

Genetic Variation Among Vegetative Compatibility Groups of *Fusarium oxysporum* f. sp. *ubense* Analyzed by DNA Fingerprinting

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ABSTRACT

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Genetic variation within a worldwide collection of 208 isolates of *Fusarium oxysporum* f. sp. *ubense*, representing physiological races 1, 2, 3, and 4 and the 20 reported vegetative compatibility groups (VCGs), was analyzed using modified DNA amplification fingerprinting. Also characterized were 133 isolates that did not belong to any of the reported VCGs of *F. oxysporum* f. sp. *ubense* including race 3 isolates from a *Heliconia* species and isolates from a symptomatic wild banana species growing in the jungle in peninsular Malaysia. The DNA fingerprint patterns were generally VCG specific, irrespective of geographic or host origin. A total of 33 different genotypes were identified within *F. oxysporum* f. sp. *ubense*; 19 genotypes were distinguished among the isolates that belonged

to the 20 reported VCGs, and 14 new genotypes were identified among the isolates that did not belong to any of the existing VCGs. DNA fingerprinting analysis also allowed differentiation of nine clonal lineages within *F. oxysporum* f. sp. *ubense*. Five of these lineages each contained numerous closely related VCGs and genotypes, and the remaining four lineages each contained a single genotype. The genetic diversity and geographic distribution of several of these lineages of *F. oxysporum* f. sp. *ubense* suggests that they have coevolved with edible bananas and their wild diploid progenitors in Asia. DNA fingerprinting analysis of isolates from the wild pathosystem provides further evidence for the coevolution hypothesis. The genetic isolation and limited geographic distribution of four of the lineages of *F. oxysporum* f. sp. *ubense* suggests that the pathogen has also arisen independently, both within and outside of the center of origin of the host.

Additional keywords: Fusarium wilt, Panama disease, *Musa*.

Fusarium wilt (Panama disease), caused by *Fusarium oxysporum* Schlechtend.:Fr. f. sp. *ubense* (E.F. Smith) W.C. Snyder & H.N. Hans. (25), is regarded as one of the most significant threats to banana (*Musa* spp.) production worldwide (19). Edible bananas originated in Asia and are now grown in virtually all areas located between 30°N and 30°S latitudes. Fusarium wilt has been reported from all banana-growing regions of the world except the South Pacific Islands, Somaliland, and countries bordering the Mediterranean Sea (21,29).

F. oxysporum f. sp. *ubense* affects species of *Musa* and *Heliconia*, and strains have been classified into four physiological races based on pathogenicity to host cultivars in the field (race 1, 'Gros Michel'; race 2, 'Bluggoe'; race 3, *Heliconia* spp.; and race 4, Cavendish cultivars and all cultivars susceptible to races 1 and 2) (19). Races of *F. oxysporum* f. sp. *ubense* are not defined genetically, but are groups of isolates that attack differential cultivars in the field. Earlier this century, race 1 of Fusarium wilt nearly destroyed the world banana export industry, which was based on the Gros Michel cultivar. Consequently, 'Gros Michel' was replaced by Cavendish cultivars, which were resistant to race 1. Although Cavendish cultivars remain resistant to race 1, another race of *F. oxysporum* f. sp. *ubense*, which is designated race 4, is capable of attacking Cavendish cultivars. Until recently, race 4 had only been recorded to cause serious losses in the subtropical regions of

Australia, South Africa, the Canary Islands, and Taiwan (23). It is thought that Cavendish cultivars in subtropical regions were predisposed to infection due to cold-induced stress during winter (12, 15). The disease also occurs in the Philippines, where Cavendish cultivars succumb in localized areas under poor edaphic conditions. Recently, Fusarium wilt has devastated Cavendish cultivars (Grande Naine and Valery) in the Asian deep tropics in peninsular Malaysia, Sumatra, Java, and Halmahera, where no predisposing factors have been identified. If these strains were to become established in the Americas, the world export industries could be severely affected, as there is no widely accepted replacement for the Cavendish cultivars. Fusarium wilt is also a major concern to bananas and plantains, which are essential to the nutritional and economic well-being of millions of people throughout the developing world.

F. oxysporum f. sp. *ubense* is considered to be a highly complex pathogen. Numerous methods have been used to characterize *F. oxysporum* f. sp. *ubense* including vegetative compatibility (5,12,14, 20,22), production of volatiles (6,13,26), electrophoretic karyotyping (4,11), random amplified polymorphic DNA (RAPD) analysis (3), and restriction fragment length polymorphism (RFLP) analysis (10). These methods have been useful for pathotype determination (17,18), for which no reliable small-plant pathogenicity test currently exists. The use of tissue culture-derived plantlets to ascertain host-pathogen responses is generally restricted to endemic isolates and has given inconsistent results. Field testing is expensive and inefficient because of the limited number of strains in any given field.

Various genetic marker systems can be used to determine genetic diversity among different isolates within a species. Since *F. oxysporum* f. sp. *ubense* is a haploid asexual pathogen, arbitrary

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primer techniques such as RAPD (33) and DNA amplification fingerprinting (DAF) (8) can be used effectively. Preliminary RAPD and DAF analysis of *F. oxysporum* f. sp. *cubense* divided isolates into two major groups, and the genetic relationships among the vegetative compatibility groups (VCGs) were determined (1, 2,3). The aims of this work were to (i) assess the genetic variation among isolates within each VCG and between different VCGs of the pathogen, (ii) determine the efficacy of DNA fingerprinting analysis to predict putative VCGs among uncharacterized isolates, and (iii) delineate clonal lineages within *F. oxysporum* f. sp. *cubense*. The racial structure and possible origins of the pathogen

are also discussed. To our knowledge, this study involves the first molecular genetic analysis of isolates from a *Heliconia* species and from a symptomatic wild banana species growing in a native situation.

MATERIALS AND METHODS

Fungal isolates. Two hundred and eight isolates of *F. oxysporum* f. sp. *cubense* from a worldwide collection and many different host genotypes, representing races 1, 2, 3, and 4 and the 20 reported VCGs, were examined (Table 1). An additional 133 isolates of unknown VCG were also characterized by DNA fingerprinting

TABLE 1. Isolates of *Fusarium oxysporum* f. sp. *cubense* analyzed in this study

