil samples from Pedra Dourada.

on PDA and grown for 7 weeks in the greenhouse (18/28 °C average night/day temperature; 12:12 h light regime), before evaluating external (yellowing of the leaves, splitting of the pseudostem and stunted growth) and internal symptoms. The vascular discolouration of the rhizome was given a rhizome discolouration score (RDS; 0: 0%; 1:1–25%; 2: 26–50%; 3: 51–75%; 4: 76–100%). When a score different from 0 was given, the isolate was considered pathogenic.

All other isolates were tested for pathogenicity at the laboratory of Phytopathology, Ghent University, Belgium. Eight-week-old tissue culture-derived plantlets of cv. Silk (accession number ITC0348) were dipped for 2 h in a spore suspension (5×10^5 spores mL^{-1}), obtained from a 2-week-old culture grown on PDA. Plantlets were grown in potting soil (Universal, type 1, M. Snebbout n.v./s.a.) in a growth room (26 °C; 16/8 h day/night light regime). After 5 weeks, plantlets were uprooted and internal symptoms were evaluated as described above.

Pathogenicity test on tomato

Pathogenicity on tomato was tested for a subset of the Foc and nonpathogenic isolates following the protocol of van der Does et al. (2008a). Four Fol isolates (race 1, 2 and 3) were included as control. Ten-day-old plantlets of cv. Moneymaker were uprooted and dipped in a spore suspension of 10^7 spores mL^{-1} for 30 min, made from a 2-week-old culture on PDA. Plantlets were planted in 200 mL pots with potting soil (Universal, type 1, M. Snebbout n.v./s.a.) and grown in a growth chamber (25 °C, 16/8 h day/

night light regime) for 3 weeks until evaluation. A disease score from 0 to 4 was attributed depending on the extent of browning of vessels: 0, no symptoms; 1, slightly swollen and/or bent hypocotyl; 2, one or two brown vascular bundles in hypocotyl; 3, at least two brown vascular bundles and growth distortion; 4, all vascular bundles are brown, plant either dead or very wilted.

DNA extraction, PCR and sequencing

Isolates were grown for 10 days on potato dextrose broth (BD Difco), crushed in liquid nitrogen and DNA extracted by use of the Wizard Genomic DNA purification kit (Promega), following the manufacturer's protocol. DNA samples were kept at −20 °C until use. The translation elongation factor-1α (EF-1α) gene was amplified using the primer EF1 and EF2 and the programme described by O'Donnell et al. (1998). The intergenic spacer region (IGS) was amplified using primers iNL1 and iCNS1 and the following programme: 95 °C for 5 min; and 30 cycles of 95 °C for 1 min, 62 °C for 1 min and 72 °C for 3 min; followed by an additional extension time at 72 °C for 10 min (O'Donnell et al., 2009). After purification with Exosap-IT (Affymetrix), PCR products were sent for sequencing to LGC genomics, Berlin, Germany with the primers mentioned above. For the sequencing of the IGS sequence, the samples were sent with two additional primers, NLa and CNSa, to obtain reliable coverage for the entire 2200 bp (O'Donnell et al., 2009).

A subset of the isolates was screened for the presence of SIX homologues. Primer pairs and amplification conditions for SIX1

to *SIX8* were as described by Meldrum *et al.* (2012). The primers and amplification conditions for *SIX9 – SIX11* and *SIX14* were taken from Laurence *et al.* (2015). Only partial fragments of the *SIX* genes were obtained. When a clear band of the expected length was obtained, the PCR product was purified with Exosap-IT, following the manufacturer's guidelines, and sent for sequencing (LGC genomics).

Data analysis

The isolates were confirmed to belong to the *F. oxysporum* species complex by comparing the *EF-1α* sequences to reference sequences from GenBank and Fusarium-ID. Additionally, IGS and *EF-1α* sequences were compared to the multilocus sequence types (MLST) as described in O'Donnell *et al.* (2009) and listed in Fusarium-ID, to identify new MLST. Sequence types, including gaps as informative characters, were attributed a number (*EF-1α*) and letter (IGS) (Table 1). Sequences identified in this work have been deposited in GenBank (Table 1).

Sequences were aligned by use of the CLUSTALW multiple alignment in BIOEDIT, and manually edited afterwards. Maximum likelihood analysis was performed in MEGA6 on all sequence types, including sequences of the MLST described by O'Donnell *et al.* (2009) that contain isolates pathogenic on banana as reference strains. The general time reversible model was applied, with the nearest-neighbour-interchange as heuristic method and partial deletion as option for gaps. Support for each branch was assessed with 1000 bootstrap replicates. Two isolates of the sister taxon of the *F. oxysporum* species complex, *F. foetens*, were used to root the tree (O'Donnell *et al.*, 2009).

Sequences of putative *SIX* genes were aligned against *SIX* homologues found in different formae speciales available on GenBank and from the Broad institute of MIT and Harvard (Fusarium comparative genome project).

Carbon-utilization pattern

The growth profile on several sugars and amino acids was characterized for a subset of the isolates based on the method derived from Steinberg *et al.* (1999). In a 96-well plate, 200 µL of minimal medium (per litre distilled water: Na_2NO_3 2 g, KH_2PO_4 1 g, $MgSO_4.7H_2O$ 2.5 g, KCl 0.5 g) and 2 mL trace element solution (per litre distilled water: citric acid 5 g, $ZnSO_4.7H_2O$ 5 g, $FeSO_4.7H_2O$ 4.75 g, $Fe(NH_4)_2(SO_4)_2.6H_2O$ 1 g, $CuSO_4.5H_2O$ 0.25 g, $MnSO_4.H_2O$ 0.05 g, H_3BO_3 0.05 g, $NaMoO_4.2H_2O$ 0.05 g) was supplemented with 5 g L^{-1} of a specific carbon source and inoculated with 50 µL of a spore suspension (2×10^5 spores mL^{-1}) obtained from 14-day-old cultures on PDA. For each carbon source, three wells were supplemented with the same isolate. The plates were incubated in the dark at 25 °C. After 4 days, the growth was assessed by turbidity measurements at 595 nm using a multiscan EX spectrophotometer (Thermo Labsystems). Hierarchical clustering (Ward's method, R package PV-CLUST) was performed on the mean optical density per carbon source to study similarities between growth profiles and the approximately unbiased *P*-values (AU-*P* value) were calculated.

