Genetic Diversity of *Fusarium oxysporum* Isolates from Cucumber: Differentiation by Pathogenicity, Vegetative Compatibility, and RAPD Fingerprinting

D. J. Vakalounakis and G. A. Fragkiadakis

National Agricultural Research Foundation (N.AG.RE.F.), Plant Protection Institute, P.O. Box 1802, 711 10 Heraklio, Crete, Greece.
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ABSTRACT

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A total of 106 isolates of *Fusarium oxysporum* obtained from diseased cucumber plants showing typical root and stem rot or Fusarium wilt symptoms were characterized by pathogenicity, vegetative compatibility, and random amplified polymorphic DNA (RAPD). Twelve isolates of other formae speciales and races of *F. oxysporum* from cucurbit hosts, three avirulent isolates of *F. oxysporum*, and four isolates of *Fusarium* spp. obtained from cucumber were included for comparison. Of the 106 isolates of *F. oxysporum* from cucumber, 68 were identified by pathogenicity as *F. oxysporum* f. sp. *radicis-cucumerinum*, 32 as *F. oxysporum* f. sp. *cucumerinum*, and 6 were avirulent on cucumber. Isolates of *F. oxysporum* f. sp. *radicis-cucumerinum* were vegetatively incompatible with *F. oxysporum* f. sp. *cucumerinum* and the other *Fusarium* isolates tested. A total of 60 isolates of *F. oxysporum* f. sp. *radicis-cucumerinum* was assigned to vegetative compatibility group (VCG) 0260 and 5 to VCG 0261, while 3 were vegetatively compatible with isolates in both VCGs 0260 and 0261 (bridging isolates). All 68 isolates of *F. oxysporum* f. sp. *radicis-cucumerinum* belonged to a single RAPD group. A total of 32 isolates of *F. oxysporum* f. sp. *cucumerinum* was assigned to eight different VCGs and two different RAPD groups, while 2 isolates were vegetatively self-incompatible. Pathogenicity, vegetative compatibility, and RAPD were effective in distinguishing isolates of *F. oxysporum* f. sp. *radicis-cucumerinum* from those of *F. oxysporum* f. sp. *cucumerinum*. Parsimony and bootstrap analysis of the RAPD data placed each of the two formae speciales into a different phylogenetic branch.

Cucumber (*Cucumis sativus* L.) is a crop of high economic importance in many countries (33). Fusarium wilt of cucumber, caused by *Fusarium oxysporum* Schlechtend.:Fr. f. sp. *cucumerinum* J.H. Owen, is a serious vascular disease worldwide (1,33,34,36). Based on disease reactions of four differential cultivars, Armstrong et al. (5) proposed three races of the pathogen, to be called races 1, 2, and 3, that occur mainly in the United States, Israel, and Japan, respectively (12,26). Root and stem rot of cucumber, incited by *F. oxysporum* f. sp. *radicis-cucumerinum* D.J. Vakalounakis, was recently described in Crete as causing severe losses in greenhouse crops (37). No races of *F. oxysporum* f. sp. *radicis-cucumerinum* have been identified (37). The genetic relatedness between these formae speciales has not yet been determined.

F. oxysporum f. sp. *radicis-cucumerinum* is distinct from *F. oxysporum* f. sp. *cucumerinum* and other *F. oxysporum* formae speciales in symptomatology, epidemiology, and cultivar susceptibility and can be differentiated by pathogenicity tests with appropriate hosts (37). However, these tests are protracted and occasionally inconclusive (42). The utilization of other laboratory methods can contribute in differentiating *F. oxysporum* isolates and can also provide information on the genetic composition of the pathogen populations (23). The vegetative, or heterokaryon, compatibility grouping is a genetically based method that can be used for this purpose (21,30). As described by Puhalla (28), nitrate nonutilizing (*nit*) mutants can be used to indirectly assess vegetative compatibility among isolates of *F. oxysporum.* These mutants can be subdivided into three phenotypes (*nit1, nit3,* and NitM) that can be differentiated by their ability to utilize various nitrogen sources (8). If *nit* mutants of two isolates anastomose to form a wild-type heterokaryon when paired on nitrate minimal medium, they are assigned to the same vegetative compatibility group (VCG). The isolates of a given VCG typically possess very similar or identical multilocus haplotypes; therefore, VCGs can be good predictors of genetic relatedness (9,18). Isolates within a given VCG usually (11) belong to the same forma specialis; however, a forma specialis may contain one or more VCGs (7,11,13,29). The method of random amplified polymorphic DNA (RAPD), which is based on polymerase chain reaction (PCR), has been used for population and phylogenetic studies in *F. oxysporum* (17,22,23,40). Fungal isolates displaying the same electrophoretic patterns of amplified DNA, when tested with several primers, have high genetic similarity (6,41).

In this study, we used pathogenicity, VCGs, and RAPD-PCR to classify 106 isolates of *F. oxysporum* obtained from diseased cucumber plants showing typical root and stem rot or Fusarium wilt symptoms, 12 isolates of other formae speciales and races of *F. oxysporum* from cucurbit hosts, 3 avirulent isolates of *F. oxysporum*, and 4 isolates of *Fusarium* spp. obtained from cucumber. Our objectives were to (i) identify by pathogenicity the 106 isolates of *F. oxysporum* from cucumber at the forma specialis level, (ii) examine the genetic relatedness among the *F. oxysporum* isolates by VCG and RAPD analysis, and (iii) assess the agreement between all three methods in differentiating these isolates.

MATERIALS AND METHODS

Fungal isolates. A total of 75 isolates of *F. oxysporum*, each from a different greenhouse, was recovered from cucumber plants showing typical root and stem rot or Fusarium wilt symptoms at 21 locations in Crete (74 isolates) and 1 location at Preveza, Greece, during 1990 to 1997 (Table 1). Isolates were identified as *F. oxysporum* by D. Brayford (International Mycological Institute, Egham, United Kingdom), K. Seifert (National Identification Service, Ot-

Corresponding author: D. J. Vakalounakis
E-mail address: vakalounakis@nefeli.imbb.forth.gr

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TABLE 1. Isolates of *Fusarium oxysporum* from cucumber