Accession number ^w	Geographic origin	Host origin and genotype ^x	Donor or collector ^y	DFG ^z	Accession number ^w	Geographic origin	Host origin and genotype ^x	Donor or collector ^y	DFG ^z
VCG 0120					VCG 0124/5				
22615	Byron Bay, Australia	Cavendish (AAA)	a	I	THAI4-2	Thailand	Kluai Namwa	f	V
23486	Wamuran, Australia	Cavendish	a	I	THAI10	Thailand	Kluai Namwa	f	V
23516	Wamuran, Australia	Inarnibal (AA)	a	I	THAI19-1	Thailand	Kluai Namwa	f	V
23539	Beerwah, Australia	Lady finger (AAB)	a	I	THAI20-2	Thailand	Kluai Namwa	f	V
23550	Wamuran, Australia	<i>Musa jaceyi</i>	a	I	THAI21	Thailand	Kluai Namwa	g	V
23551	Wamuran, Australia	SH3362 (AA)	a	I	F9129	Taiwan	Latundan	d	V
23598	Wamuran, Australia	Cavendish	a	I	RP19(DAVAO)	Philippines	Silk (AAB)	c	V
23599	Wamuran, Australia	Cavendish	a	I	RP20 (Ph2)	Philippines	Latundan	c	V
23607	Wamuran, Australia	SH3362	a	I	RP21 (PhL1)	Philippines	Latundan	c	V
MD401	Wamuran, Australia	Cavendish	a	I	RP22 (PhL2)	Philippines	Latundan	c	V
N5631	Landsborough, Australia	Cavendish	a	I	RP24 (T1)	Taiwan	Gros Michel	c	V
-42F	Wamuran, Australia	Cavendish	a	I	VCG 0124				
W91-307	Eungella, Australia	Lady finger	a	I	23485	Mena Creek, Australia	Lady finger	a	IV
W91-345	Mullumbimby, Australia	Lady finger	a	I	23532	Ormeau, Australia	Lady finger	a	IV
23987	Wamuran, Australia	Cavendish	a	I	23534	Ormeau, Australia	Lady finger	a	IV
INDO14	West Java, Indonesia	Pisang Ambon Putih (AAA)	b	I	23536	Brookfield, Australia	Ducasse (ABB)	a	IV
RP1 (STGM1)	Costa Rica	Gros Michel (AAA)	c	I	23538	Agnes Waters, Australia	Lady finger	a	IV
RP2 (STGM2)	Costa Rica	Gros Michel	c	I	23567	Moresby, Australia	Ducasse	a	IV
RP3 (FCJ7)	Jamaica	Lacatan (AA)	c	I	23603	Tallebudgera, Australia	Lady finger	a	IV
RP4 (NH)	Natal, South Africa	Cavendish cv. Williams (AAA)	c	I	23734	Ormeau, Australia	Lady finger	a	IV
RP5 (GAL1)	Canary Islands	Dwarf Cavendish (AAA)	c	I	THAI14	Kanjanaburi, Thailand	Kluai Namwa	f	IV
RP6 (SA6)	South Africa	Dwarf Cavendish	c	I	RP25 (A35)	Brazil	Unknown	c	IV
RP3S1	Honduras	Highgate (AAA)	c	I	RP26 (STD1)	Honduras	Highgate	c	IV
RPSTH1	Honduras	Highgate	c	I	RP27 (STD2)	Honduras	Highgate	c	IV
RPIC2	Canary Islands	Dwarf Cavendish	c	I	RP29 (FCJ3)	Jamaica	Unknown	c	IV
F9131	South Africa	Cavendish cv. Williams	d	I	RP30 (MW30)	Malawi	Harare	c	IV
VCG 0121					RP31 (MW67)	Malawi	Kholobowa	c	IV
F9130	Taiwan	Cavendish	d	III	RP32 (MW80)	Malawi	Harare	c	IV
RP7 (T3)	Taiwan	Cavendish	c	III	RP33 (STN2)	Nicaragua	Bluggoe (ABB)	c	IV
RP8 (F9130)	Taiwan	Cavendish	c	III	RP34 (STPA2)	Tanzania	Pisang awak	c	IV
RP9 (GM)	Taiwan	Cavendish	c	III	RP35 (B1)	United States	Burro (ABB)	c	IV
RP10 (ML)	Taiwan	Cavendish	c	III	VCG 0125				
RP11 (TBR)	Taiwan	Cavendish	c	III	8605	Tallebudgera, Australia	Lady finger	a	IV
RP12 (SKC)	Taiwan	Gros Michel	c	III	8611	Tomewin, Australia	Lady finger	a	IV
VCG 0122					22468	Currumbin, Australia	Lady finger	a	IV
PHIL10	Philippines	Cavendish cv. Grande Naine (AAA)	e	II	23477	Tallebudgera, Australia	Lady finger	a	IV
RP13 (Ph2)	Philippines	Cavendish	c	II	23480	Tallebudgera, Australia	Lady finger	a	IV
RP14 (P18)	Philippines	Cavendish	c	II	23482	Currumbin, Australia	Lady finger	a	IV
RP15 (P79)	Philippines	Cavendish	c	II	23487	Currumbin, Australia	Lady finger	a	IV
RP16 (LAP)	Philippines	Cavendish	c	II	23488	Tallebudgera, Australia	Lady finger	a	IV
RP17 (SABA)	Philippines	Saba (BBB)	c	II	23529	South Johnstone, Australia	Ducasse	a	IV
RP18 (PW3)	Philippines	Cavendish	c	II	23604	Petches Creek, Australia	Lady finger	a	IV
VCG 0123					M5386	Mareeba, Australia	Ducasse	a	IV
MAL5	Malaysia	Pisang awak (ABB)	?	V	23906	Pimpama, Australia	Lady finger	a	IV
PHIL3	Philippines	Latundan (AAB)	e	V	INDIA1	India	Mysore (AAB)	?	IV
PHIL8	Philippines	Latundan	e	V	INDIA2	India	Mysore	?	IV
PHIL13	Philippines	Latundan	e	V	(continued on the next page)				
PHIL16	Philippines	Latundan	e	V					
PHIL17	Philippines	Abaca (<i>Musa textilis</i>)	e	V					
PHIL19	Philippines	Latundan	e	V					
THAI1-2	Thailand	Kluai Namwa (ABB)	f	V					
THAI2-1	Thailand	Kluai Namwa	f	V					
THAI3-1	Thailand	Kluai Namwa	f	V					

^w Isolates with the prefix RP were kindly donated by R. Ploetz, Tropical Research and Education Center, University of Florida. INDO = Indonesia; MAL = Malaysia; PHIL = the Philippines; and THAI = Thailand.

^x Host genotypes are inter- and intraspecific hybrids of *Musa acuminata* (A) and *M. balbisiana* (B). ? = genomic constitution unknown.

^y Donors or collectors of isolates are a, K. Pegg and N. Moore, Queensland Department of Primary Industries, Indooroopilly, Australia; b, I. Djatnika; c, R. Ploetz; d, L. Burgess; e, L. Magnaye; f, N. Singburadom; g, D. Jones; h, I. Buddenhagen; i, R. Shivas; j, J. C. Bartlett; k, J. Sinurat; l, H. Stover; m, G. P. Salingay; and n, Y. Doon. ? = donor or collector unknown.

^z DFG = DNA fingerprint group.

(Table 2). Isolates were classified as 'VCG unknown' either because they had not yet been typed by VCG analysis prior to DNA fingerprinting or they were not compatible with the available set of NitM testers representing the 20 currently recognized VCGs (N. Y. Moore, *unpublished data*). The isolates that had not been analyzed prior to DNA fingerprinting, but were compatible with the existing VCGs (N. Y. Moore, *unpublished data*), were initially treated as VCG unknown to test the ability of DNA fingerprinting to determine putative VCG. Isolates were stored as monoconidial cultures that had been grown on sterile moist filter paper that was dried and stored at 4°C. Each isolate was analyzed at least twice by DNA fingerprinting.

DAF and data analysis. The modified DAF system described by Bentley and Bassam (1) was used with the following minor modifications. For DNA purification, isolates of *F. oxysporum* f. sp. *cubeuse* were cultured on carnation leaf agar plates at 25°C for 4 to 5 days. These cultures were used to inoculate 250-ml Erlenmeyer flasks containing 200 ml of quarter-strength potato dextrose broth and incubated at room temperature without shaking for no longer than 7 days. The DNA amplification reactions, thermocycling, and electrophoresis conditions are described by Bentley and Bassam (1). Primer sequences used are listed in Table 3. The similarity between different isolates of *F. oxysporum* f. sp. *cubeuse*

TABLE 1. (continued from the preceding page)