Results

Isolation of *F. oxysporum* and pathogenicity on banana

In total, 71 isolates of *F. oxysporum* were collected (Table 1). From the farm in Pedra Dourada, 43 isolates were collected from soil, different graminoids, pseudostem and roots of banana cultivars Ouro and Prata and pseudostem of banana cv. Maçã. Symptoms of fusarium wilt were only observed in cv. Maçã. In addition, 26 isolates were collected from the root, rhizome and pseudostem of tissue culture-derived plantlets of banana cv. Maçã grown for 3 months in the greenhouse in soil collected at different spots on the farm in Pedra Dourada. Finally, two isolates were obtained from the rhizome of clearly affected banana cv. Maçã on two farms in Araponga.

All isolates were tested for pathogenicity on banana cv. Silk, a cultivar susceptible to Foc race 1. Pathogenicity was exclusively observed in isolates obtained from banana cv. Maçã. None of the isolates collected from a non-host were pathogenic on banana, including the isolates collected from a moderately susceptible (cv. Prata) or resistant (cv. Ouro) banana cultivar. From the farm in Pedra Dourada, nine pathogenic isolates were obtained, which were also tested for pathogenicity on cv. Grande Naine. One of the pathogenic isolates, FoxPDa3, also caused clear symptoms on cv. Grande Naine in the greenhouse assay (Deltour *et al.*, 2017). In addition, four isolates that were recovered from banana cv. Maçã plantlets grown in soil samples from the farm in Pedra Dourada were pathogenic to banana cv. Silk. Also the two isolates obtained from affected cv. Maçã on farms in Araponga were pathogenic to banana cv. Silk, resulting in a total of 15 pathogenic isolates and 56 non-pathogenic isolates (Table 1).

Population diversity based on *EF-1α* and IGS

Sixteen different *EF-1α* sequence types, named St1 to St16 (approximately 653 bp), including insertions/deletions (indels) as informative characters, were identified. Forty-eight sites were polymorphic, with 42 single nucleotide polymorphisms and six indels. Of the whole population, 52% belonged to the lineage containing St1 to St5. This lineage differs by a maximum of one single nucleotide or indel (Fig. 1).

Twenty-five different IGS haplotypes, named StA to StY, were found. Of the approximately 1922 bp IGS fragment, 285 single nucleotide polymorphisms and 22 indels were found. Topological incongruence was found between the trees based on the *EF-1α* and IGS sequence. The dominant *EF-1α* lineage (St1 to St5) segregated into two different monophyletic lineages and two separate isolates in the phylogenetic tree based on the IGS sequences. Also isolates of the lineages St12 to St15 and St7, both monophyletic, were segregated in the tree based on IGS (Fig. 1).

Thirty-two unique *EF-1α*/IGS MLST were found, 22 of which comprised only one isolate. Only *EF-1α*/IGS sequence types St1/StB, St9/StQ, St7/StS and St16/StP belonged to a MLST (MLST 28, 25, 20 and 16, respectively) already described by O'Donnell *et al.* (2009).

The pathogenic isolates were divided into two different sequence types. The first group, belonging to St9/StQ,
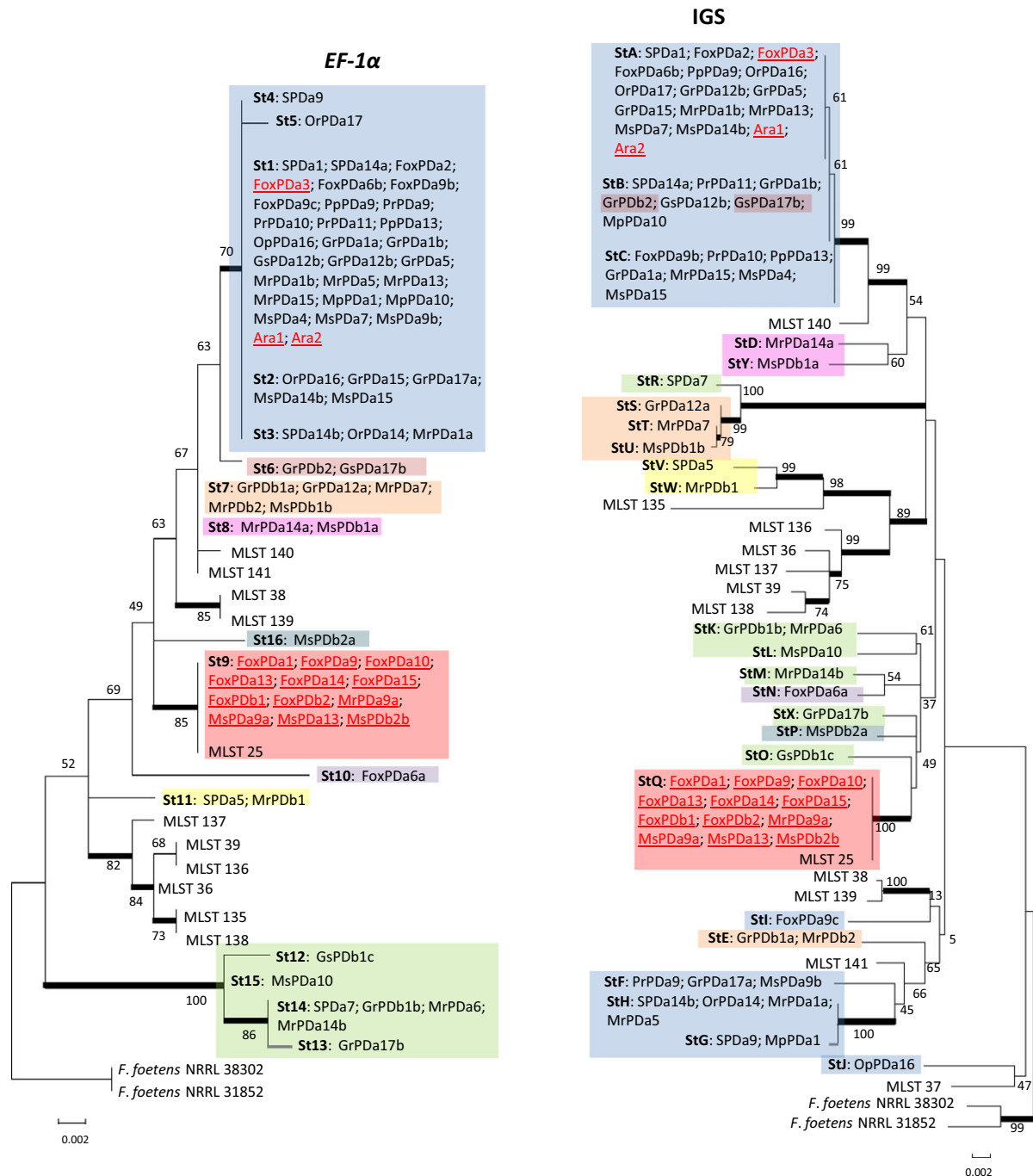
Figure 1 Trees with the highest log likelihood based on the *EF-1α* and IGS sequences generated using the maximum likelihood method, using 1000 bootstraps. The tree is rooted with *Fusarium foetens* isolates NRRL 38302 and NRRL 31852. The numbers at the nodes show the percentage of trees in which the associated taxa clustered together. The multilocus sequence types (MLST) containing f. sp. *cubense* as described by O'Donnell *et al.* (2009) were added as reference strains. Strains are grouped in their respective sequence types and strains pathogenic to bananas of subgroup Silk are indicated in red and underlined. Coloured boxes show the grouping of the isolates based on the *EF-1α* allele and their respective position on the tree based on the IGS allele. Well-supported lineages (bootstrap >70%) are indicated in bold.