Isolate	Symptom picture[a]	Origin (source)[b]	Year	Disease index[c] 17°C	Disease index[c] 29°C	RAPD group[d]
F. oxysporum f. sp. *radicis-cucumerinum* VCG 0260[e] (60 isolates)						
AF1-b	RSR	Vainia, Crete (A)	1990	3.0 (100)	0.5 (37)	I
AZ1-e	RSR	Stomio, Crete (A)	1990	3.0 (100)	0.3 (25)	I
AFu-9	RSR	Stomio, Crete (A)	1992	3.0 (100)	0.3 (17)	I
AFu-11	RSR	Stomio, Crete (A)	1992	3.0 (100)	1.5 (55)	I
AFu-35	RSR	Stomio, Crete (A)	1993	3.0 (100)	1.4 (58)	I
AFu-42	RSR	Stomio, Crete (A)	1993	3.0 (100)	0.0 (0)	I
AF-2	RSR	Xerokampos, Crete (A)	1991	3.0 (100)	2.8 (92)	I
AFu-8	RSR	Xerokampos, Crete (A)	1992	2.5 (83)	1.1 (42)	I
AFu-10	RSR	Xerokampos, Crete (A)	1992	3.0 (100)	0.0 (0)	I
AFu-14	RSR	Xerokampos, Crete (A)	1992	3.0 (100)	2.0 (67)	I
AFu-33	RSR	Xerokampos, Crete (A)	1993	3.0 (100)	1.9 (82)	I
AFu-37	RSR	Xerokampos, Crete (A)	1993	1.1 (42)	0.4 (17)	I
AFu-38	RSR	Xerokampos, Crete (A)	1993	1.7 (67)	0.0 (0)	I
AF-4	RSR	Kalamaki, Crete (A)	1991	3.0 (100)	2.3 (100)	I
AFu-1	RSR	Kalamaki, Crete (A)	1992	3.0 (100)	3.0 (100)	I
AFu-2	RSR	Kalamaki, Crete (A)	1992	3.0 (100)	1.0 (50)	I
AFu-44	RSR	Kalamaki, Crete (A)	1993	2.7 (100)	0.0 (0)	I
AFu-3	RSR	Ierapetra, Crete (A)	1992	3.0 (100)	0.0 (0)	I
AFu-6	RSR	Ierapetra, Crete (A)	1992	3.0 (100)	0.3 (8)	I
AFu-7[f]	RSR	Ierapetra, Crete (A)	1992	3.0 (100)	1.6 (58)	I
AFu-24	RSR	Ierapetra, Crete (A)	1992	2.7 (100)	2.2 (100)	I
AFu-27	RSR	Ierapetra, Crete (A)	1992	3.0 (100)	1.3 (42)	I
AFu-29	RSR	Ierapetra, Crete (A)	1993	3.0 (100)	0.0 (0)	I
AFu-34	RSR	Ierapetra, Crete (A)	1993	2.8 (100)	1.2 (55)	I
AFu-40	RSR	Ierapetra, Crete (A)	1993	1.0 (33)	0.3 (8)	I
AFu-41	RSR	Ierapetra, Crete (A)	1993	3.0 (100)	0.4 (25)	I
AFu-66	RSR	Ierapetra, Crete (A)	1997	3.0 (100)	1.7 (58)	I
AFu-12	RSR	Anatoli, Crete (A)	1992	1.1 (42)	0.2 (18)	I
AFu-13	RSR	Anatoli, Crete (A)	1992	3.0 (100)	0.0 (0)	I
AFu-36	RSR	Anatoli, Crete (A)	1993	2.8 (100)	0.3 (11)	I
AFu-15	RSR	Ferma, Crete (A)	1992	2.0 (100)	1.2 (50)	I
AFu-18	RSR	Ferma, Crete (A)	1992	2.8 (100)	0.5 (27)	I
AFu-78	RSR	Ferma, Crete (A)	1997	3.0 (100)	1.8 (80)	I
AFu-16	RSR	Achlia, Crete (A)	1992	3.0 (100)	1.3 (44)	I
AFu-19	RSR	Achlia, Crete (A)	1992	1.0 (33)	0.0 (0)	I
AFu-17	RSR	Koutsouras, Crete (A)	1992	3.0 (100)	0.3 (25)	I
AFu-48	RSR	Koutsouras, Crete (A)	1993	3.0 (100)	0.3 (8)	I
AFu-60	RSR	Koutsouras, Crete (A)	1996	3.0 (100)	1.7 (63)	I
AFu-72	RSR	Koutsouras, Crete (A)	1997	3.0 (100)	0.9 (58)	I
AFu-73	RSR	Koutsouras, Crete (A)	1997	2.3 (100)	2.3 (78)	I
AFu-75	RSR	Koutsouras, Crete (A)	1997	2.6 (92)	2.6 (92)	I
AFu-20	RSR	Kalo Nero, Crete(A)	1992	2.8 (100)	1.3 (50)	I
AFu-28	RSR	Goudouras, Crete (A)	1993	2.8 (100)	2.7(100)	I
AFu-74	RSR	Goudouras, Crete (A)	1997	3.0 (100)	2.3 (83)	I
AFu-31	RSR	Gra Ligia, Crete (A)	1993	3.0 (100)	0.0 (0)	I
AFu-32	RSR	Gra Ligia, Crete (A)	1993	3.0 (100)	0.0 (0)	I
AFu-68	RSR	Gra Ligia, Crete (A)	1997	3.0 (100)	0.4 (17)	I
AFu-58	RSR	Tympaki, Crete (A)	1996	3.0 (100)	2.8 (92)	I

(*continued on the next page*)

[a] RSR = root and stem rot symptoms and FW = Fusarium wilt symptoms on diseased plant from which the isolate was recovered.

[b] A = Authors, B = M. Gerlagh and M. Grijger, C = R. D. Martyn, D = American Type Culture Collection (ATCC), E = P. H. Williams, F = D. Netzer, G = T. Katan, H = T.C. Paulitz, I = H. Komada, J = H. Shiomi, and K = W. R. Jarvis.

[c] Disease index was the mean disease severity value on artificially inoculated seedlings, 'Knossos'. Disease severity for root and stem rot was assessed with a 0 to 3 visual scale, in which 0 = no symptoms; 1 = light yellowing of cotyledons, light or moderate rot on taproot and secondary roots, and crown rot; 2 = moderate or severe yellowing of cotyledons with or without wilting, stunting, severe rot on taproot and secondary roots, crown rot with or without hypocotyl rot, and vascular discoloration in the stem; and 3 = dead seedlings. Disease severity for Fusarium wilt was assessed with a different 0 to 3 visual scale, in which 0 = no symptoms; 1 = light vascular discoloration in the stem with or without stunting; 2 = vascular discoloration in the stem, stunting, wilting with or without yellowing of cotyledons; and 3 = dead seedlings. Numbers in parentheses indicate percentage of diseased seedlings having a score ≥1. Pathogenicity tests were carried out at least twice for each isolate.

[d] As determined by random amplified polymorphic DNA polymerase chain reaction using 10 arbitrary primers.

[e] Vegetative compatibility groups (VCGs) as determined in this study and numbered according to the adopted by Puhalla (28) and further standardized by Kistler et al. (18) VCG numbering system, in which 026# = VCG code for *F. oxysporum* f. sp. *radicis-cucumerinum* as suggested in this study; 018# = VCG code for *F. oxysporum* f. sp. *cucumerinum* as suggested by Katan (13); 018– = an artificial group containing single-member VCGs of *F. oxysporum* f. sp. *cucumerinum*; 018-HSI = an artificial group containing heterokaryon self-incompatible isolates that did not form heterokaryons upon pairing with themselves or with any of the other isolates. Six arbitrary VCGs (1-A to 1-F), not following the above VCG numbering system, have been reported for a number of *F. oxysporum* f. sp. *cucumerinum* isolates from Korea (1) not included in this study.