Accession number ^w	Geographic origin	Host origin and genotype ^x	Donor or collector ^y	DFG ^z	Accession number ^w	Geographic origin	Host origin and genotype ^x	Donor or collector ^y	DFG ^z
THAI7-1	Thailand	Kluai Namwa	f	IV	MAL18	Malaysia	Pisang Raja (AAB)	?	III
RP42 (1S?)	Bodles, Jamaica	Unknown	c	IV	MAL20	Malaysia	Pisang Berangan (AA)	?	III
RP43 (STPA3)	Uganda	Pisang awak	c	IV	MAL32	Malaysia	Pisang Rastali (AAB)	h	III
RP44 (STNP5)	Zaire	Ney poovan (AB)	c	IV	INDO26	Indonesia	Pisang Kepok (BBB)	l	III
VCG 0126					INDO30	Indonesia	Pisang Susu (AAA)	l	III
PHIL6	Philippines	Latundan	e	II	INDO32	Indonesia	Pisang Berangan	l	III
PHIL7	Philippines	Cardaba (BBB)	e	II	RPJAK1	Indonesia	Unknown	c	III
INDO33	Indonesia	Pisang Manurung (ABB)	h	II	INDO34	Indonesia	Pisang Berangan	h	III
INDO38	Indonesia	Pisang Rubus (?)	i	II	INDO52	Indonesia	Cavendish cv. Grande Naine	h, j	III
INDO40	Indonesia	Pisang Manurung	h, j	II	INDO56	Indonesia	Cavendish cv. Valery (AAA)	h, m	III
INDO41	Indonesia	Pisang Manurung	h, j	II	VCG 01214				
INDO42	Indonesia	Unknown	h, j	II	RPMW40	Malawi	Harare	c	VII
INDO43	Indonesia	Pisang Manurung	h, j	II	VCG 01215				
INDO44	Indonesia	Pisang Manurung	h, j	II	RPCR1-1	Costa Rica	Gros Michel	c	I
INDO45	Indonesia	Pisang Manurung	h, j	II	VCG 01216				
INDO57	Indonesia	Pisang Manurung	j, k	II	INDO39	Indonesia	Pisang Berangan	h	III
INDO58	Indonesia	Pisang Manurung	j, k	II	INDO47	Indonesia	Cavendish cv. Grande Naine	h, j	III
INDO59	Indonesia	Pisang Manurung	j, k	II	INDO48	Indonesia	Pisang Berangan	h, j	III
INDO60	Indonesia	Pisang Manurung	j, k	II	INDO50	Indonesia	Cavendish cv. Grande Naine	h, j	III
INDO63	Indonesia	Highgate	j, k	II	INDO53	Indonesia	Cavendish cv. Grande Naine	h, j	III
INDO72	Indonesia	Pisang Puju (?)	j, k	II	MAL1	Malaysia	Pisang Raja	?	III
RPJAK2	Indonesia	Highgate	c	II	MAL4	Malaysia	Pisang Raja	?	III
RPJAK4	Indonesia	Highgate	c	II	MAL5	Malaysia	Pisang Raja	?	III
RP45 (S1)	Honduras	Highgate	c	II	MAL11	Malaysia	Pisang Mas	?	III
RP46 (STM3)	Honduras	Maqueño (AAB)	c	II	MAL14	Malaysia	Pisang Kebatu (ABB)	?	III
RP48 (STB2)	Honduras	Highgate	c	II	MAL21	Malaysia	Pisang Berangan	g	III
RP49 (4S1)	Honduras	Maqueño	c	II	MAL22	Malaysia	Cavendish cv. Grande Naine	g	III
RP50 (5S1)	Honduras	Maqueño	c	II	MAL26	Malaysia	Cavendish	n	III
VCG 0128					MAL27	Malaysia	Pisang Rastali	n	III
22993	South Johnstone, Australia	Blue Java (ABB)	a	IV	MAL28	Malaysia	Unknown	n	III
22994	South Johnstone, Australia	Bluggoe	a	IV	MAL29	Malaysia	Pisang Berangan	?	III
23909	Kamerunga, Australia	Bluggoe	a	IV	MAL31	Malaysia	Pisang Rastali	h	III
23996	Kamerunga, Australia	Bluggoe	a	IV	MAL34	Malaysia	Pisang Rastali	h	III
24235	Australia	Unknown	a	IV	MAL36	Malaysia	Cavendish cv. Williams	h	III
24246	South Johnstone, Australia	Monthan (ABB)	a	IV	MAL37	Malaysia	Cavendish cv. Williams	h	III
24247	South Johnstone, Australia	Tuu Gia (AA)	a	IV	MAL38	Malaysia	Pisang Serendah (AAA)	h	III
24249	South Johnstone, Australia	Dwarf Yawa (ABB)	a	IV	MAL39	Malaysia	Cavendish cv. Grande Naine	h	III
24250	South Johnstone, Australia	Kluai Nui Mue Nang (ABB)	a	IV	MAL40	Malaysia	Cavendish cv. Grande Naine	h	III
24251	South Johnstone, Australia	Blue Java	a	IV	MAL41	Malaysia	Cavendish cv. Grande Naine	h	III
24253	South Johnstone, Australia	Tukuru (ABB)	a	IV	VCG 01217				
24255	South Johnstone, Australia	Silver Bluggoe (ABB)	a	IV	MAL7	Malaysia	Pisang Rastali	?	V
VCG 0129					MAL8	Malaysia	Pisang Rastali	?	V
8617	Mooloolah, Australia	Cavendish	a	V	MAL23	Malaysia	Pisang Kebatu	g	V
24234	Mooloolah, Australia	Cavendish	a	V	MAL30	Malaysia	Pisang Rastali	h	V
23509	Gunalda, Australia	Lady finger	a	V	MAL43	Malaysia	Pisang Rastali	h	V
23510	Gympie, Australia	Lady finger	a	V	23775	Malaysia	Pisang Rastali	a	V
23512	Wappa Dam, Australia	Lady finger	a	V	VCG 01218				
23518	Kin Kin, Australia	Lady finger	a	V	INDO5	Indonesia	Pisang Siem (ABB)	b	VI
VCG 01210					VCG 01219				
RP51 (A1-1)	Florida, United States	Apple	c	II	INDO25	Indonesia	Pisang Ambon (AAA)	l	II
RP52 (A2-1)	Florida, United States	Apple	c	II	INDO35	Indonesia	Pisang Raja Sereh (AAB)	h	II
RP53 (GG1)	Florida, United States	Apple	c	II	INDO36	Indonesia	Pisang Garing (?)	h	II
RP54 (JC4)	Florida, United States	Apple	c	II	INDO37	Indonesia	Pisang Ambon Putih	h	II
VCG 01211					VCG 01220				
23631	Wamuran, Australia	SH3142 (AA)	a	I	24200	Camaron, Western Australia	Cavendish cv. Williams	a	IV
RP57 (13721)	?	Unknown	c	I	24208	Camaron, Western Australia	Cavendish cv. Williams	a	IV
VCG 01212					24218	Camaron, Western Australia	Cavendish cv. Williams	a	IV
RP58 (STNP1)	Tanzania	Ney poovan	c	IV	24220	Camaron, Western Australia	Cavendish cv. Williams	a	IV
VCG 01213					24211	Camaron, Western Australia	Cavendish cv. Williams	a	IV
MAL15	Malaysia	Pisang Mas (AA)	?	III	24219	Camaron, Western Australia	Cavendish cv. Williams	a	IV
MAL17	Malaysia	Pisang Mas	?	III					

was determined using Gel Compar v. 3.1 (1). A total of 483 fragments was scored for the isolates from known VCGs with all 10 primers; 351 were polymorphic (Table 3). To determine the relationships between the VCGs based on all primers, a similarity matrix that represents

the average of the values from the 10 similarity matrices was clustered by the unweighted pair group method with arithmetic means (UPGMA) using the SAHN module of NTSYSpc v. 2.0. The goodness-of-fit of the phenogram was determined by

TABLE 2. Isolates of *Fusarium oxysporum* f. sp. *cubense* of unknown vegetative compatibility group (VCG) identified by DNA fingerprinting analysis

Accession number ^v	Geographic origin	Host origin and genotype ^w	Donor or collector ^x	DFG ^y	Genotype ^z
INDO10	West Java, Indonesia	Pisang Jimbluk (?)	a	V	01218
INDO16	West Java, Indonesia	Pisang Ambon Putih (AAA)	a	II	2
INDO54	East Java, Indonesia	Pisang Susu (AAB)	b, c	II	3
INDO65	West Sumatra, Indonesia	Kinalun/Gajah (?)	d	III	0121
INDO67	West Sumatra, Indonesia	Pisang Buai (AAA?)	d	III	0121
INDO69	West Sumatra, Indonesia	Pisang Kepok (BBB)	d	IV	0124/5
INDO77	Sumatra, Indonesia	Cavendish cv. Williams (AAA)	e, f	III	01213/16
INDO78	Sumatra, Indonesia	Cavendish (AAA)	e, f	III	01213/16
INDO79	Sumatra, Indonesia	Umalag (AAA)	e, f	III	01213/16
INDO80	East Java, Indonesia	Pisang Susu	b, c	V	0123
INDO81	East Java, Indonesia	Pisang Ambon (AAA)	b, c	I	0120/15
INDO82	East Java, Indonesia	Pisang Berangan (AA)	b, c	III	01213/16
INDO83	East Java, Indonesia	Pisang Ambon Lumut (AAA)	b, c	III	01213/16
INDO84	East Java, Indonesia	Cavendish cv. Williams	b, c	III	01213/16
INDO85	East Java, Indonesia	Pisang Ambon Kuning (?)	b, c	III	01213/16
INDO87	Sulawesi, Indonesia	Pisang Raja Sereh (AAB)	b, c	III	01213/16
INDO88	Sulawesi, Indonesia	Unknown	b, c	II	0126
INDO89	Sulawesi, Indonesia	Pisang Puju (?)	b, c	II	0126
INDO90	Sulawesi, Indonesia	Pisang Puju	b, c	II	0126
INDO91	Sulawesi, Indonesia	Pisang Ambon	b, c	II	0126
INDO92	Halmahera, Indonesia	Sangate (AAA?)	b, c	II	0126
INDO93	Halmahera, Indonesia	Cavendish cv. Grande Naine (AAA)	b, c	I	0120
INDO94	Halmahera, Indonesia	Cavendish	b, c	III	01213/16
INDO95	Halmahera, Indonesia	Mulubebe (?)	b, c	III	0121
INDO96	Halmahera, Indonesia	Sangate	b, c	II	0126
INDO97	Halmahera, Indonesia	Cavendish	b, c	III	01213/16
INDO98	Halmahera, Indonesia	Cavendish	b, c	III	01213/16
INDO99	Halmahera, Indonesia	Unknown	b, c	II	0126
INDO100	East Java, Indonesia	Pisang Putot (?)	b, c	III	01213/16
INDO101	Sulawesi, Indonesia	Pisang Ambon Lumut	c, g	II	0126
INDO102	Sulawesi, Indonesia	Pisang Ambon Lumut	c, g	II	0126
THAI1-1	Petchabun, Thailand	Kluai Namwa (ABB)	h	V	0123
THAI22	Nong Khai, Thailand	Kluai Namwa	i	V	01218
THAI23	Phuket Island, Thailand	Kluai Namwa	i	IV	0124/5
THAI24	Chumporn, Thailand	Kluai Namwa	i	V	01218
THAI25	Chiang Rai Province, Thailand	Kluai Namwa	j	V	0123
THAI26	Chiang Mai Province, Thailand	Kluai Namwa	j	V	0124/5
THAI27	Chiang Mai Province, Thailand	Kluai Namwa	j	IV	01220
THAI28	Chiang Mai Province, Thailand	Kluai Namwa	j	IV	01220
THAI29	Nan Province, Thailand	Kluai Namwa	j	IV	0123
THAI30	Phrae Province, Thailand	Kluai Namwa	j	V	0123
THAI31	Uttaradit Province, Thailand	Kluai Namwa	j	V	0123
THAI32	Uttaradit Province, Thailand	Kluai Namwa	j	V	0123
THAI33	Chiang Rai Province, Thailand	Kluai Namwa	j	V	0123
THAI34	Chiang Rai Province, Thailand	Kluai Namwa	j	V	0123
THAI35	Chiang Rai Province, Thailand	Kluai Namwa	j	V	12
THAI36	Chiang Rai Province, Thailand	Kluai Namwa	j	V	0123
THAI37	Chiang Rai Province, Thailand	Kluai Namwa	j	V	0123
THAI38	Chiang Rai Province, Thailand	Kluai Namwa	j	V	0123
THAI39	Chiang Rai Province, Thailand	Kluai Namwa	j	IV	0124/5
MAL4	Kota Sarang Semut, Malaysia	Pisang awak (AAB)	?	IV	4
MAL44	Selangor, Malaysia	Pisang Lilin (AA)	k	III	01213/16
MAL45	Selangor, Malaysia	Pisang Lilin	k	III	01213/16
MAL46	Selangor, Malaysia	Pisang Lilin	k	III	01213/16
MAL47	Selangor, Malaysia	Cavendish cv. Williams	b, l, m	III	01213/16
MAL48	Selangor, Malaysia	Bluggoe (ABB)	b, l, m	III	01213/16
MAL49	Selangor, Malaysia	Gros Michel (AAA)	b, l, m	III	01213/16
MAL50	Selangor, Malaysia	Pisang Mas (AA)	b, l, m	III	01213/16
MAL51	Selangor, Malaysia	Kuda (AA)	b, l, m	III	01213/16
MAL52	Pahang, Malaysia	Unknown (AAB)	b, l, m	III	01213/16
MAL53	Pahang, Malaysia	Unknown (AAB)	b, l, m	III	01213/16