contained all pathogenic isolates from Pedra Dourada with the exception of one, and contained no non-pathogenic isolates. The second group, belonging to St1/StA, contained a single pathogenic isolate from Pedra Dourada (FoxPDa3), and the two isolates from Araponga (Ara1 and Ara2). St1/StA contained many non-pathogenic isolates that form the locally dominant group.

SIX genes

A subset of the population comprising six Foc isolates (three isolates of St9/StQ and three from St1/StA) and six nonpathogenic *F. oxysporum* isolates (three isolates of St1/StA, one of St1/StB and two of St1/StC) was screened for homologues of the *SIX* genes. Isolates of Fol race 2 and 3 were included as reference, and resulted in clear bands of the expected length for all *SIX* genes, except *SIX4*. Clear amplicons with identical length to the references were observed only in *SIX1*, *SIX3* and *SIX8* (Table 2). Other primers resulted in multiple nonspecific PCR products (*SIX2*, *SIX5*, *SIX6*, *SIX7*, *SIX9*, *SIX10*, *SIX11* and *SIX14*) and no amplicon was found after amplification with *SIX4* primers.

PCR amplification with the *SIX1* primers resulted in a 260 bp band for the Fol controls and all Foc isolates. No band with similar length was found in the non-pathogenic isolates. The nucleotide sequences of *SIX1* of all Foc isolates were identical (accession no. KY296398), and were identical to *SIX1* of Australian Foc isolates described by Meldrum *et al.* (2012) and Laurence *et al.* (2015).

Amplification with the *SIX3* primers showed a 555 bp fragment for the Fol controls and for all Foc isolates, except for FoxPDa3. An amplicon was also observed for two nonpathogenic isolates (FoxPDa6b and PrPDa11). Two different homologues were found. A first homologue (accession no. KY296399) was found in FoxPDa1, FoxPDa15, FoxPDb1 and Ara2 and was identical over the full length to the sequences of Fol race 3 isolate IPO3. The second homologue (accession no. KY296400), found in Ara1, FoxPDA6b and PrPDa11 was identical to the sequences of Fol race 3 isolate FOL-MM10 (Fig. 2). To the authors' knowledge, to date these *SIX3* sequences have been found exclusively in Fol isolates, and are used for race determination within Fol. Despite the presence of homologues of *SIX3* identical to sequences found in Fol race 3, the isolates caused no vascular discolouration or growth distortions in tomato plants cv. Moneymaker, which is susceptible to all Fol races (Fig. 3).

A homologue of *SIX8* was detected in all tested pathogenic isolates and in two nonpathogenic isolates (FoxPDa2 and FoxPDa9b). The 233 bp amplicon stretches along the third exon as proposed by Fraser-Smith *et al.* (2014). At least two homologues of multi-copy gene *SIX8* were found. The pathogenic isolates FoxPDb1, FoxPDa1, FoxPDa15, Ara1, Ara2 and non-pathogenic FoxPDa9b shared the same unique sequence (accession no. KY296401; Fig. 4). This sequence showed high similarity with a *SIX8* sequence of isolate Fol4287 (Fig. 5). Two different sequence types seemed to be present in nonpathogenic FoxPDa2 (accession no. KY296402), but in different ratios: a high copy number of a sequence highly similar to homologues of *SIX8* observed in Foc subtropical race 4 (Meldrum *et al.*, 2012) and the homologues described as *Foc-SIX8a* in Fraser-Smith *et al.* (2014), and a low copy-number of the sequence type found in FoxPDb1 (Fig. 4). In pathogenic isolate FoxPDa3, a high frequency of ambiguous bases was found (Figs 4 & 5). The sequence of FoxPDa3 (accession no. KY296403) appears to correspond to an approximately equal copy number of the main sequence of FoxPDa2 and the sequence of the other isolates (Fig. 4).

Carbon utilization pattern

The three pathogenic isolates belonging to St9/StQ had a similar growth profile shown by a strongly supported cluster (AU-*P* value = 99), characterized by better growth on sugars, proline and threonine. The growth profile of pathogenic isolate FoxPDa3 (St1/StA) and non-pathogenic isolate FoxPDa2 (St1/StA) also clustered together with strong support (AU-*P* value = 99). The growth profile of the other isolates (pathogenic isolates: Ara1, Ara2 and nonpathogenic isolate: FoxPDa9b) did not form a strongly supported cluster with other isolates (Fig. 6).

Table 2 Screening for *SIX* genes in a subset of *Fusarium* isolates.

Isolate	PCR amplicon			Accession no.		
	SIX1	SIX3	SIX8	SIX1	SIX3	SIX8
Fusarium oxysporum f. sp. *lycopersici*						
BFOL-70	+	+	+	—	—	—
IPO3	+	+	+	—	—	—
F. oxysporum f. sp. *cubense*						
FoxPDa15	+	+	+	KY296398	KY296399	KY296401
FoxPDb1	+	+	+	KY296398	KY296399	KY296401
FoxPDa1	+	+	+	KY296398	KY296399	KY296401
Ara1	+	+	+	KY296398	KY296400	KY296401
Ara2	+	+	+	KY296398	KY296399	KY296401
FoxPDa3	+	–	+	KY296398	—	KY296403
Nonpathogenic *F. oxysporum*						
FoxPDa2	–	–	+	—	—	KY296402
FoxPDa6b	–	+	–	—	KY296400	—
PpPDa9	–	–	–	—	—	—
FoxPDa9b	–	–	+	—	—	KY296401
PrPDa10	–	–	–	—	—	—
PrPDa11	–	+	–	—	KY296400	—

```
BFOL-51     -GACGGGGTAACCCATATTGCGTGTTTCCCGGCCGCCGCACGTCTTCTACT-
14844       -GACGGGGTAACCCATATTGCGTGTTTCCCGGCCACCGCACGTCTTCTACT-
IPO3        -GACGGGGTAACCCATATTGCGTGTTTCCCGGCCGCCCCACGTCTTCTACT-
FOL-MM10    -GACGGGGTAACCCATATTGCATGTTTCCCGGCCGCCGCACGTCTTCTACT-
FoxPDb1     -GACGGGGTAACCCATATTGCGTGTTTCCCGGCCGCCCCACGTCTTCTACT-
FoxPDa6b    -GACGGGGTAACCCATATTGCATGTTTCCCGGCCGCCGCACGTCTTCTACT-
```