[f] Isolates included in previous publications: AFu-4 (37), AFu-7 (37), AF1-a (37), ATCC 16416 (4,5,16,37), ATCC 36330 (5,26,35,36,38), ATCC 36332 (5,37), Cu:1-1 (25), Cu:2-1 (25), NETH 10782 (16), NETH 11179 (16), NETH 20286 (16), PD 85/453B (10), PD 85/463-3 (10), PHW 231 (34,35,36,38), PSU 1086 (16), PSU 1098 (16), PSU 1265 (16), and PSU 1266 (16).

TABLE 1. (*continued from the preceding page*)

Isolate	Symptom picture[a]	Origin (source)[b]	Year	Disease index[c] 17°C	Disease index[c] 29°C	RAPD group[d]
AFu-61	RSR	Tympaki, Crete (A)	1996	2.7 (92)	1.5 (58)	I
AFu-59	RSR	Antiskari, Crete (A)	1996	2.3 (75)	0.8 (25)	I
AFu-62	RSR	K. Pirgos, Crete (A)	1996	3.0 (100)	1.4 (64)	I
AFu-67	RSR	Arvi, Crete (A)	1997	3.0 (100)	1.3 (58)	I
AFu-69	RSR	Arvi, Crete (A)	1997	3.0 (100)	0.9 (42)	I
AFu-70	RSR	Arvi, Crete (A)	1997	2.9 (100)	1.6 (58)	I
AFu-71	RSR	Arvi, Crete (A)	1997	2.8 (100)	2.7 (92)	I
AFu-76	RSR	Makrigialos, Crete (A)	1997	2.4 (100)	0.4 (43)	I
AFu-77	RSR	Makrigialos, Crete (A)	1997	3.0 (100)	1.3 (58)	I
PD 85/453B[f]	Unknown	Grenp, Netherlands (B)	1985	2.6 (100)	2.2 (80)	I
NETH 30196	Unknown	Netherlands (B)	1986	2.8 (100)	1.0 (50)	I
NETH 20286[f]	Unknown	Barendrecht, Netherlands (C)	Unknown	3.0 (100)	1.5 (50)	I
VCG 0261						
AK-1	RSR	Ferma, Crete (A)	1990	2.5 (83)	1.5 (67)	I
AK-2	RSR	Ferma, Crete (A)	1990	3.0 (100)	2.0 (83)	I
AF-5	RSR	Anatoli, Crete (A)	1991	1.8 (58)	0.8 (25)	I
AFu-4[f]	RSR	Keratokampos, Crete (A)	1992	3.0 (100)	2.0 (83)	I
AFu-26	RSR	Keratokampos, Crete (A)	1992	2.3 (100)	1.5 (50)	I
VCG 0260/261 (bridging group)						
AF1-a[f]	RSR	Vainia, Crete (A)	1990	3.0 (100)	2.0 (67)	I
AZ1-d	RSR	Stomio, Crete (A)	1990	3.0 (100)	0.3 (8)	I
PD 86/463-3[f]	Unknown	Netherlands (B)	1986	2.8 (100)	0.5 (50)	I
Fisher's LSD (P = 0.05)				0.49	0.87	
F. oxysporum f. sp. *cucumerinum*						
VCG 0180 (18 isolates)						
AFu-21	FW	Heraklio, Crete (A)	1992	0.4 (25)	2.8 (100)	II
AFu-50	FW	Heraklio, Crete (A)	1994	1.0 (75)	3.0 (100)	II
AFu-57	FW	Heraklio, Crete (A)	1995	0.3 (8)	2.1 (100)	II
AFu-79	FW	Heraklio, Crete (A)	1997	0.3 (33)	3.0 (100)	II
AFu-46	FW	Kavrochori, Crete (A)	1993	0.8 (58)	2.8 (100)	II
AFu-49	FW	Kavrochori, Crete (A)	1994	0.3 (8)	2.8 (100)	II
AFu-51	FW	Kavrochori, Crete (A)	1994	0.8 (20)	2.7 (100)	II
AFu-52	FW	Kavrochori, Crete (A)	1994	1.0 (3)	3.0 (100)	II
AFu-53	FW	Gazi, Crete (A)	1994	0.0 (0)	1.8 (83)	II
ATCC 16416[f]	FW	Webster, Florida (D)	1956	0.0 (0)	2.8 (100)	II
PSU 1086[f]	FW	Florida (C)	1981	0.7 (25)	3.0 (100)	II
PSU 1098[f]	FW	Florida (C)	1981	1.0 (33)	2.9 (100)	II
PHW 231[f]	FW	Florida (E)	1965	1.8 (83)	3.0 (100)	II
ATCC 36330[f]	FW	Samaria, Israel (D)	1970	0.5 (17)	2.8 (100)	II
FOC-N	FW	Israel (F)	Unknown	1.1 (42)	3.0 (100)	II
FOCU-56D	FW	Israel (G)	Unknown	0.8 (25)	3.0 (100)	II
TCP-1	Unknown	Canada (H)	Unknown	0.4 (12)	2.3 (75)	II
TCP-2	Unknown	Canada (H)	Unknown	1.5 (50)	3.0 (100)	II
VCG 0181						
NETH 10782[f]	Unknown	Netherlands (C)	Unknown	0.3 (8)	2.1 (73)	III
NETH 11179[f]	Unknown	Nootdorp, Netherlands (C)	1979	2.3 (83)	3.0 (100)	III
Cu:4-1	FW	Japan (I)	Unknown	0.0 (0)	2.8 (100)	III
Cu:7-0	FW	Japan (I)	Unknown	0.2 (8)	2.7 (100)	III
VCG 0182						
ATCC 36332[f]	FW	Japan (D)	1973	1.2 (42)	1.4 (75)	III
F. oxy. 90-01	FW	Japan (I)	Unknown	0.0 (0)	2.8 (100)	III
VCG 0183						
FOC-1	FW	Japan (I)	Unknown	0.6 (42)	2.8 (100)	III
Cu:5-0	FW	Japan (I)	Unknown	2.8 (100)	2.6 (100)	III
VCG 018-						
Cu:6-0	FW	Japan (I)	Unknown	1.8 (83)	2.6 (100)	III
Tf-213	FW	Japan (J)	1985	1.5 (50)	2.8 (100)	III
Tf-304	FW	Japan (J)	1985	1.2 (58)	2.8 (100)	III
Tf-579	FW	Japan (J)	1996	1.5 (50)	2.8 (100)	III
018-HSI						
Cu:1-1[f]	FW	Fukuoka, Japan (I)	Unknown	1.7 (70)	2.1 (82)	III
Cu:2-1[f]	FW	Japan (I)	Unknown	2.0 (67)	3.0 (100)	III
Fisher's LSD (P = 0.05)				0.87	0.51	
Avirulent isolates of *F. oxysporum* from cucumber						
AFu-39	RSR	Gra Ligia, Crete (A)	1993	0.0 (0)	0.0 (0)	I
AFu-65	RSR	Preveza, Epirus (A)	1993	0.0 (0)	0.0 (0)	V
PSU 1265[f]	FW	Alberta, Canada (C)	Unknown	0.0 (0)	0.0 (0)	IV
PSU 1266[f]	FW	Canada (C)	Unknown	0.0 (0)	0.0 (0)	IV
cc#28	RSR	Leamington, Canada (K)	Unknown	0.0 (0)	0.0 (0)	VI
NETH 652	Unknown	Barendrecht, Netherlands (K)	1979	0.0 (0)	0.0 (0)	VII