(continued on the next page)

^v HOND = Honduras; INDO = Indonesia; MAL = Malaysia; MEX = Mexico; PHIL = the Philippines; THAI = Thailand; and VIET = Viet Nam.

^w Host genotypes are inter- and intraspecific hybrids of *Musa acuminata* (A) and *M. balbisiana* (B). ? = genomic constitution unknown.

^x Donors or collectors of isolates are a, I. Djatnika; b, I. Buddenhagen; c, J. C. Bartlett; d, Jumjunidang; e, Stefanus; f, G. P. Salingay; g, J. Sinurat; h, N. Singburaudom; i, D. Jones; j, S. Kooariyakul; k, S. H. Jamuluddin; l, N. Moore; m, S. Bentley; n, T. Y. Hock; o, L. Magnaye; p, E. Aguilar; q, S. Medina; r, H. H. Nhi; s, D. T. Thanh; t, J. Stanton; u, K. Pegg; v, R. Caid; and w, J. Duff. ? = donor or collector unknown.

^y DFG = DNA fingerprint group.

^z The new genotypes identified in this study were numbered consecutively, whereas genotypes that were identical to an existing VCG were referred to by their VCG code.

computing a cophenetic value matrix using the COPH module and comparing this matrix with the SAHN tree matrix using the MX-COMP module. A cophenetic correlation of $r > 9.0$ is considered a very good fit.

RESULTS

Genetic variation within each VCG of *F. oxysporum* f. sp. *cubense*. Isolates within each VCG generally produced an identi-

TABLE 2. (continued from the preceding page)

Accession number ^v	Geographic origin	Host origin and genotype ^w	Donor or collector ^x	DFG ^y	Genotype ^z
MAL54	Pahang, Malaysia	Unknown (AAB)	b, l, m	III	01213/16
MAL55	Pahang, Malaysia	Pisang awak	b, l, m	V	0123
MAL57	Negeri Sembilan, Malaysia	Pisang Rastali (AAB)	b, l, m	V	01217
MAL58	Negeri Sembilan, Malaysia	Pisang Rastali	b, l, m	V	01217
MAL59	Negeri Sembilan, Malaysia	Cavendish cv. Williams	b, l, m	III	01213/16
MAL60	Negeri Sembilan, Malaysia	Cavendish cv. Williams	b, l, m	III	01213/16
MAL61	Negeri Sembilan, Malaysia	Pisang Rastali	b, l, m	III	01213/16
MAL62	Negeri Sembilan, Malaysia	Pisang Rastali	b, l, m	V	01217
MAL63	Negeri Sembilan, Malaysia	Pisang Rastali	b, l, m	V	01217
MAL64	Negeri Sembilan, Malaysia	Pisang Rastali	b, l, m	V	01217
MAL65	Negeri Sembilan, Malaysia	<i>Musa acuminata</i> subsp. <i>malaccensis</i>	b, l, m	V	5
MAL66	Negeri Sembilan, Malaysia	<i>Musa acuminata</i> subsp. <i>malaccensis</i>	b, l, m	V	5
MAL67	Negeri Sembilan, Malaysia	<i>Musa acuminata</i> subsp. <i>malaccensis</i>	b, l, m	V	5
MAL68	Negeri Sembilan, Malaysia	Pisang Kapas (?)	b, l, m	III	01213/16
MAL69	Near Melaka, Malaysia	Unknown (AAB)	b, l, m	III	01213/16
MAL70	Johor, Malaysia	Cavendish cv. Grande Naine	b, l, m	III	01213/16
MAL71	Johor, Malaysia	Cavendish cv. Grande Naine	b, l, m	III	01213/16
MAL72	Johor, Malaysia	Cavendish cv. Grande Naine	b, l, m	III	01213/16
MAL73	Johor, Malaysia	Pisang Rastali	b, l, m	V	0123
MAL74	Johor, Malaysia	Cavendish cv. Grande Naine	b, l, m	III	0121
MAL75	Johor, Malaysia	Cavendish cv. Grande Naine	b, l, m	III	0121
MAL76	Johor, Malaysia	Cavendish cv. Grande Naine	b, l, m	III	0121
MAL77	Johor, Malaysia	Cavendish cv. Grande Naine	b, l, m	III	0121
MAL78	Johor, Malaysia	Cavendish cv. Grande Naine	b, l, m	III	01213/16
MAL80	Johor, Malaysia	Cavendish cv. Grande Naine	b, l, m	III	01213/16
MAL81	Johor, Malaysia	Cavendish cv. Grande Naine	b, l, m	III	01213/16
MAL82	Johor, Malaysia	Cavendish cv. Grande Naine	b, l, m	III	01213/16
MAL83	Johor, Malaysia	Cavendish	n	III	01213/16
PHIL1	Mindanao, Philippines	Latundan (AAB)	o	II	7
PHIL4	Mindanao, Philippines	Latundan	o	IV	11
PHIL18	Luzon, Philippines	Latundan	o	II	8
PHIL22	Luzon, Philippines	Katali (ABB)	o	IV	11
PHIL23	Luzon, Philippines	Siusok (ABB)	o	IV	11
PHIL24	Luzon, Philippines	Latundan	p, q	IV	9
PHIL26	Luzon, Philippines	Latundan	p, q	IV	10
INDIA7	India	Poovan (AAB)	?	IV	01220
VIET1	So' n La Province, Viet Nam	Chuôi ngôp cao (?)	r, s, t	V	13
VIET3	Tu Liem, Hanôï, Viet Nam	Chuôi xiêm (ABB)	b, l, u	IV	14
VIET4	Vinh Phú Province, Viet Nam	Chuôi xiêm	b, l, u	IV	14
VIET5	Vinh Phú Province, Viet Nam	Chuôi xiêm	b, l, u	IV	14
VIET6	Vinh Phú Province, Viet Nam	Chuôi xiêm	b, l, u	IV	14
VIET7	Vinh Phú Province, Viet Nam	Chuôi xiêm	b, l, u	IV	14
VIET8	Thúa Thiên Huê Province, Viet Nam	Chuôi xiêm	b, l, u	V	12
VIET9	Thúa Thiên Huê Province, Viet Nam	Chuôi xiêm	b, l, u	V	12
VIET10	Thúa Thiên Huê Province, Viet Nam	Chuôi xiêm	b, l, u	V	12
VIET11	Thúa Thiên Huê Province, Viet Nam	Chuôi xiêm	b, l, u	V	12
VIET12	Thúa Thiên Huê Province, Viet Nam	Chuôi xiêm	b, l, u	V	12
VIET13	Thúa Thiên Huê Province, Viet Nam	Chuôi xiêm	l, u	V	12
VIET14	Tiên Giang Province, Viet Nam	Chuôi xiêm	u	IV	0124/5
VIET15	Tiên Giang Province, Viet Nam	Chuôi xiêm	b	V	12
VIET16	Tiên Giang Province, Viet Nam	Chuôi xiêm	b, l	V	12
VIET17	Cân Tho Province, Viet Nam	Chuôi xiêm	b, l, u	IV	0124/5
VIET18	Cân Tho Province, Viet Nam	Chuôi xiêm	b, l, u	IV	0124/5
VIET19	Cân Tho Province, Viet Nam	Chuôi xiêm	b, l, u	V	12
VIET20	Vinh Long Province, Viet Nam	Chuôi xiêm	b, l, u	IV	0124/5
VIET21	Vinh Long Province, Viet Nam	Chuôi xiêm	b, l, u	IV	0124/5
VIET22	Tiên Giang Province, Viet Nam	Chuôi xiêm	b, l, u	V	12
MEX1	Nayarit, Mexico	Silk (Manzano) (AAB)	b	VIII	6
MEX2	Nayarit, Mexico	Silk (AAB)	b	VIII	6
MEX3	Nayarit, Mexico	Silk	b	VIII	6
MEX4	Nayarit, Mexico	Silk	b	VIII	6
MEX5	Nayarit, Mexico	Silk	b	VIII	6
MEX6	Nayarit, Mexico	Silk	b	VIII	6
MEX7	Nayarit, Mexico	Silk	b	VIII	6
HOND1	La Esparanza, Peña, Honduras	Gros Michel	v	IV	0124/5
HOND2	La Esparanza, Peña, Honduras	Gros Michel	v	IV	0124/5
HOND3	La Esparanza, Peña, Honduras	Gros Michel	v	IV	0124/5
HOND4	El Progreso, Honduras	Chato (ABB)	v	IV	0124/5
HOND5	El Progreso, Honduras	Chato	v	IV	0124/5
24405	Darwin, Australia	<i>Heliconia chartacea</i>	w	IX	1
24406	Darwin, Australia	<i>Heliconia chartacea</i>	w	IX	1