Figure 2 Sequence alignments of part of the *SIX3* genes where single nucleotide polymorphisms were found. Comparison of isolates from this study with *Fusarium oxysporum* f. sp. *lycopersici* (Fol) strains from Lievens *et al.* (2009). BFOL-51 shows the sequence of Fol race 1 and race 2, while isolates 14844, IPO3 and FOL-MM10 represent the different sequence types of Fol race 3. Variable nucleotides are indicated in bold and marked in grey. Isolates FoxPDa15, FoxPDa1 and Ara2 have the same sequence as FoxPDb1; isolates Ara1 and PrPDa11 have the same sequence as FoxPDa6b.
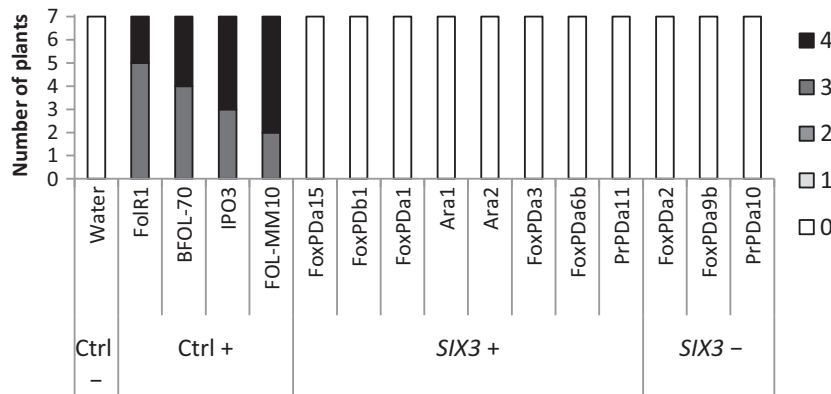

Figure 3 Disease scores of the different strains on tomato plants, cv. Moneymaker (susceptible to all races of *Fusarium oxysporum* f. sp. *lycopersici*). Isolates FolR1, BFOL-70, IPO3 and FOL-MM10, *F. oxysporum* f. sp. *lycopersici* race 1, 2, 3 and 3, respectively, were included as controls.
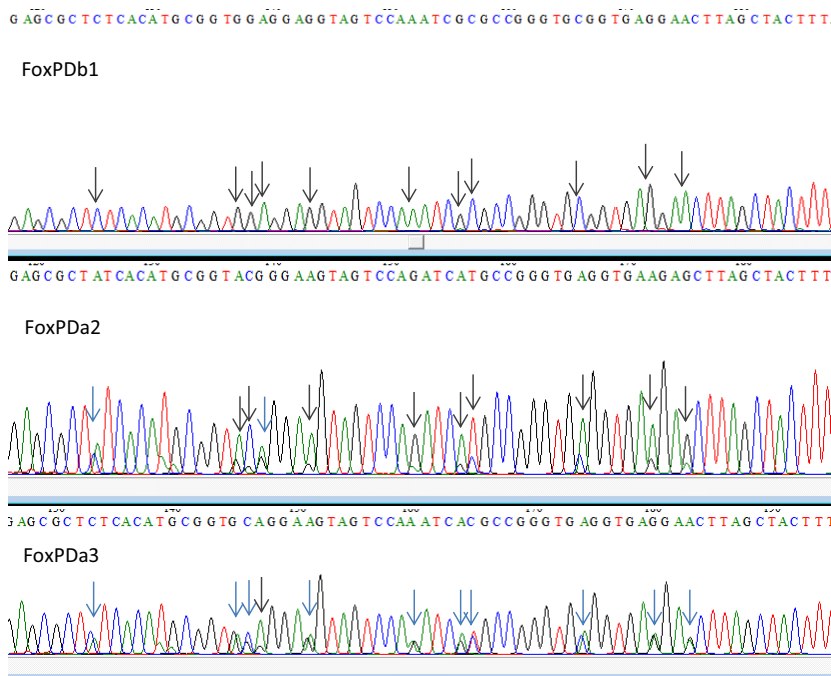

Figure 4 Illustration of the electropherogram showing ambiguous base pairs in a *SIX8* fragment, bp 149–218, of the isolates FoxPDb1 (Foc, group St9/StQ), FoxPDa2 (nonpathogenic *F. oxysporum*) and FoxPDa3 (Foc, group St1/StA). Blue arrows indicate clear ambiguous base pairs, grey arrows show corresponding base pairs with little or no ambiguity.

Discussion

In this study, evidence is shown of the introduction of pathogenicity on banana in a local *F. oxysporum* population in Brazil, probably through HGT.