tawa, Canada), J. Juba (Fusarium Research Center, Pennsylvania State University, University Park), and D. J. Vakalounakis. In all, 31 isolates of *F. oxysporum* from cucumber, 12 isolates of other formae speciales and races of *F. oxysporum* from cucurbit hosts, 3 avirulent isolates of *F. oxysporum*, 1 isolate of *F. solani* f. sp. *cucurbitae*, and 3 isolates of *Fusarium* spp. obtained from cucumber were provided by foreign colleagues or culture collections (Tables 1 and 2). The three isolates of *Fusarium* spp. collected from cucumber stem (cc#23 and cc#49) and roots (cc#274) in Canada (Table 2) were identified as *F. proliferatum*, *F. solani*, and *F. equiseti*, respectively, by J. Juba. Isolate PT-1C from Crete, recovered from melon (Table 2), was identified as *F. oxysporum* by K. Seifert. A single microconidial culture was prepared from each isolate. Potato dextrose agar (PDA) and broth (PDB) were used during fungal isolation and inoculum preparation for pathogenicity tests, respectively (37).

Pathogenicity tests. The pathogenicity of each isolate was tested on cucumber seedlings at the one-true-leaf stage. Their roots were dipped into a conidial suspension (10^7 microconidia per ml) of the test isolate for 30 min, after which seedlings were transplanted into sterilized organic substrate Belplanto (Klasmann-Deilmann GmbH, Geeste, Germany) in plastic pots (8.5 cm in diameter) and kept in growth chambers with a 12-h photoperiod under mixed fluorescent and incandescent lamps. The photosynthetic photon-flux density (PPFD) at plant canopy was 150 μmol s^{-1} m^{-2}. Seedlings treated similarly and dipped in tap water served as a control. Plants were watered daily, and no fertilizers were applied.

Two different kinds of pathogenicity tests were carried out. In the first, two different groups of seedlings of the local cultivar Knossos (susceptible to both *F. oxysporum* f. sp. *radicis-cucumerinum* and *F. oxysporum* f. sp. *cucumerinum*) (37) were inoculated with each isolate. One group was incubated at 17°C and the other at 29°C, with 12 replicate seedlings per temperature and isolate. These temperatures can distinguish the two formae speciales; root and stem rot develops better at 17°C, whereas Fusarium wilt develops better at 29°C (37). In the second test, seedlings of the cultivars SMR-18 (resistant to *F. oxysporum* f. sp. *cucumerinum* and susceptible to *F. oxysporum* f. sp. *radicis-cucumerinum*) (35,37) and Knossos were inoculated with each isolate and then incubated at 22°C with 12 replicate seedlings per cultivar and isolate. At 22°C, both pathogens induce symptoms on cucumber (37). Disease assessment was made 15 and 20 days after inoculation in the first and the second procedure, respectively. For each isolate, both tests were conducted at least twice. The symptoms observed were classified into two categories. In the first category, the symptoms resembled those caused by *F. oxysporum* f. sp. *radicis-cucumerinum* (root and hypocotyl rot, yellowing, and wilting with or without vascular discoloration in the stem), while in the second, they resembled those caused by *F. oxysporum* f. sp. *cucumerinum* (yellowing, wilting, and vascular discoloration in the stem without any root or stem rot) (37).

Vegetative compatibility tests. *nit* mutants were generated on minimal medium (MM) amended with 1.5% potassium chlorate (MMC) (28), characterized as *nit1*, *nit3*, or NitM (8), and complemented on MM at 27°C in the dark (28). Heterokaryon (complementation) was evident after 5 to 20 days by the formation of a wild-type mycelium at the contact zone between two *nit* mutants (28). Absence of wild-type growth between *nit* mutants derived from the same parent isolate indicated allelic, overlapping, or otherwise noncomplementary mutations or vegetative self-incompatibility (15), while absence of wild-type growth between *nit* mutants from different parent isolates indicated either noncomplementarity or inability to form heterokaryons due to lack of vegetative compatibility (15).

A total of 12 to 15 mutants were generated from each of seven isolates of *F. oxysporum* f. sp. *radicis-cucumerinum* (AFu-3, AFu-4, AFu-7, AFu-14, AFu-15, AFu-20, and AFu-24) and four isolates of *F. oxysporum* f. sp. *cucumerinum* (ATCC 16416, ATCC 36330, ATCC 36332, and PHW 231). The *nit* mutants of each isolate were paired and two complementary mutants, which formed strong heterokaryons, were used to determine compatibility with other isolates. When *nit* mutants from distinct parent isolates were complementary, these isolates were placed in the same VCG. Two complementary *nit* mutants from each VCG that formed robust heterokaryons with many mutants in the same VCG were selected as VCG testers. The complementary testers FOCU-56L/12 and FOCU-57N/18 of VCG 0180 of *F. oxysporum* f. sp. *cucumerinum* were donated by T. Katan (Volcani Center, Israel).

Three *nit* mutants from each of the remaining isolates were paired with the VCG testers of both pathogens. If complementation was established, the isolate was assigned to a certain VCG. If not, four additional *nit* mutants were tested. If the reactions were again negative, the phenotypes of the seven mutants were identified and the mutants were paired among themselves. If complementation was established and at least one of the complementary mutants was NitM (20), the isolate was considered as belonging to a new VCG. If the 7 mutants did not form a heterokaryon among them-