cal DNA fingerprint and were closely related, regardless of geographic origin or host source. The genetic similarity between isolates within each VCG was determined based on primer ILOE (Table 4). All isolates within each of the VCGs 0121, 0122, 0123, 0128, 0129, 01210, 01213, 01216, 01217, 01219, and 01220 produced an identical DNA fingerprint pattern and, therefore, had a genetic similarity of 100% (Table 4). All isolates within VCG 0120 produced an identical DNA fingerprint, except for isolates RP1, RP2, and 23607 (Fig. 1). These three isolates all produced one DNA fragment at a reduced intensity compared with the other isolates in VCG 0120, and isolate 23607 also differed by the intensity of one other fragment. The average genetic similarity among isolates within VCG 0120 was 99% (Table 4). Isolates within VCG 0126 were very similar to each other, except that isolates PHIL7 and INDO72 were missing one fragment that was present in the DNA fingerprints of the other isolates within this VCG. The two isolates examined within VCG 01211 differed by one fragment. The genetic similarity within the VCGs 01212, 01214, 01215, and 01218 could not be determined, as only one isolate was examined from each of these VCGs. Among isolates in VCGs 0124, 0125, 0128, 01212, and 01220, five genotypes were identified that did not always correlate with the VCG. Within this group, some isolates in the same VCG produced different DNA fingerprints, and some isolates belonging in different VCGs produced identical DNA fingerprints. The DNA fingerprints of isolates in these VCGs differed by the

presence or absence of one or two fragments, and the genetic similarity among isolates in these VCGs ranged from 96 to 100%.

Genetic variation between different VCGs of *F. oxysporum* f. sp. *ubense*. As there was minimal variation among isolates within each VCG, an isolate was selected to represent each VCG, and the relationships between the VCGs were determined by comparing these representative isolates using 10 different arbitrary primers (Fig. 2). Each of the primers grouped the isolates similarly based on the respective DNA fingerprint patterns they generated. Although each primer produced similar results, it was possible to differentiate closely related VCGs more easily with some primers than with others. For each of the 10 primers, a similarity matrix was generated based on the Jaccard coefficient using Gel Compar. To determine the relationships between the VCGs based on all primers, a similarity matrix that represented the average of the values from the 10 similarity matrices (Table 5) was clustered by the UPGMA method using the SAHN module of NTSYSpc v. 2.0 (Fig. 3). The obtained cophenetic correlation value of $r = 0.99$ indicated that the UPGMA cluster analysis was statistically significant.

The DNA fingerprint patterns generated by most primers revealed a distinct genotype for each different VCG, except for VCGs 01213 and 01216, which produced an identical DNA fingerprint pattern with all primers, i.e., 19 genotypes were differentiated among the 20 reported VCGs of *F. oxysporum* f. sp. *ubense*. In most instances, each VCG could be distinguished by the presence or absence of more than one fragment. Some VCGs, however, often produced an identical genotype with the majority of primers including VCGs 0120 and 01215, VCGs 0124 and 0125, and VCGs 0129 and 01211.

Identification of isolates of unknown VCG. The isolates of unknown VCG were from Australia, Honduras, India, Indonesia, Malaysia, Mexico, the Philippines, Thailand, and Viet Nam (Table 2). For all isolates that belonged to an existing VCG, the putative VCG determined by DNA fingerprinting agreed with the actual VCG designation (Table 2). Among isolates of unknown VCG, several produced a DNA fingerprint characteristic of an existing VCG, but the isolates were not compatible with that VCG based on tests using the currently available NitM testers. The difference that determines vegetative incompatibility may be only minor, and further screening with more primers may differentiate these iso-

TABLE 3. Primer sequences and number of polymorphic fragments generated

Primer	Sequence 5' to 3'	Total number of fragments scored	Number of polymorphic fragments
DINQ	CTG GCC CA	51	41
DJDH	ACC AGC CA	48	31
EHKJ	GCT CAC GA	47	29
HIRH	ACG TCC AC	50	33
ILOE	GAT GAG CC	54	41
IMBE	GAA ACG CC	49	32
IMBR	GTA ACG CC	49	38
NRKI	CCT CGT GG	46	40
NROI	CCT GGT GG	48	35
RKMI	CCC GTC GT	41	31
Total		483	351

TABLE 4. Genetic similarity among isolates within each vegetative compatibility group (VCG) of *Fusarium oxysporum* f. sp. *ubense* based on DNA fingerprinting analysis with primer ILOE^y

VCG	Number of isolates analyzed	Average genetic similarity (%)
0120	26	99
0121	7	100
0122	7	100
0123	21	100
0124	19	96
0125	18	96
0126	23	99
0128	12	100
0129	6	100
01210	4	100
01211	2	98
01212	1	... ^z
01213	12	100
01214	1	...
01215	1	...
01216	24	100
01217	6	100
01218	1	...
01219	4	100
01220	6	100

^y The value given is the average of the values determined for all isolates within a VCG based on the Jaccard similarity coefficient using Gel Compar analysis.

^z The genetic similarity could not be determined as only one isolate was examined from each of these VCGs.

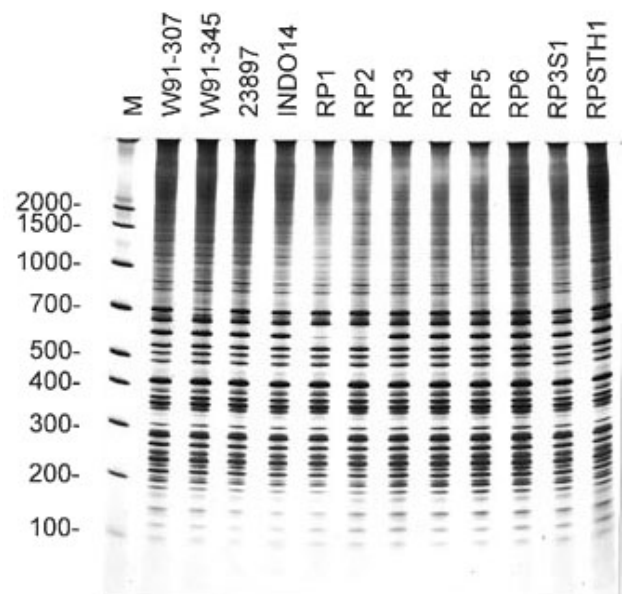


Fig. 1. Genetic variation within vegetative compatibility group (VCG) 0120 of *Fusarium oxysporum* f. sp. *ubense*. DNA fingerprints were generated using primer ILOE. The isolate accession numbers are indicated at the top of the figure. The sizes in base pairs of the molecular weight marker are indicated to the left of the figure.

lates or further vegetative compatibility testing with additional NitM testers may confirm the putative VCG classification. Among the isolates that were not compatible with any of the 20 currently recognized VCGs, 14 new genotypes were identified (Tables 2 and 6). These new genotypes were numbered consecutively (1 to 14), whereas genotypes that represented an existing VCG were referred to by their VCG code.

Clonal lineages within *F. oxysporum* f. sp. *cupense*. Comparison of the DNA fingerprints, both visually and by phenetic analysis, subdivided isolates of *F. oxysporum* f. sp. *cupense* into nine clonal lineages (Table 7). The lineages within *F. oxysporum* f. sp. *cupense* were referred to as DNA fingerprint groups (DFGs). Five of these lineages each contained numerous closely related VCGs and genotypes, and the remaining four lineages each contained only a single VCG or genotype. The genetic similarity of the VCGs and genotypes within each lineage ranged from 80 to 100%. The similarity

of the VCGs within each lineage was also evidenced by the presence of VCG cross-compatible isolates within some lineages. There were no VCGs common to more than one lineage.