The local *F. oxysporum* population obtained from the investigated farm is dominated by one group with highly similar *EF-1α* sequence types. The over-representation of a single sequence type suggests a clonal population structure, which has been observed frequently in local
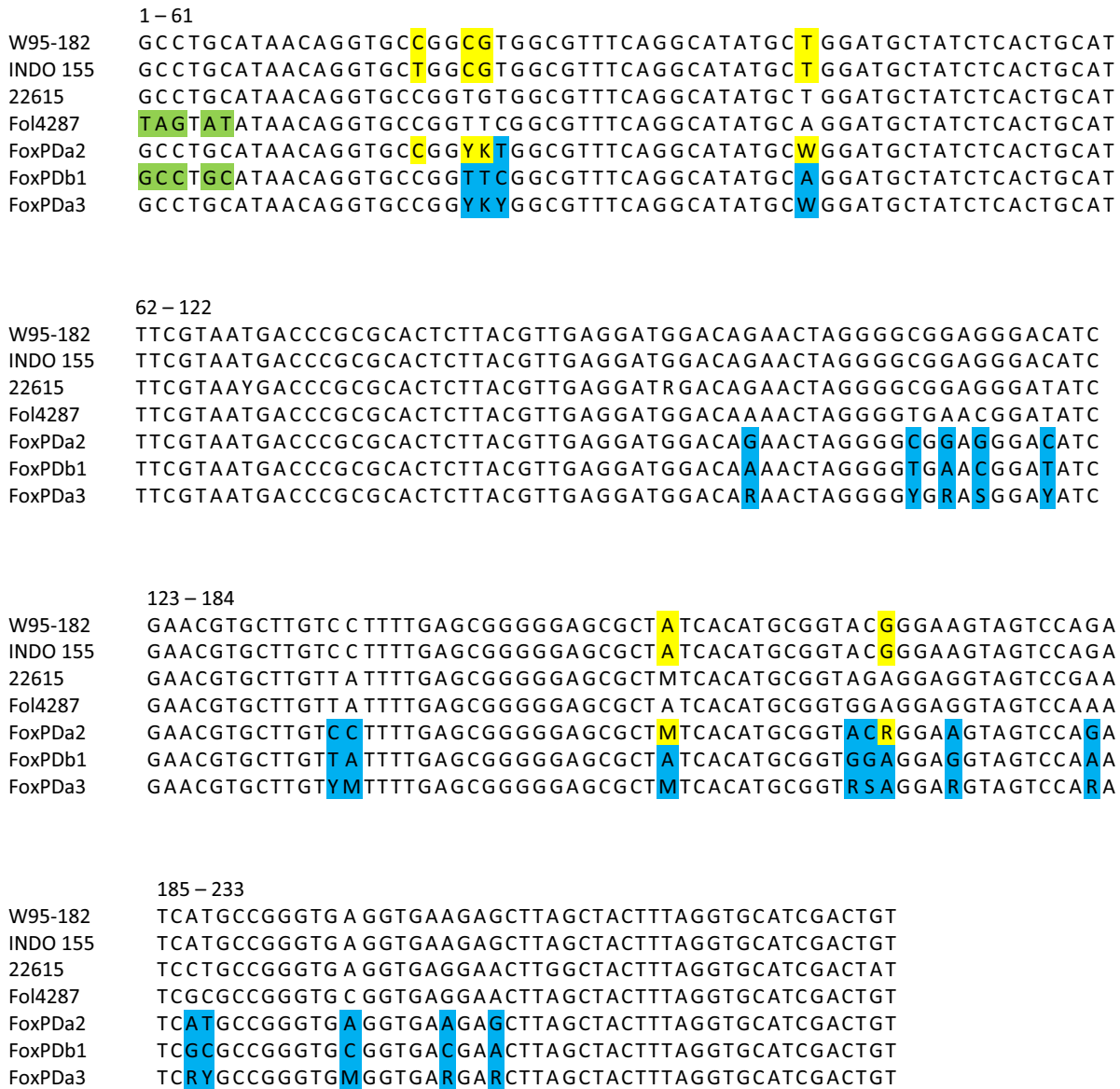
```
             1 – 61
W95-182      GCCTGCATAACAGGTGCCGGCGTGGCGTTTCAGGCATATGCTGGATGCTATCTCACTGCAT
INDO 155     GCCTGCATAACAGGTGCTGGCGTGGCGTTTCAGGCATATGCTGGATGCTATCTCACTGCAT
22615        GCCTGCATAACAGGTGCCGGGTGTGGCGTTTCAGGCATATGCTGGATGCTATCTCACTGCAT
Fol4287      TAGTATATAACAGGTGCCGGTTCGGCGTTTCAGGCATATGCAGGATGCTATCTCACTGCAT
FoxPDa2      GCCTGCATAACAGGTGCCGGYKTGGCGTTTCAGGCATATGCWGGATGCTATCTCACTGCAT
FoxPDb1      GCCTGCATAACAGGTGCCGGTTCGGCGTTTCAGGCATATGCAGGATGCTATCTCACTGCAT
FoxPDa3      GCCTGCATAACAGGTGCCGGYKYGGCGTTTCAGGCATATGCWGGATGCTATCTCACTGCAT

             62 – 122
W95-182      TTCGTAATGACCCGCGCACTCTTACGTTGAGGATGGACAGAACTAGGGGCGGAGGGACATC
INDO 155     TTCGTAATGACCCGCGCACTCTTACGTTGAGGATGGACAGAACTAGGGGCGGAGGGACATC
22615        TTCGTAAYGACCCGCGCACTCTTACGTTGAGGATRGACAGAACTAGGGGCGGAGGGATATC
Fol4287      TTCGTAATGACCCGCGCACTCTTACGTTGAGGATGGACAAAACTAGGGGTGAACGGATATC
FoxPDa2      TTCGTAATGACCCGCGCACTCTTACGTTGAGGATGGACAGAACTAGGGGCGGAGGGACATC
FoxPDb1      TTCGTAATGACCCGCGCACTCTTACGTTGAGGATGGACAAAACTAGGGGTGAACGGATATC
FoxPDa3      TTCGTAATGACCCGCGCACTCTTACGTTGAGGATGGACARAACTAGGGGYGRASGGAYATC

             123 – 184
W95-182      GAACGTGCTTGTCCTTTTGAGCGGGGGGAGCGCTATCACATGCGGTACGGGAAGTAGTCCAGA
INDO 155     GAACGTGCTTGTCCTTTTGAGCGGGGGGAGCGCTATCACATGCGGTACGGGAAGTAGTCCAGA
22615        GAACGTGCTTGTTATTTTGAGCGGGGGGAGCGCTMTCACATGCGGTAGAGGAGGTAGTCCGAA
Fol4287      GAACGTGCTTGTTATTTTGAGCGGGGGGAGCGCTATCACATGCGGTGGAGGAGGTAGTCCAAA
FoxPDa2      GAACGTGCTTGTCCTTTTGAGCGGGGGGAGCGCTMTCACATGCGGTACRGGAAGTAGTCCAGA
FoxPDb1      GAACGTGCTTGTTATTTTGAGCGGGGGGAGCGCTATCACATGCGGTGGAGGAGGTAGTCCAAA
FoxPDa3      GAACGTGCTTGTYMTTTTGAGCGGGGGGAGCGCTMTCACATGCGGTRSAGGARGTAGTCCARA

             185 – 233
W95-182      TCATGCCGGGTGAGGTGAAGAGCTTAGCTACTTTAGGTGCATCGACTGT
INDO 155     TCATGCCGGGTGAGGTGAAGAGCTTAGCTACTTTAGGTGCATCGACTGT
22615        TCCTGCCGGGTGAGGTGAGGAACTTGGCTACTTTAGGTGCATCGACTAT
Fol4287      TCGCGCCGGGTGCGGTGAGGAACTTAGCTACTTTAGGTGCATCGACTGT
FoxPDa2      TCATGCCGGGTGAGGTGAAGAGCTTAGCTACTTTAGGTGCATCGACTGT
FoxPDb1      TCGCGCCGGGTGCGGTGACGAACTTAGCTACTTTAGGTGCATCGACTGT
FoxPDa3      TCRYGCCGGGTGMGGTGARGARCTTAGCTACTTTAGGTGCATCGACTGT
```