TABLE 2. Isolates of *Fusarium* spp. from cucurbits and soil

Isolate[a]	Classification	Origin (source)[b]	RAPD group[c]
Nd.pl.2	*F. oxysporum* f. sp. *benincasae*	Netherlands (D)	VIII
PT-1	*F. oxysporum* f. sp. *melonis*	Ierapetra, Crete (A)	ND[d]
FOM-T-0	*F. oxysporum* f. sp. *melonis* race 0	Almeria, Spain (B)	IX
FOM-T-1	*F. oxysporum* f. sp. *melonis* race 1	Murcia, Spain (B)	IX
FOM-T-2	*F. oxysporum* f. sp. *melonis* race 2	Almeria, Spain (B)	X
FON-T-0	*F. oxysporum* f. sp. *niveum* race 0	Valencia, Spain (B)	IX
FON-T-2	*F. oxysporum* f. sp. *niveum* race 2	Almeria, Spain (B)	ND
Tf-209	*F. oxysporum* f. sp. *niveum*	Japan (C)	XI
SUF-124-1	*F. oxysporum* f. sp. *luffae*	Japan (E)	XII
SUF-363-2	*F. oxysporum* f. sp. *luffae*	Japan (E)	ND
FOM0-1	*F. oxysporum* f. sp. *momordicae*	Taiwan (F)	XIII
FOM0-2	*F. oxysporum* f. sp. *momordicae*	Taiwan (F)	ND
92M1	*F. oxysporum* (from melon)	France (G)	XIV
Fo47[e]	*F. oxysporum* (from soil cultivated with melon)	Chateaurenard, France (G)	XV
91440	*F. oxysporum* (from noncultivated soil)	France (G)	XVI
SH-2	*F. solani* f. sp. *cucurbitae* race 1	Italy (H)	XVII
cc#23	*F. proliferatum* (from cucumber)	Leamington, Canada (I)	XVIII
cc#49	*F. solani* (from cucumber)	Canada (I)	XIX
cc#274	*F. equiseti* (from cucumber)	Canada (I)	XX

[a] These isolates were avirulent to cucumber, except isolate SH-2, which was virulent.
[b] A = authors, B = R. Gonzalez-Torres, C = H. Shiomi, D = M. Gerlagh and M. Grijger, E = H. Nagao, F = S. K. Sun, G = C. Steinberg, H = V. M. Stravato, and I = W. R. Jarvis.
[c] As determined by random amplified polymorphic DNA polymerase chain reaction using 10 arbitrary primers.
[d] Not determined.
[e] Isolate included in previous publications (19) and used commercially as a biological control agent under the trade name Fusaclean.

selves, 13 *nit* mutants of the same isolate along with the first 7 were paired. If, again, no heterokaryon formed and the 20 mutants included at least 1 from each phenotypic class (*nit1*, *nit3*, and NitM), the isolate was considered heterokaryon self-incompatible (HSI).

DNA preparation and RAPD analysis. Total DNA was extracted at least twice from each isolate using different mycelial batches (43). For RAPD analysis, the primers OPA-01, OPB-01 to OPB-20, and OPF-05 (Operon Technologies, Inc., Alameda, CA) were used. Each amplification reaction was set at 25 µl. DNA (~25 ng) was put into 0.5-ml Eppendorf tubes and primer (50 ng), reaction buffer (final concentration: 10 mM Tris-HCl, pH 8.8; 50 mM KCl; 2.5 mM $MgCl_2$; and 0.1% Triton X-100), 2′-deoxynucleoside 5′-triphosphate (0.2 mM final concentration for each nucleotide; Pharmacia Biotech AB, Uppsala, Sweden), and *Taq* DNA polymerase (1.2 units; Promega Corporation, Madison, WI) were added. Amplification conditions in a thermocycler (Perkin-Elmer Corporation, Norwalk, CT) were 5 min at 94°C, followed by 45 cycles of 2 min at 94°C, 1 min at 34°C, and 2 min at 72°C, with a final application of 72°C for 15 min. The isolates that could not be distinguished from each other by the electrophoretic patterns (2% agarose) of the amplified DNA (31) were classified into the same RAPD group. RAPD reproducibility was confirmed by repeating the reactions at least twice and including control isolates.

Data analysis. All pathogenicity tests were conducted in a completely randomized design. Disease index data for each experiment were analyzed using the Fisher's least significant difference test ($P = 0.05$) (although there is no implied linearity in the disease scales used) (32,39). Replicate experiments gave similar results. To record RAPD patterns, a binary data matrix was prepared for each isolate and primer, on which each intense RAPD band was scored as 1 when present or 0 when absent. Bands of the same size, generated from *F. oxysporum* isolates by a single primer, were considered identical. The binary data sets were pooled for each isolate separately and used to calculate the genetic distances with the following formula based on Jaccard's coefficient (2,3): $D = 1 - (B/M)$, in which D is the genetic distance between two *F. oxysporum* isolates, B is the common RAPD bands, and M is the total RAPD bands in these patterns. Dendrograms were constructed using the software package PHYLIP 3.5 (J. Felsenstein, Washington University, Seattle). For parsimony analysis, the binary data were analyzed by the programs MIX (Wagner parsimony method with 100 different input orders of the data) and CONSENSE (strict consensus tree method). A data set containing only the value "0" was used as an outgroup (25). The genetic distance data were subjected to cluster analysis by the programs NEIGHBOR (unweighted pair-group method with arithmetic averages, random input order) and CONSENSE. To determine the statistical significance of the dendrogram branches, the data were bootstrapped with 1,000 replications using the program SEQBOOT.

RESULTS

Pathogenicity tests. A total of 64 of the 66 isolates of *F. oxysporum* from Greece, obtained over an 8-year period (1990 to 1997) from greenhouse cucumbers with root and stem rot symptoms, as well as 4 isolates from the Netherlands (PD 85/453B, PD 86/466-3, NETH 20286, and NETH 30196), was virulent on both cucumber 'Knossos' and 'SMR-18' (Table 1), causing symptoms resembling those incited by *F. oxysporum* f. sp. *radicis-cucumerinum* (37). The aggressiveness of these isolates was generally high (Table 1). For most isolates (62 out of 68), the disease index and number of seedlings affected was higher at 17 than at 29°C (Table 1). Based on disease symptoms under natural infection and artificial inoculation conditions, the differential effect of temperature on disease development, and the virulence on 'SMR-18' (37), the 68 isolates were identified as *F. oxysporum* f. sp. *radicis-cucumerinum*.

Nine isolates of *F. oxysporum* from Greece obtained over a 6-year period (1992 to 1997) from greenhouse cucumbers with Fusarium wilt symptoms, as well as 25 foreign isolates of *F. oxysporum* f. sp. *cucumerinum*, were avirulent on 'SMR-18' but virulent on 'Knossos' (Table 1), causing symptoms resembling those incited by *F. oxysporum* f. sp. *cucumerinum* (37). Aggressiveness of the isolates was variable but generally high (Table 1). For most isolates (30 out of 32), both the disease index and number of seedlings affected were higher at 29 than at 17°C (Table 1). Based on disease symptoms under natural infection and artificial inoculation conditions, the differential effect of temperature on disease development and the avirulence on 'SMR-18' (37), the 28 isolates were identified as *F. oxysporum* f. sp. *cucumerinum*.