DISCUSSION

Relationships between DNA fingerprints and VCGs. In general, there was little or no genetic variation among isolates within each VCG of *F. oxysporum* f. sp. *cupense*, irrespective of host or geographic origin. Only one DNA fingerprint pattern that represented a unique genotype was distinguished for each VCG, except for VCGs 0120, 0126, and 01211. These VCGs each contained one or two isolates that differed by the intensity or absence of a single DNA fragment. Because the DNA fingerprints were generally VCG specific, it was possible to quickly determine a putative VCG classification for isolates prior to vegetative compatibility analysis. In

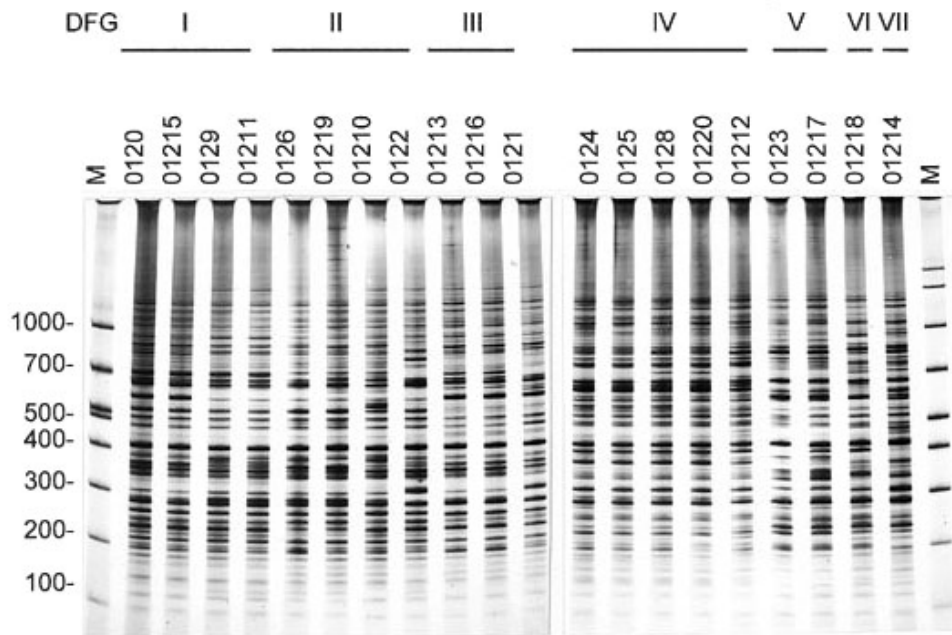


Fig. 2. Genetic variation between different vegetative compatibility groups (VCGs) of *Fusarium oxysporum* f. sp. *cupense*. DNA fingerprints were generated using primer ILOE. The VCG codes and DNA fingerprint group (DFG) numbers are indicated at the top of the figure. The sizes in base pairs of the molecular weight marker are indicated to the left of the figure.

TABLE 5. Genetic similarity (%) between different vegetative compatibility groups (VCGs) of *Fusarium oxysporum* f. sp. *cupense*²

VCG	VCG																				
	0120	01215	0129	01211	0126	01219	01210	0122	0121	01213	01216	0124	0125	0128	01220	01212	0123	01217	01214	01218	
0120	100																				
01215	98	100																			
0129	85	85	100																		
01211	86	86	99	100																	
0126	83	85	85	87	100																
01219	82	82	82	83	88	100															
01210	82	80	82	84	84	84	100														
0122	79	78	81	82	84	83	80	100													
0121	76	74	76	76	76	76	75	75	100												
01213	73	73	74	75	76	75	76	74	88	100											
01216	76	74	74	75	74	75	75	74	88	100	100										
0124	58	59	59	60	60	61	60	59	62	61	61	100									
0125	58	59	59	59	61	60	59	60	61	60	60	99	100								
0128	57	58	59	59	59	60	59	59	61	61	60	91	92	100							
01220	59	61	58	53	62	62	60	60	63	61	61	91	91	94	100						
01212	57	58	59	58	62	61	60	60	62	61	61	81	82	84	85	100					
0123	59	59	58	57	58	59	56	57	58	60	60	69	67	68	68	69	100				
01217	57	57	58	58	58	59	57	58	59	60	59	68	68	69	67	69	94	100			
01214	58	57	57	56	60	59	58	58	63	59	61	67	66	68	68	67	72	73	100		
01218	56	56	58	57	59	58	58	58	61	61	61	66	67	66	67	67	73	73	71	100	

² Each value is the average of the values determined for 10 different primers using the Jaccard similarity coefficient.

this study, there was near complete correlation between the putative VCG classification determined by DNA fingerprinting and the actual VCG designation. The exception was that several isolates of unknown VCG produced a DNA fingerprint characteristic of an existing VCG but were not compatible with that VCG based on tests using the currently available NitM testers. Further vegetative compatibility testing of these isolates with additional NitM testers has since confirmed the putative VCG classification (N. Y. Moore, unpublished data). DNA fingerprinting also enabled characterization of isolates that were not compatible with any of the existing VCGs. The identification of genetically similar isolates of unknown VCG may facilitate the establishment of new VCGs, as genetically similar isolates are more likely to belong in the same VCG.

Although vegetative compatibility is a useful means of grouping genetically similar isolates, it does not provide any indication of the genetic relatedness between incompatible isolates, such as those from different VCGs and formae speciales, and heterokaryon self-incompatible isolates. A mutation at a single *vic* locus could result in closely related isolates being vegetatively incompatible and, thus, clonally related isolates may occur in different VCGs. Using DNA fingerprinting analysis, we have determined the genetic variation among isolates in different VCGs of *F. oxysporum* f. sp. *ubense* including many isolates of unknown VCG. Several different VCGs generated identical DNA fingerprints with the majority of primers including VCGs 0120 and 01215, VCGs 0124 and 0125, VCGs 0129 and 01211, and VCGs 01213 and 01216. These pairs of VCGs have also been shown to include "bridging" or cross-compatible isolates (N. Y. Moore, unpublished data). The bridging isolates are capable of forming heterokaryons with NitM testers representing both VCGs. VCGs 01213 and 01216 produced identical DNA fingerprint patterns with all the arbitrary primers tested and were considered to be the same genotype. This genotype is significant in that it is capable of severely damaging Cavendish cultivars in the tropics (tropical race 4).

The VCGs and genotypes in DFG IV (VCGs 0124, 0125, 0128, 01212, and 01220; genotypes 4, 9, 10, 11, and 14) were also closely related based on their DNA fingerprint patterns. Within this lineage, some isolates in different VCGs produced identical DNA fingerprint, and some isolates in the same VCG produced different DNA fingerprints. The genotypes identified within this lineage correlated with geographic origin rather than VCG or host origin. For example, the isolate in VCG 0124 from Thailand was identical to isolates in VCG 0125 from Thailand, but was different from isolates in VCG 0124 from other countries. Similarly, isolates from the Vinh Phú Province in North Viet Nam belonging in VCGs 0124/5 and 0125 were identical to each other, but distinct from VCG 0124/5 isolates from the Tiên Giang, Cần Tho, and Vinh Long Provinces in

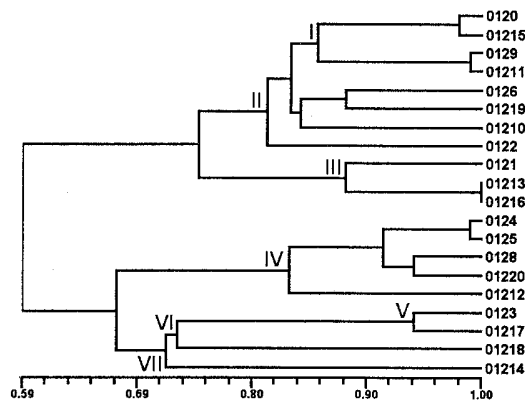


Fig. 3. Genetic similarity between the reported vegetative compatibility groups (VCGs) of *Fusarium oxysporum* f. sp. *ubense*. Phenogram represents UPGMA cluster analysis of the average of the similarity values determined for 10 arbitrary primers using the Jaccard similarity coefficient. DNA fingerprint groups (DFGs) identified in this study are indicated on each branch of the phenogram.

South Viet Nam. Based on the similarity of the DNA fingerprint patterns of the genotypes within DFG IV, more rigorous VCG testing with more NitM testers may indicate that other genotypes within this lineage are also cross-compatible.

Clonal lineages within *F. oxysporum* f. sp. *ubense*. We have identified nine major lineages within *F. oxysporum* f. sp. *ubense*. The genetic similarity of the VCGs and genotypes within each lineage ranged from 80 to 100%. Within each lineage of *F. oxysporum* f. sp. *ubense*, the DNA fingerprint pattern of each genotype differed by only one or a few fragments. The similarity of the DNA fingerprint patterns suggests that the genotypes of *F. oxysporum* f. sp. *ubense* have evolved by mutation within each clonal lineage. The occurrence of VCG cross-compatible isolates within several lineages and the absence of VCGs and genotypes common to different lineages are further evidence that each lineage is clonally derived. Furthermore, the lineages within *F. oxysporum* f. sp. *ubense* were as different from each other as they were from other formae speciales of *F. oxysporum* (S. Bentley, unpublished data). These results suggest that *F. oxysporum* f. sp. *ubense* is polyphyletic, which is in agreement with previous studies by Koenig et al. (10) and O'Donnell et al. (16) based on RFLP analysis and DNA sequencing of nuclear and mitochondrial genes, respectively.