Figure 5 DNA sequence alignment of the *SIX8* homologues from isolates of this study compared to homologues described in the literature. Differences between isolate W95-182, INDO 155 (*Foc-SIX8a*) and FoxPDa2 are indicated in yellow. Differences between isolate Fol4287 and FoxPDb1 are indicated in green. Differential nucleotides between FoxPDa2, FoxPDa3 and FoxPDb1 are indicated in blue. W95-182: *Fusarium oxysporum* f. sp. *cubense* subtropical race 4, VCG 0120 (Meldrum *et al.*, 2012). INDO 155: *F. oxysporum* f. sp. *cubense* tropical race 4, VCG 01213, *Foc-SIX8a* homologue (Fraser-Smith *et al.*, 2014). 22615: *F. oxysporum* f. sp. *cubense* subtropical race 4, VCG 0120, *Foc-SIX8b* homologue (Fraser-Smith *et al.*, 2014). Fol4287: *F. oxysporum* f. sp. *lycopersici* race 2 (Ma *et al.*, 2010).

F. oxysporum populations (Edel *et al.*, 2001; Skovgaard *et al.*, 2002; Nel *et al.*, 2006; Balmas *et al.*, 2010; Demers *et al.*, 2015). Pathogenic strains were only found in two different *EF-1α*/IGS sequence types. The main group (St9/StQ) contained no nonpathogenic isolates, and several Foc isolates collected around the world have the same sequence type (MLST 25 by O'Donnell *et al.*, 2009), suggesting that this is an introduced pathogen. However, one pathogenic strain from the farm and two from a neighbouring farm shared the same sequence type (St1/StA) with the dominant group of nonpathogenic

isolates, indicating a potential exchange of pathogenicity from an individual of the St9/StQ group to a local former nonpathogenic isolate of group St1/StA.

Effector genes generally lie on a lineage-specific chromosome, which contains no housekeeping genes (Ma *et al.*, 2010). As *EF-1α* is a housekeeping gene, and the IGS region lies between housekeeping genes, they are a part of the core genome. The presence of putative effector genes was investigated, to get insight in the diversity of the lineage-specific parts of the genome in these isolates. Of all *SIX* genes tested, only *SIX1* was found

Figure 6 Heatmap of the growth of different *Fusarium oxysporum* isolates on minimal medium supplemented with a single carbon source (optical density at 595 nm). The standard deviation is given between brackets (*n* = 3). Pathogenic isolates are indicated in bold and the *EF-1α* and IGS sequence type is shown. The cluster dendrogram is based on Ward's method. The numbers at the nodes give the approximately unbiased *P*-value, based on 1000 bootstraps.

exclusively in all pathogenic isolates, indicating its potential importance for the pathogenicity on banana. The *SIX1* homologues were identical in all pathogenic isolates tested and identical to sequences of *SIX1* in Australian Foc isolates of race 1, 2 and tropical race 4 (Meldrum *et al.*, 2012; Laurence *et al.*, 2015). The identical sequence of *SIX1* among the Brazilian Foc isolates and Foc from a geographically distinct region supports the hypothesis of HGT. The sequences of Australian Foc subtropical race 4 differed by one base pair (Meldrum *et al.*, 2012). To the authors' knowledge, an identical sequence of *SIX1* has not been found in other formae speciales.

Putative effector gene *SIX8* is a multicopy gene, which occurs in 3 to 13 copies on lineage-specific and telomeric regions of Fol strains (de Sain & Rep, 2015). In the pathogenic isolates of group St9/StQ, a single sequence type of *SIX8* seems to be present. In nonpathogenic isolate FoxPDa2, which belongs to St1/StA, a combination of at least two different sequence types seems to be present, one similar to the sequence type found in the group St9/StQ, and a distinct one which seems to be present in a higher copy number than the *SIX8* homologue found in group St9/StQ. Pathogenic isolate FoxPDa3 seems to contain more or less equal proportions of the homologues found in the St9/StQ group and the main homologue of FoxPDa2, supporting the suggestion that FoxPDa3 has evolved from an isolate similar to FoxPDa2 by gaining a part of the *SIX8* copies of a Foc

from group St9/StQ. This observation contributes to the hypothesis of HGT. However, cloning or full genome sequencing is needed to distinguish the sequence types of *SIX8* and identify their copy number.

The hypothesis that FoxPDa3 has evolved from an isolate similar to FoxPDa2 is further supported by the similarity in the growth profile of FoxPDa3 and FoxPDa2. In addition, the clonal nature of the three tested pathogenic isolates belonging to St9/StQ appears to be confirmed by their similar growth profile.

Putting together the information about the population based on *EF-1α* and IGS, *SIX1* and *SIX8* homologues and the growth profile, it is suggested that isolate FoxPDa3 has gained its pathogenicity through HGT. This could also be the case for the isolates Ara1 and Ara2. The interplay of the exchanged pathogenicity genes with a different core genome, which is adapted to local conditions, could explain the higher virulence of isolate FoxPDa3 compared to the other Foc isolates of group St9/StQ, as observed in the greenhouse assay (Deltour *et al.*, 2017).

Topological incongruence between phylogenetic trees based on different marker genes is a strong indication of HGT (Fitzpatrick, 2012). Contradiction between the topologies of the trees based on *EF-1α* and IGS, which was found in this study and previously by O'Donnell *et al.* (2009), suggests that HGT might also take place between parts of the core genome. Vlaardingerbroek *et al.* (2016) observed horizontal transfer of chromosome

8 and a part of chromosome 7, both part of the core genome, between two *F. oxysporum* strains. HGT of both lineage-specific and core chromosomes makes the evolutionary potential of FOSC complex challenging to study.