The isolates AFu-39, AFu-65, PSU 1265, PSU 1266, Fo47, 91440, and 92M1 (Tables 1 and 2) of *F. oxysporum*, cc#23 of *F. proliferatum*, cc#49 of *F. solani*, and cc#274 of *F. equiseti*, as well as the other formae speciales of *F. oxysporum* tested (Table 2), were avirulent on either cucumber 'Knossos' or 'SMR-18'. *F. solani* f. sp. *cucurbitae* was virulent on both 'Knossos' and 'SMR-18'.

VCGs of *F. oxysporum* f. sp. *radicis-cucumerinum*. Among the 64 isolates of *F. oxysporum* f. sp. *radicis-cucumerinum* from Greece, one predominant VCG containing 57 isolates and a second VCG containing 5 isolates were detected (Table 1). In all cases, the *nit* mutants of the tested isolates and the tester strains formed strong heterokaryons. According to the numbering system adopted by Puhalla (28) and further standardized by Kistler et al. (18), it is suggested that these VCGs be designated 0260 and 0261. The complementary *nit* mutants, chosen as testers of VCG 0260, were AFu-14A/4 (NitM, parental isolate AFu-14) and AFu-15B/2 (NitM, parental isolate AFu-15); testers of VCG 0261 were AFu-4C/8 (NitM, parental isolate AFu-4) and AFu-4C/9 (*nit1*, parental isolate AFu-4). All three *nit* mutants generated from the remaining two isolates (AF1-a and AZ1-d from Greece) were observed to form strong heterokaryons only with the tester AFu-15B/2 of VCG 0260 and the tester AFu-4C/8 of VCG 0261 (bridging isolates) (Table 1). The presence of bridging strains has also been reported in other formae speciales of *F. oxysporum* (7,15). Isolates PD 85/453B, NETH 20286, and NETH 30196 from the Netherlands formed strong heterokaryons with both testers of VCG 0260, while isolate PD 86/466-3 formed strong heterokaryons with the testers of VCGs 0261 and 0261 (bridging isolate) (Table 1).

VCGs of *F. oxysporum* f. sp. *cucumerinum*. One predominant VCG (0180) containing 18 isolates of *F. oxysporum* f. sp. *cucumerinum* from Greece, the United States, Israel, and Canada (among them the isolates ATCC 16416 and ATCC 36330, which are the reference strains of races 1 and 2, respectively) (5), was detected (Table 1). A total of 12 isolates from Japan and the Netherlands belonged to seven VCGs designated 0181 (four isolates), 0182 (two isolates), and 0183 (two isolates), while the remaining four single-member VCGs were grouped into the artificial VCG 018– (Table 1). Two isolates from Japan (Cu:1-1 and Cu:2-1) were HSI (Table 1). The complementary *nit* mutants chosen as testers of VCG 0180 were FOCU-56L/12 and FOCU-57N/18 (both NitM, parental isolates FOCU-56 and FOCU-57, respectively); testers of VCG 0181 were NETH 10782A/2 and NETH 10782A/5 (both NitM, parental isolate NETH 10782); testers of VCG 0182 were ATCC 36332B/1 and ATCC 36332B/2 (NitM and *nit1*, respectively, parental isolate ATCC 36332); and testers of VCG 0183 were FOC-1A/1 and FOC-1A/4 (both NitM, parental isolate FOC-1). In all eight VCGs, heterokaryons were strong, except the *nit* mutants from the isolates TCP-1 and TCP-2 (Table 1), which reacted weakly with the testers of VCG 0180, forming dots of mycelial tufts. These two isolates may constitute a subgroup of VCG 0180, as described in other formae speciales of *F. oxysporum* (7,14,15). The avirulent isolates PSU 1265 and PSU 1266, previously reported as *F. oxysporum* f. sp. *cucumerinum* by other investigators (16), formed strong heterokaryons between them, but not with the testers of all VCGs of either *F. oxysporum* f. sp. *radicis-cucumerinum* or *F. oxysporum* f. sp. *cucumerinum*.

No heterokaryon formation was observed among the testers of VCGs of *F. oxysporum* f. sp. *radicis-cucumerinum*, *F. oxysporum* f. sp. *cucumerinum*, and the testers of the other *Fusarium* isolates studied.

RAPD-PCR analysis. Of the 22 arbitrary primers tested, 10 primers generating reproducible banding patterns were selected for RAPD-PCR (Table 3). A total of 107 distinct bands, consistent in repeated amplifications, were amplified using the DNAs from 121 *F. oxysporum* isolates as templates; 97 of these bands were polymorphic (Table 3). The size of the RAPDs ranged from 0.2 to 2.3 kb (Fig. 1). The RAPD groups defined were composed of isolates with identical RAPD patterns when tested with the same primer and were assigned the Roman numbers I to XX (Tables 1 and 2). RAPD-PCR analysis showed that each of the primers could distinguish *F. oxysporum* f. sp. *radicis-cucumerinum* from *F. oxysporum* f. sp. *cucumerinum* (Fig. 1). The isolates of the former were classified into a single RAPD group (I) that also contained the four isolates from the Netherlands (PD 85/453B, PD 86/466-3, NETH 20286, and NETH 30196) (Table 1). The isolates of *F. oxysporum* f. sp. *cucumerinum* were classified into two RAPD groups (II and III) (Table 1). RAPD group II contained isolates ATCC 16416 and ATCC 36330 (Table 2), which are the reference strains for races 1 and 2, respectively (5). All *F. oxysporum* f. sp. *cucumerinum* isolates originating from Japan, as well as two isolates from the Netherlands (NETH 10782 and NETH 11179), belonged to the same RAPD group (III) as the isolate ATCC 36332 (Table 1), which is the reference strain for race 3 (5). The control isolates of the various formae speciales of *F. oxysporum* in cucurbits and all the avirulent isolates of *F. oxysporum* could be also distinguished, with the exception of AFu-39, which belonged to the same RAPD group I as the isolates of *F. oxysporum* f. sp. *radicis-cucumerinum* (Tables 1 and 2).

Genetic relationships based on RAPD data. The genetic distance between *F. oxysporum* f. sp. *radicis-cucumerinum* (RAPD group I) and *F. oxysporum* f. sp. *cucumerinum* (RAPD groups II and III), calculated from the binary RAPD data using a Jaccard's formula, was 47 to 48% (Table 4). The distance between RAPD groups II and III was 30% (Table 4). Parsimony analysis of the binary RAPD data using the Wagner parsimony method and the strict consensus tree method of PHYLIP 3.5 showed that *F. oxysporum* f. sp. *radicis-cucumerinum* and *F. oxysporum* f. sp. *cucumerinum* were grouped into distinct phylogenetic branches (Fig. 2). Bootstrapping (bootstrap value 99%) supported the separation of these branches (Fig. 2). Cluster analysis of the genetic distance matrix (Table 4) also separated the two formae speciales into distinct branches supported by bootstrapping (data not shown). Both the genetic distance (Table 4) and the parsimony analysis (Fig. 2) indicated that *F. oxysporum* f. sp. *radicis-cucumerinum* and *F. oxysporum* f. sp. *cucumerinum* were more closely related to the control isolates of other formae speciales of *F. oxysporum* than to each other.