The largest lineage (DFG IV) contained 10 different genotypes of worldwide distribution (Fig. 4). The second largest lineage (DFG II) consisted of eight genotypes, all of which originated from Indonesia and the Philippines, except for an isolated population from Florida (VCG 01210). Four of the lineages of *F. oxysporum* f. sp. *ubense* each contained a single VCG or genotype and were of limited geographic origin. DFG VI was represented by VCG 01218, and although in this study only one isolate was examined, further analysis of more isolates has confirmed the genetic relationship of this VCG to the other VCGs of *F. oxysporum* f. sp. *ubense* (S. Bentley, unpublished data). DFG VII was represented

TABLE 6. Geographical distribution of genotypes of *Fusarium oxysporum* f. sp. *ubense*

Country	No. of isolates of FOC	Genotypes identified by DNA fingerprint pattern ^z	Total no. of genotypes	Lineages represented
Australia	62	0120, 0124, 0125, 0128, 0129, 01211, 01220, 1 (24405, 24406)	8	I, IV, IX
Brazil	1	0124	1	IV
Canary Islands	2	0120	1	I
Costa Rica	3	0120, 01215	2	I
Honduras	14	0120, 0124, 0124/5, 0125, 0126	3	I, II, IV
India	3	0125	2	IV
Indonesia	65	0120, 0121, 0124/5, 0126, 01213/16, 01218, 01219, 2 (INDO16), 3 (INDO54)	10	I, II, III, IV, V, VI
Irian Jaya	1	0126	1	II
Jamaica	3	0120, 0124, 0125	3	I, IV
Malawi	9	0124, 0124/5, 01214	3	I, V, VII
Malaysia	71	0121, 0123, 01213/16, 01217, 4 (MAL4), 5 (MAL65-67)	6	III, IV, V
Mexico	7	6 (MEX1-7)	1	VIII
Nicaragua	1	0124	1	IV
Philippines	26	0122, 0123, 0126, 7 (PHIL1), 8 (PHIL18), 9 (PHIL24), 10 (PHIL26), 11 (PHIL4, 22, 23)	8	II, III, IV, V
South Africa	3	0120	1	I
Taiwan	9	0121, 0123	2	III, V
Tanzania	2	0124, 01212	2	IV
Thailand	30	0123, 0124, 0124/5, 0125, 01218, 01220, 12 (THAI35)	7	IV, V, VI
Uganda	1	0125	1	IV
United States	6	0124, 0124/5, 01210	3	II, IV
Viet Nam	21	0124/5, 12 (VIET8-13, 15, 16, 19, 22), 13 (VIET1), 14 (VIET3-7)	4	IV, V
Zaire	1	0125	1	IV
Total	340	19	33	9

^z The new genotypes identified in this study were numbered consecutively; whereas genotypes that represented an existing VCG were referred to by their VCG code. The accession numbers of isolates that represent each new genotype are given in parenthesis.

by VCG 01214. Although only a single isolate was available for analysis in this study, Koenig et al. (10) also found this genotype to be genetically distinct from other isolates of *F. oxysporum* f. sp. *ubense*. Isolates within VCG 01214 are from Malawi and are atypical in that chlamydospores are not produced (10). DFG VIII was represented by one of the new genotypes (genotype 6) that was found attacking the Manzano ('Silk', AAB) cultivar in Nayarit, Mexico, and DFG IX included the race 3 isolates of *F. oxysporum* f. sp. *ubense* (genotype 1) from a *Heliconia* species in Australia (Fig. 5).

Previous studies of diversity within *F. oxysporum* f. sp. *ubense* have divided isolates into two major groups based on volatile analysis (13,17), pectic enzyme analysis (18), electrophoretic karyotyping (4), DNA fingerprinting (1,2,3), RFLP analysis (10), and DNA sequence analysis of nuclear and mitochondrial genes (16).

TABLE 7. Clonal lineages of *Fusarium oxysporum* f. sp. *ubense* determined by DNA fingerprinting analysis

DNA fingerprint group	Genotypes ^z
I	0120, 0129, 01211, 01215
II	0122, 0126, 01210, 01219, 2, 3, 7, 8
III	0121, 01213, 01216
IV	0124, 0125, 0128, 01212, 01220, 4, 9, 10, 11, 14
V	0123, 01217, 5, 12, 13
VI	01218
VII	01214
VIII	6
IX	1

^z The new genotypes identified in this study were numbered consecutively; whereas genotypes that represented an existing VCG were referred to by their VCG code.

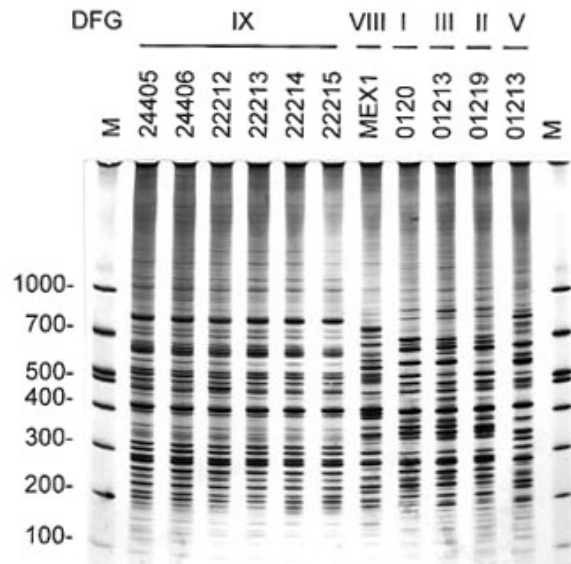


Fig. 5. Comparison of race 3 isolates of *Fusarium oxysporum* f. sp. *ubense* from *Heliconia* species to isolates from banana from Mexico and some vegetative compatibility group (VCG) representative isolates by DNA fingerprinting analysis using primer ILOE. The isolate accession numbers or VCG codes and DNA fingerprint group (DFG) numbers are indicated at the top of the figure. The sizes in base pairs of the molecular weight marker are indicated to the left of the figure.

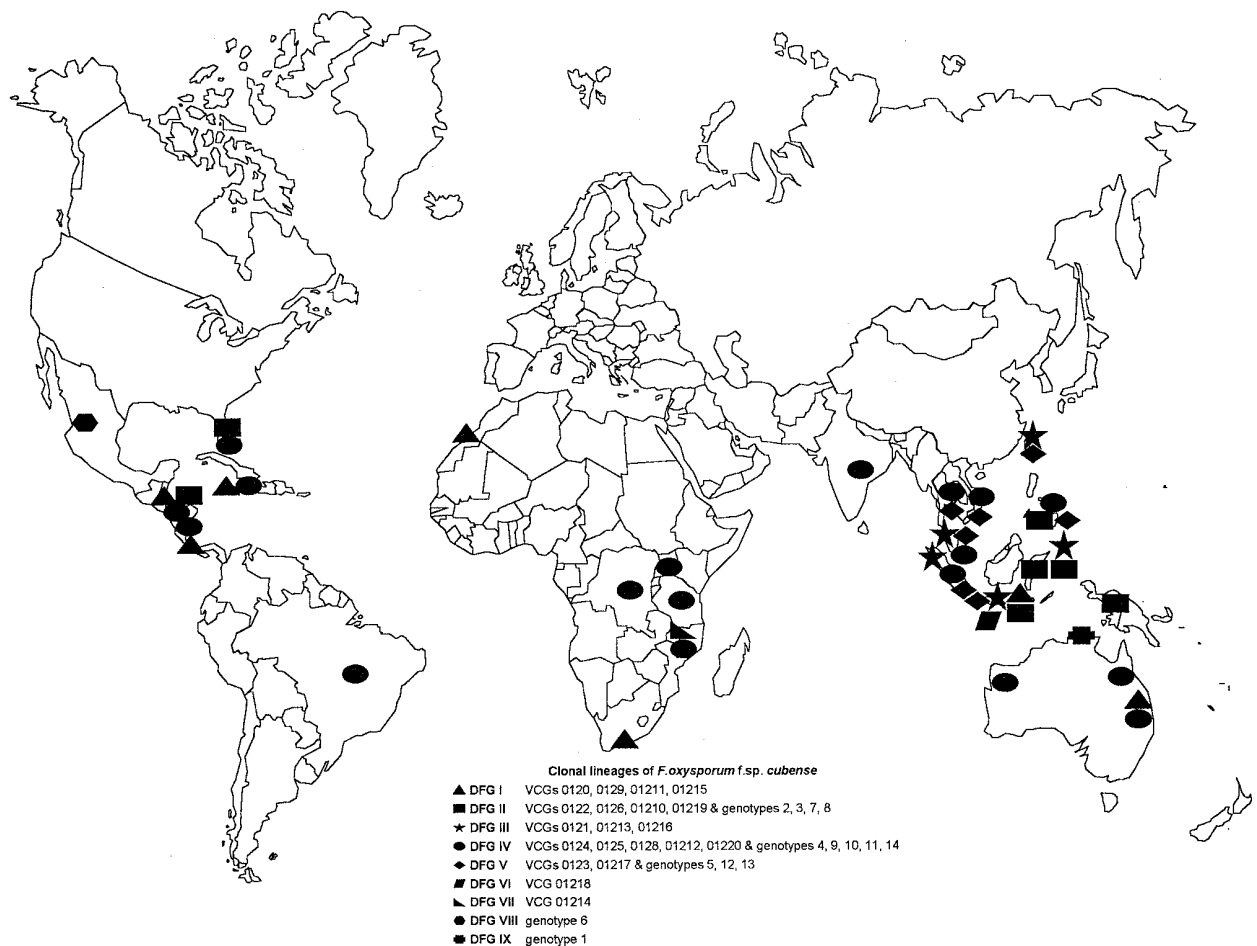


Fig. 4. Global distribution of DNA fingerprint group (DFG) lineages within *Fusarium oxysporum* f. sp. *ubense*.