A sexual cycle has never been observed in *F. oxysporum*, but other mechanisms, such as transfer of active transposable elements and heterokaryosis after a parasexual cycle, can be involved in genetic change and exchange in *F. oxysporum* (Gordon & Martyn, 1997). Although the results here support the hypothesis of HGT, it cannot be excluded that FoxPDa3 has been introduced and that the dominant nonpathogenic St1/StA population has developed from an ancestor of FoxPDa3 that has lost its pathogenicity. Complete genome sequencing of the isolates of this study is necessary to confirm the event of HGT and is an interesting way to expand the understanding of virulence mechanisms in forma specialis *cubense*. Considering the limited geographic scale of this study, assumptions cannot be made on the frequency of HGT in nature. Therefore, the associated risk of dissemination of pathogenicity genes via this mechanism cannot be estimated. Ma *et al.* (2010) obtained HGT under specific experimental conditions with very high spore densities. It might be interesting to investigate if specific *in planta* conditions may favour HGT.

Surprisingly, the *SIX3* homologues found in this study were identical to *SIX3* homologues of Fol race 3 as described in Lievens *et al.* (2009). Until now, they have only been found in Fol isolates. However, none of the isolates containing a *SIX3* homologue caused symptoms in tomato plants. Other factors essential for pathogenicity on tomato (van der Does *et al.*, 2008b; Michielse *et al.*, 2009; de Sain & Rep, 2015) may be lacking in these isolates. Primers have been developed based on the sequences of *SIX3* and *SIX8* for race identification in Fol and Foc, respectively (Lievens *et al.*, 2009; Fraser-Smith *et al.*, 2014). A *SIX8* homologue highly similar to the homologue Foc-*SIX8a*, described as specific for Foc subtropical race 4, was found in an isolate that is nonpathogenic on banana (Fraser-Smith *et al.*, 2014). The present results call for careful use of those primers for diagnostics without complementing pathogenicity tests. As both *SIX3* and *SIX8* homologues were found in isolates incapable of causing disease in banana, their role as single factors in pathogenicity on banana is doubtful.

The isolates in the present study were initially collected to explore how frequently Foc could be found in non-hosts. As Foc could not be isolated from hosts other than banana cv. Maçã, Foc seems to colonize non-hosts rather seldomly. In a study conducted in an Australian banana plantation, only 6 out of 154 isolates from weeds were identified as Foc tropical race 4 (Hennessy *et al.*, 2005). In contrast, studies on other formae speciales report isolation frequencies in the range of 50% to 94% of all isolates collected from non-hosts (Helbig & Carroll, 1984; Elmer & Lacy, 1987; Gordon *et al.*, 1989; Altinok, 2013; Scott *et al.*, 2014). The sample size of the present study does not allow firm conclusions to be made on capacity and frequency of Foc to colonize non-hosts, and more extensive sampling is necessary.

Although banana is an introduced crop in Brazil, pathogenic isolates with marker genes identical to the main local nonpathogenic population have been found. This suggests that Foc populations are dynamic, and local pathogenic strains can evolve from introduced pathogens, probably through HGT. Through sequencing of two marker genes of the core genome and putative effector genes, isolates could be identified that probably received pathogenicity genes via HGT. Full genome sequencing of those isolates is necessary to confirm HGT and is an excellent opportunity to further investigate genes involved in virulence on banana. Of the putative effector genes, only *SIX1* seems to be linked with the disease on banana, as *SIX3* and *SIX8* homologues were also found in nonpathogenic isolates.

Acknowledgements

References

Altinok HH, 2013. Fusarium species isolated from common weeds in eggplant fields and symptomless hosts *of Fusarium oxysporum* f. sp. *melongenae* in Turkey. *Journal of Phytopathology* **161**, 335–40.

Balmas V, Migheli Q, Scherm B *et al.*, 2010. Multilocus phylogenetics show high levels of endemic fusaria inhabiting Sardinian soils (Tyrrhenian Islands). *Mycologia* **102**, 803–12.

Chakrabarti A, Rep M, Wang B, Ashton A, Dodds P, Ellis J, 2011. Variation in potential effector genes distinguishing Australian and non-Australian isolates of the cotton wilt pathogen *Fusarium oxysporum* f. sp. *vasinfectum*. *Plant Pathology* **60**, 232–43.

Costa SN, Bragan CAD, Ribeiro LR *et al.*, 2015. Genetic structure of *Fusarium oxysporum* f. sp. *cubense* in different regions from Brazil. *Plant Pathology* **64**, 137–46.

Cunha CMS, Hinz RH, Pereira A, Tcacenco FA, Stadnik MJ, 2015. Aggressiveness and genetic diversity of *Fusarium oxysporum* f. sp. *cubense* from Santa Catarina, southern Brazil. *Tropical Plant Pathology* **5**, 326–34.

van Dam P, Fokkens L, Schmidt SM *et al.*, 2016. Effector profiles distinguish *formae speciales* of *Fusarium oxysporum*. *Environmental Microbiology* **18**, 4087–102.

De Langhe E, Vrydaghs L, de Maret P, Perrier X, Denham T, 2009. Why bananas matter: an introduction to the history of banana domestication. *Ethnobotany Research & Applications* **7**, 165–77.

Deltour P, França SC, Pereira OL *et al.*, 2017. Disease suppressiveness to Fusarium wilt of banana in an agroforestry system: Influence of soil characteristics and plant community. *Agriculture, Ecosystems and Environment* **239**, 173–81.

Demers JE, Gugino BK, Jiménez-Gasco Mdel M, 2015. Highly diverse endophytic and soil *Fusarium oxysporum* populations associated with field-grown tomato plants. *Applied and Environmental Microbiology* **81**, 81–90.

van der Does HC, Lievens B, Claes L, Houterman PM, Cornelissen BJC, Rep M, 2008a. The presence of a virulence locus discriminates

Fusarium oxysporum isolates causing tomato wilt from other isolates. *Environmental Microbiology* **10**, 1475–85.

van der Does HC, Duyvesteijn RGE, Goltstein PM *et al.*, 2008b. Expression of effector gene *SIX1* of *Fusarium oxysporum* requires living plant cells. *Fungal Genetics and Biology* **45**, 1257–64.

Edel V, Steinberg C, Gautheron N, Recorbet G, Alabouvette C, 2001. Genetic diversity of *Fusarium oxysporum* populations isolated from different soils in France. *FEMS Microbiology Ecology* **36**, 61–71.

Elmer WH, Lacy WL, 1987. Effects of crop residues and colonization of plant tissues on propagule survival and soil populations of *Fusarium oxysporum* f. sp. *apii* race 2. *Phytopathology* **77**, 381–7.

Fitzpatrick DA, 2012. Horizontal gene transfer in fungi. *FEMS Microbiology Letters* **329**, 1–8.