DISCUSSION

The distinction of *F. oxysporum* f. sp. *radicis-cucumerinum* from *F. oxysporum* f. sp. *cucumerinum* was initially based on differences in disease symptoms and temperature optima for disease development (37). A major objective of this study was to determine if these differences could be correlated with distinct VCGs and RAPDs. The results clearly revealed a strong correlation between these characteristics, indicating that these two formae speciales of *F. oxysporum* (37) are genetically distinct. Therefore, the VCG and RAPD groupings defined can be valuable diagnostic tools in population studies of *F. oxysporum* from cucumber.

The pathogenicity, VCG, and RAPD tests showed that 64 isolates of *F. oxysporum* from Greece obtained from cucumber with typical root and stem rot symptoms, 3 isolates from the Netherlands (PD 85/453B, PD 86/466-3, and NETH 20286) previously considered *F. oxysporum* f. sp. *cucumerinum* (10,16), and the isolate NETH 30196 should be classified as *F. oxysporum* f. sp. *radicis-cucumerinum.* Results from previous studies (10,16) are comparable and support the new classification of the isolates PD 85/453B, PD 86/466-3, and NETH 20286. Thus, our view possibly explains the clustering of the isolate NETH 20286 to a phylogenetic branch distinct from those of the *F. oxysporum* f. sp. *cucumerinum* isolates tested with mtDNA-restriction fragment length polymorphisms (RFLPs) by Kim et al. (16), as well as its extensive genetic divergence (more than 67% genetic distance) from these isolates (16). In addition, the host range described by Vakalounakis (37) for *F. oxysporum* f. sp. *radicis-cucumerinum* (cucumber, melon, and watermelon for seedlings and cucumber and melon for adult plants) was similar to that previously found for the isolates PD 85/453B and PD 86/466-3 (10). Host specificity similar to that of *F. oxysporum* f. sp. *radicis-cucumerinum* was also reported for a number of *F. oxysporum* isolates obtained from wilted cucumber plants in the Bahamas (24). The relatedness of these isolates with *F. oxysporum* f. sp. *radicis-cucumerinum* remains to be tested.

The *F. oxysporum* f. sp. *radicis-cucumerinum* isolates from the Netherlands were collected from various locations during 1985 to 1986; the root and stem rot outbreak appeared in the Ierapetra area of Crete in the growing season 1989 to 1990 (37). Considering the fact that agricultural supplies such as seeds and organic growth

TABLE 3. Primers used in this study and random amplified polymorphic DNA fragments generated from the *Fusarium oxysporum* isolates

Primer code	Primer sequence	Amplified fragments	Polymorphic fragments
OPA-01	5′-CAGGCCCTTC-3′	11	10
OPB-01	5′-GTTTCGCTCC-3′	14	14
OPB-02	5′-TGATCCCTGG-3′	12	12
OPB-03	5′-CATCCCCCTG-3′	11	8
OPB-04	5′-GGACTGGAGT-3′	8	7
OPB-07	5′-GGTGACGCAG-3′	9	7
OPB-08	5′-GTCCACACGG-3′	12	10
OPB-14	5′-TCCGCTCTGG-3′	9	9
OPB-20	5′-GGACCCTTAC-3′	7	6
OPF-05	5′-CCGAATTCCC-3′	14	14

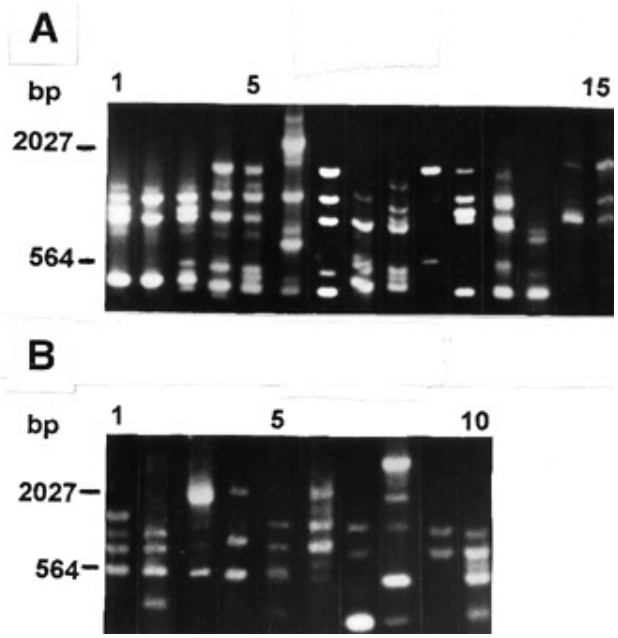

Fig. 1. Agarose electrophoresis showing the random amplified polymorphic DNA (RAPD) banding patterns amplified with two of the arbitrary primers used in this study (Table 3). Numbers on the left indicate band size in kilobase pairs. **A,** Primer OPF-05. Lane 1 presents the pattern of RAPD groups I, V, VI, and VIII; lane 2, VII; lane 3, XIV and XV; lane 4, II; lane 5, III; lane 6, IV; lane 7, IX and XVI; lane 8, X; lane 9, XI; lane 10, XII; lane 11, XIII; lane 12, XVII; lane 13, XVIII; lane 14, XIX; and lane 15, XX. **B,** Primer OPB-20. Lane 1 presents the pattern of RAPD groups I, VI, VII, VIII, XIV, and XV; lane 2, V; lane 3, II and XI; lane 4, III, XII, XIII, and XVI; lane 5, IV; lane 6, IX and XX; lane 7, XVII; lane 8, XVIII; lane 9, XIX; and lane 10, XX. Note RAPD groups I, II, and III. RAPD group I, the only group of *Fusarium oxysporum* f. sp. *radicis-cucumerinum*, consistently appeared with banding patterns different from RAPD groups II and III of *F. oxysporum* f. sp. *cucumerinum*.

substrates are imported to Crete mainly from the Netherlands, *F. oxysporum* f. sp. *radicis-cucumerinum* could have been introduced to Crete, possibly with contaminated cucumber seeds or substrates. Root and stem rot does not represent a major cucumber disease in the Netherlands, probably because most greenhouse crops are grown in soilless cultures and under better sanitary conditions (H. Van Kooten and A. A. Klapwijk, *personal communication*).

The genetic relationships among *F. oxysporum* f. sp. *cucumerinum* isolates were complex, as reflected by the multiple VCGs and the two RAPD groups. This observation is in agreement with the results of Kim et al. (16), who reported that *F. oxysporum* f. sp. *cucumerinum* is the most diverse among the formae speciales of *F. oxysporum*, which infect cucurbits. The proposed classification of isolates ATCC 16416, PSU 1086, and PSU 1098 and isolates NETH 10782 and NETH 11179 by Kim et al. (16) into two distinct genetic groups (IX and I, respectively) using mtDNA-RFLPs is in agreement with our classification of the same isolates into two groups (II and III, respectively), using RAPDs. In addition, the reported genetic distance between mtDNA-RFLP groups IX and I (36 to 58%) (16) is comparable to the genetic distance (30%) found between RAPD groups II and III in our study.