In this study, we have further differentiated the two major groups within *F. oxysporum* f. sp. *cubense* into nine clonal lineages based on analysis of more isolates (a total of 341) and more molecular markers (a total of 483) than previous studies. There was broad agreement between the lineages based on DNA fingerprinting analysis and those of Koenig et al. (10) based on RFLP analysis. Koenig et al. (10) described 10 clonal lineages within *F. oxysporum* f. sp. *cubense* based on RFLP analysis using anonymous single-copy probes. Among 165 isolates, 72 haplotypes were identified, five of which accounted for nearly half the isolates examined. In this study, we have analyzed an additional five VCGs not studied by Koenig et al. (10) and 133 isolates not belonging to the currently defined VCGs. FOC I described by Koenig et al. (10) corresponded to DFG IV, except Koenig et al. (10) found that VCG 01212 was genetically distinct (FOC VIII) from VCGs 0124, 0125, and 0128, but we found that VCG 01212 was 85% genetically similar to these VCGs. FOC II (VCGs 0120, 0126, 0129, and 01215) and FOC IX (VCG 01211) (10) corresponded to DFG I (VCGs 0120, 0129, 01211, and 01215) except for VCG 0126. We grouped VCG 0126 in DFG II along with VCGs 0122, 01210, and 01219, but Koenig et al. (10) classified VCGs 0122 and 01210 as distinct clades (FOC IV and VI, respectively) based on RFLP analysis. We found that VCGs 0121, 01213, and 01216 formed a distinct cluster (genetic similarity of 88%); however, Koenig et al. (10) found that VCGs 0121 and 01213, together with three isolates from VCG 0120/01215, formed a clade (FOC III) of weak bootstrap support (53%) that could not be confidently differentiated from several other VCGs. In agreement with Koenig et al. (10), we also found VCG 01214 (FOC V and DFG VII) to represent a genetically distinct population. Koenig et al. (10) found that the seven isolates they analyzed from VCG 0123 fell into either of two clades (FOC VII and FOC X), and the multilocus haplotype of these isolates appeared to represent a combination of alleles from the two main lineages, FOC I and II. In contrast, we analyzed 23 isolates belonging in VCG 0123 and found no genetic variation in the DNA fingerprints of these isolates. Isolates in VCG 0123 were found to be closely related to isolates in VCG 01217, and several new genotypes including the isolates from the wild banana plants (*M. acuminata* subsp. *malaccensis*). Also in contrast to Koenig et al. (10), we did not find VCGs or genotypes common to more than one lineage.

Relationships between clonal lineages and races. The lineages within *F. oxysporum* f. sp. *cubense* corresponded with pathogenic race in that DFGs I to III contained all race 4 isolates, DFGs IV to VIII contained all race 1 and 2 isolates, and DFG IX was represented by race 3 isolates. A notable exception were the isolates in VCG 01220, which attacked Cavendish cultivar Williams in Carnarvon, Western Australia (18). Although these isolates were assigned to race 4 because they attacked Cavendish cultivars, they were more similar to race 1 and 2 isolates based on volatile, pectic enzyme and DNA fingerprinting analysis (18). The Cavendish plants were thought to be predisposed to infection by both waterlogging and drought stress. Race designation with this host-pathogen interaction is extremely difficult because of the inherent variability of the pathosystem as well as the influence of climate, edaphic conditions, and type of planting material used. For example, in subtropical regions such as Australia and South Africa, isolates in VCG 0120 are designated race 4, as they are capable of affecting Cavendish cultivars; however in tropical regions such as Costa Rica and Honduras, VCG 0120 isolates do not cause wilt in Cavendish cultivars and are, therefore, referred to as race 1. Consequently, isolates of the same genotype are classified as different races. In this study, isolates with virulence to Cavendish cultivars in the subtropics were restricted to DFG I, and isolates with virulence to Cavendish cultivars in the tropics to DFG III. The absence of Cavendish-virulent strains from the other lineages of *F. oxysporum* f. sp. *cubense* suggests that race 4 has not evolved from races 1 or 2, as previously suggested (20). The genetic distance among the

races suggests that races 1 and 2 have evolved together, whereas race 3 and race 4 are both of separate origin.

It is not known if the Australian isolates from *Heliconia* are a unique strain or the same strain that originated in Central America (32) that was perhaps introduced to Australia with infected planting material. No cultures of the original Central American strain were available for analysis, and more recent attempts to isolate *F. oxysporum* f. sp. *cubense* from *Heliconia* in tropical America have been unsuccessful. The original strains of race 3 were deliberately sought from *Heliconia* in Latin America as the possible origin of Fusarium wilt in banana ('Gros Michel'), after it was found that the bacterium that causes Moko disease of banana (*Ralstonia solanacearum* race 2) had originated in *Heliconia* (7). It would be interesting to reisolate *F. oxysporum* f. sp. *cubense* from *Heliconia* in the tropical American jungle for comparison with the Australian isolates. Further examination of isolates from remnant plants of 'Gros Michel', 'Silk', and 'Bluggoe' in these jungle areas is also warranted.

Origin of *F. oxysporum* f. sp. *cubense*. There are two hypotheses for the origin of *F. oxysporum* f. sp. *cubense*. The first hypothesis proposed that the pathogen coevolved with banana in Asia and has been distributed to other countries in infected banana rhizomes and attached soil (27,28,30). The second hypothesis is that the pathogen evolved independently from local populations of *F. oxysporum* in different countries to attack an introduced host plant (24). Our results indicate that while most lineages of *F. oxysporum* f. sp. *cubense* have probably coevolved with banana in Asia, several lineages have probably arisen independently. The genetic isolation and limited geographic distribution of DFGs VI, VII, VIII, and IX indicate they have probably developed independently both within (DFG VI) and outside of (DFGs VII, VIII, and IX) the center of origin of the host.

If the coevolution hypothesis is correct, it is expected that there will be greatest diversity within populations of the pathogen at the center of origin of the host (31). In this study, DFGs II, IV, and V were the most divergent. Although DFG IV contained the most genotypes, the genotypes within this lineage were closely related, as evidenced by the similarity of their DNA fingerprint patterns and the presence of cross-compatible isolates among the VCGs. In contrast, there was greatest variability within DFGs II and V based on their DNA fingerprint patterns and also the absence of cross-compatible isolates among the different VCGs and genotypes within each of these lineages. Isolates in DFGs II and V were predominantly from Indonesia, Malaysia, and the Philippines (the exception was VCG 01210, which was unique to Florida). The fact that the most variable genotypes were of Asian origin supports the hypothesis of coevolution and subsequent distribution to other countries in infected banana rhizomes or attached soil (27). Further evidence that suggests *F. oxysporum* f. sp. *cubense* has coevolved with banana in Asia is the genetic similarity (95%) between the Malaysian isolates (genotype 5) from the wilted wild banana plants (*M. acuminata* subsp. *malaccensis*) and isolates in DFG V. Besides these three affected plants of this species, no disease was observed (based on external symptoms) in the many other wild banana plants surveyed by some of the authors in the jungles of Indonesia, Malaysia, Thailand, and Viet Nam. This suggests either that the pathogen was not present or, more likely, that *F. oxysporum* f. sp. *cubense* was present but was not capable of causing disease in the wild banana hosts. Gordon and Martyn (9) described *F. oxysporum* as nonpathogenic in native situations. If pathogenesis does develop in a native plant community, it is expected to be short-lived, because the fungus faces renewed competition from other microorganisms and the search for a new host is restricted spatially (9). More information on the interaction between *F. oxysporum* f. sp. *cubense* and wild banana hosts would be obtained by analyzing more isolates from the wild pathosystem including isolates from the roots and rhizome of symptomless wild banana plants. The coevolution hypothesis has important implications in the selection

of banana cultivars with resistance to *Fusarium* wilt, as resistant cultivars are most likely to be present in regions where there is greatest diversity within the host and the pathogen (31). It is apparent that further collection and genetic analysis of *F. oxysporum* f. sp. *ubense* strains from Asia, the center of origin and domestication of *Musa*, is necessary.

In summary, we have identified 33 different genotypes within *F. oxysporum* f. sp. *ubense*. Knowledge of the host range and geographic distribution of these genotypes may facilitate the selection of resistant cultivars for particular locations, depending on the endemic strains present. The known geographic distribution of these genotypes may also be useful in establishing quarantine zones to limit the spread, both nationally and internationally, of the more virulent strains of the pathogen.

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