Fraser-Smith S, Czislowskia E, Meldrum RA *et al.*, 2014. Sequence variation in the putative effector gene *SIX8* facilitates molecular differentiation of *Fusarium oxysporum* f. sp. *cubense*. *Plant Pathology* **63**, 1044–52.

Gawehns F, Ma L, Bruning O *et al.*, 2015. The effector repertoire of *Fusarium oxysporum* determines the tomato xylem proteome composition following infection. *Frontiers in Plant Science* **6**, 967.

Gordon TR, Martyn RD, 1997. The evolutionary biology of *Fusarium oxysporum*. *Annual Review of Phytopathology* **35**, 111–28.

Gordon TR, Okamoto D, Jacobson DJ, 1989. Colonization of muskmelon and nonsusceptible crops by *Fusarium oxysporum* f. sp. *melonis* and other species of *Fusarium*. *Phytopathology* **79**, 1095–100.

Helbig JB, Carroll RB, 1984. Dicotyledonous weeds as a source of *Fusarium oxysporum* pathogenic on soybean. *Plant Disease* **68**, 694–6.

Hennessy C, Walduck G, Daly A, Padovan A, 2005. Weed hosts of *Fusarium oxysporum* f. sp. *cubense* tropical race 4 in northern Australia. *Australasian Plant Pathology* **34**, 115–7.

Kashiwa T, Inami K, Fujinaga M *et al.*, 2013. An avirulence gene homologue in the tomato wilt fungus *Fusarium oxysporum* f. sp. *lycopersici* race 1 functions as a virulence gene in the cabbage yellows fungus *F. oxysporum* f. sp. *conglutinans*. *Journal of General Plant Pathology* **79**, 412–21.

Laurence MH, Summerell BA, Liew ECY, 2015. *Fusarium oxysporum* f. sp. *canariensis*: evidence for horizontal gene transfer of putative pathogenicity genes. *Plant Pathology* **64**, 1068–75.

Lievens B, Houterman PM, Rep M, 2009. Effector gene screening allows unambiguous identification of *Fusarium oxysporum* f. sp. *lycopersici* races and discrimination from other *formae speciales*. *FEMS Microbiology Letters* **300**, 201–15.

Lori G, Edel-Hermann V, Gautheron N, Alabouvette C, 2004. Genetic diversity of pathogenic and nonpathogenic populations of *Fusarium oxysporum* isolated from carnation fields in Argentina. *Phytopathology* **94**, 661–8.

Ma LJ, 2014. Horizontal chromosome transfer and rational strategies to manage Fusarium vascular wilt diseases. *Molecular Plant Pathology* **15**, 763–6.

Ma LJ, van der Does HC, Borkovich KA *et al.*, 2010. Comparative genomics reveals mobile pathogenicity chromosomes in *Fusarium*. *Nature* **464**, 367–73.

Meldrum RA, Fraser-Smith S, Tran-Nguyen LTT, Daly AM, Aitken EAB, 2012. Presence of putative pathogenicity genes in isolates of

Fusarium oxysporum f. sp. *cubense* from Australia. *Australasian Plant Pathology* **41**, 551–7.

Michielse CB, van Wijk R, Reijnen L *et al.*, 2009. The nuclear protein Sge1 of *Fusarium oxysporum* is required for parasitic growth. *PLoS Pathogens* **5**, e1000637.

Nel B, Steinberg C, Labuschagne N, Viljoen A, 2006. Isolation and characterization of nonpathogenic *Fusarium oxysporum* isolates from the rhizosphere of healthy banana plants. *Plant Pathology* **55**, 207–16.

O'Donnell K, Kistler HC, Cigelnig E, Ploetz RC, 1998. Multiple evolutionary origins of the fungus causing Panama disease of banana: concordant evidence from nuclear and mitochondrial gene genealogies. *Proceedings of the National Academy of Sciences of the United States of America* **95**, 2044–9.

O'Donnell K, Gueidan C, Sink S *et al.*, 2009. A two-locus DNA sequence database for typing plant and human pathogens within the *Fusarium oxysporum* species complex. *Fungal Genetics and Biology* **46**, 936–48.

Ordonez N, Seidl MF, Waalwijk C *et al.*, 2015. Worse comes to worst: bananas and Panama disease – when plant and pathogen clones meet. *PLoS Pathogens* **11**, e1005197.

Pereira JCR, Pereira JR, Castro MEA, Gasparotto L, 2005. Ocorrência do Mal-do-Panamá em bananeira do subgrupo figo, em Piau, Minas Gerais. *Fitopatologia Brasileira* **30**, 554.

Ploetz RC, 1990. *Fusarium Wilt of Banana*. St Paul, MN, USA: APS Press.

de Sain M, Rep M, 2015. The role of pathogen-secreted proteins in fungal vascular wilt diseases. *International Journal of Molecular Sciences* **16**, 23970–93.

Sasaki K, Nakahara K, Tanaka S, Shigyo M, Ito S, 2015. Genetic and pathogenic variability of *Fusarium oxysporum* f. sp. *cepae* isolated from onion and welsh onion in Japan. *Phytopathology* **105**, 525–32.

Schmidt SM, Houterman PM, Schreiver I *et al.*, 2013. MITEs in the promoters of effector genes allow prediction of novel virulence genes in *Fusarium oxysporum*. *BMC Genomics* **14**, 119.

Scott JC, McRoberts DN, Gordon TR, 2014. Colonization of lettuce cultivars and rotation crops by *Fusarium oxysporum* f. sp. *lactucae*, the cause of fusarium wilt of lettuce. *Plant Pathology* **63**, 548–53.

Skovgaard K, Bødker L, Rosendahl S, 2002. Population structure and pathogenicity of members of the *Fusarium oxysporum* complex isolated from soil and root necrosis of pea (*Pisum sativum* L.). *FEMS Microbiology Ecology* **42**, 367–74.

Steinberg C, Whipps JM, Wood DA, Fenlon J, Alabouvette C, 1999. Effects of nutritional sources on growth of one non-pathogenic strain and four strains of *Fusarium oxysporum* pathogenic on tomato. *Mycological Research* **103**, 1210–6.

Thatcher LF, Gardiner DM, Kazan K, Manners JM, 2012. A highly conserved effector in *Fusarium oxysporum* is required for full virulence on *Arabidopsis*. *Molecular Plant–Microbe Interactions* **25**, 180–90.

Vlaardingerbroek I, Beerens B, Rose L, Fokkens L, Cornelissen BJ, Rep M, 2016. Exchange of core chromosomes and horizontal transfer of lineage-specific chromosomes in *Fusarium oxysporum*. *Environmental Microbiology* **18**, 3702–13.