More than half of the isolates of *F. oxysporum* f. sp. *cucumerinum* we studied belonged to VCG 0180, which included nine isolates from Greece, three from Israel, four from the United States, and two from Canada. These isolates all belong to the same clonal lineage, presumably originating from a common progenitor. Although geographically separated, the above countries have close agricultural contacts that could allow frequent redistribution of genetically similar isolates. The grouping of the reference isolates of both races 1 and 2 of *F. oxysporum* f. sp. *cucumerinum* under VCG 0180 and RAPD group II shows a very high genetic similarity and possibly indicates that one of these races gave rise to the other (11). Based on pathogenicity tests and nuclear repetitive DNA fingerprinting, Namiki et al. (25) proposed a high genetic similarity for several isolates of *F. oxysporum* f. sp. *cucumerinum* from Japan. All Japanese isolates of *F. oxysporum* f. sp. *cucumerinum* used in this study belonged to the same RAPD group as isolate ATCC 36332, the reference strain for race 3, which is predominant in Japan (3). However, vegetative compatibility tests revealed the presence of additional genetic polymorphism.

The isolates PSU 1265 and PSU 1266 were observed in this study to be avirulent to 'Knossos', which was susceptible to *F. oxysporum* f. sp. *cucumerinum* isolates. However, since other investigators (16) identified them as *F. oxysporum* f. sp. *cucumerinum*, they might have lost their virulence as a result of prolonged storage in culture. The probability that these isolates belong to a race to which 'Knossos' is not susceptible also can not be excluded. The high genetic distance (approximately 70%) observed in our study between these two isolates and the other tested is in agreement with the results of Kim et al. (16), who, using mtDNA RFLPs, reported a 2 to 32% similarity between these two isolates and several *F. oxysporum* isolates from cucurbits.

The RAPD technique easily distinguished the isolates of *F. oxysporum* f. sp. *radicis-cucumerinum* from those of *F. oxysporum* f. sp. *cucumerinum*. Further research is in progress involving Southern blot analysis and sequence characterization of the DNA regions from which the RAPD fragments, differentiating the RAPD patterns of the two formae speciales, were derived. This work should reveal the genetic basis of these potential diagnostic markers (17,27) and provide more insights into the molecular evolution of the formae speciales of *F. oxysporum* that infect cucumber.

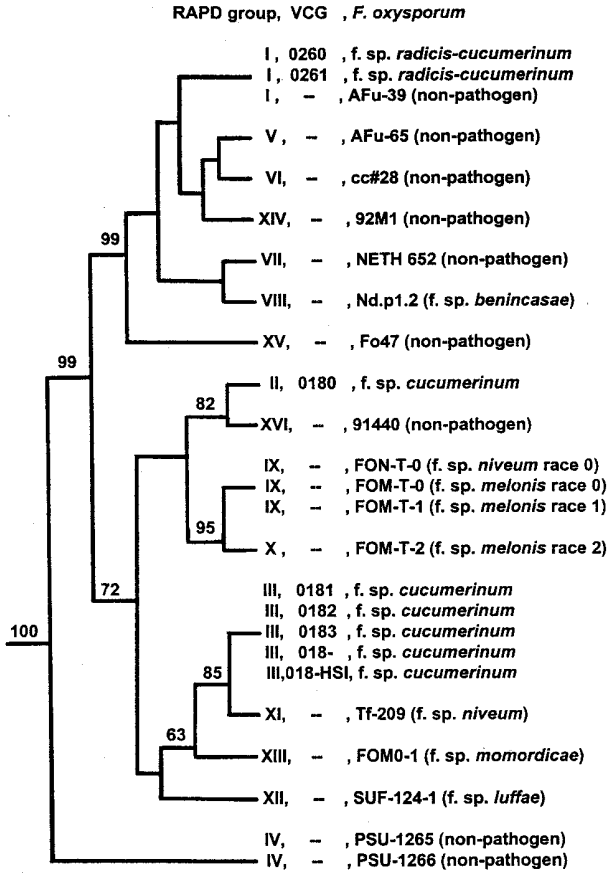

Fig. 2. Dendrogram showing genetic relationships of *Fusarium oxysporum* isolates studied. The most parsimonious trees and the strict consensus tree (unrooted) were produced by PHYLIP 3.5. The numbers on the branches represent the confidence intervals generated by bootstrapping with 1,000 replications.

TABLE 4. Genetic distance values calculated from random amplified polymorphic DNA (RAPD) patterns of the *Fusarium oxysporum* isolates used in this study[a]

RAPD group	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	XVI
I	0.00	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…
II	0.47	0.00	…	…	…	…	…	…	…	…	…	…	…	…	…	…
III	0.48	0.30	0.00	…	…	…	…	…	…	…	…	…	…	…	…	…
IV	0.74	0.67	0.75	0.00	…	…	…	…	…	…	…	…	…	…	…	…
V	0.20	0.50	0.55	0.74	0.00	…	…	…	…	…	…	…	…	…	…	…
VI	0.06	0.44	0.48	0.75	0.19	0.00	…	…	…	…	…	…	…	…	…	…
VII	0.17	0.48	0.49	0.75	0.29	0.19	0.00	…	…	…	…	…	…	…	…	…
VIII	0.10	0.44	0.48	0.74	0.22	0.12	0.15	0.00	…	…	…	…	…	…	…	…
IX	0.50	0.22	0.28	0.72	0.57	0.56	0.49	0.48	0.00	…	…	…	…	…	…	…
X	0.53	0.29	0.35	0.74	0.60	0.59	0.53	0.52	0.09	0.00	…	…	…	…	…	…
XI	0.57	0.38	0.19	0.75	0.57	0.55	0.52	0.47	0.36	0.41	0.00	…	…	…	…	…
XII	0.56	0.31	0.32	0.75	0.62	0.56	0.60	0.57	0.28	0.32	0.41	0.00	…	…	…	…
XIII	0.48	0.35	0.23	0.74	0.56	0.49	0.48	0.48	0.33	0.40	0.30	0.26	0.00	…	…	…
XIV	0.14	0.44	0.47	0.78	0.27	0.17	0.24	0.17	0.47	0.51	0.55	0.52	0.46	0.00	…	…
XV	0.18	0.52	0.52	0.67	0.27	0.14	0.27	0.21	0.49	0.54	0.58	0.58	0.53	0.20	0.00	…
XVI	0.47	0.17	0.35	0.68	0.56	0.51	0.50	0.48	0.26	0.30	0.40	0.32	0.39	0.48	0.55	0.00

[a] The isolates used in this study are represented by the RAPD groups under which they were classified.

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