Characterization of *Fusarium oxysporum* Isolates Obtained from Cucumber in China by Pathogenicity, VCG, and RAPD

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ABSTRACT

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Thirty-four isolates of *Fusarium oxysporum*, obtained in China from cucumber plants showing either Fusarium wilt (*F. oxysporum* f. sp. *cucumerinum*) or root and stem rot (*F. oxysporum* f. sp. *radicis-cucumerinum*) symptoms, were characterized by pathogenicity, vegetative compatibility, and random amplified polymorphic DNA (RAPD). Of these, 23 isolates were identified by pathogenicity as *F. oxysporum* f. sp. *cucumerinum*, and one as *F. oxysporum* f. sp. *radicis-cucumerinum*, while 10 isolates were avirulent on cucumber, melon, sponge gourd, and pumpkin. The Chinese isolates of *F. oxysporum* f. sp. *cucumerinum* were assigned to RAPD groups III and XXI and to vegetative compatibility group (VCG) 0183, four new VCGs, 0184 to 0187, and a single-member VCG included in the artificial VCG 018-. The Chinese isolate of *F. oxysporum* f. sp. *radicis-cucumerinum* was assigned to RAPD group I and bridging VCG 0260/0261. The occurrence of *F. oxysporum* f. sp. *radicis-cucumerinum* on cucumber is reported for the first time in China.

Cucumber (*Cucumis sativus* L.) is a crop of significant economic importance in many countries (21). It is affected by several fungal pathogens, and *Fusarium oxysporum* Schlechtend.:Fr. is among the most important (21). On cucumber, two formae speciales of *F. oxysporum* have been described, *F. oxysporum* f. sp. *cucumerinum* J.H. Owen, causing Fusarium wilt (12), and *F. oxysporum* f. sp. *radicis-cucumerinum* D.J. Vakalounakis, causing root and stem rot (16,22-24).

Fusarium wilt of cucumber is a serious vascular disease worldwide, including in Greece and China (1,21). Armstrong et al. (2) proposed three races of *F. oxysporum* f. sp. *cucumerinum*, to be called races 1, 2, and 3, mainly occurring in the United States, Israel, and Japan, respectively (7,15). Root and stem rot of cucumber was described for the first time during 1989-90 in Crete as causing severe losses in greenhouse crops (22). The disease was also recorded in Canada in 1994 (4,18), in France in 1998 (19), and in Spain in 2000

(14), where significant yield losses were encountered (23,24). No races of *F. oxysporum* f. sp. *radicis-cucumerinum* have yet been identified (22-24).

The genetic variation of 106 isolates of *F. oxysporum* from cucumber has been studied with respect to pathogenicity, vegetative compatibility grouping, and random amplified polymorphic DNA (RAPD) analysis (23). Isolates of *F. oxysporum* f. sp. *radicis-cucumerinum* were assigned to vegetative compatibility groups (VCGs) 0260, 0261, and 0260/0261 (bridging isolates) as well as to a single RAPD group (I) (23). Isolates of *F. oxysporum* f. sp. *cucumerinum* were assigned to VCGs 0180 to 0183 and to four single-member VCGs (018-) as well as to two different RAPD groups (II, III), while vegetatively self-incompatible (HIS) isolates were also recorded (23).

In the present study, we used pathogenicity, VCGs, and RAPD-PCR to characterize 34 isolates of *F. oxysporum* from China obtained from cucumber plants showing symptoms of either Fusarium wilt (33 isolates) or root and stem rot (one isolate). Our objectives were to: (i) identify these isolates at the forma specialis level, (ii) investigate whether *F. oxysporum* f. sp. *radicis-cucumerinum* occurs in China, and (iii) examine genetic relatedness among the Chinese isolates of *F. oxysporum* from cucumber, and compare them with previ-

ously characterized isolates of *F. oxysporum* from cucumber, by VCG and RAPD analyses.

MATERIALS AND METHODS

Fungal isolates. Isolates of *F. oxysporum* were recovered from diseased cucumber plants, showing either typical Fusarium wilt or root and stem rot symptoms, from seven provinces of China, which represent different geographical regions, between the years 1998 and 2000 (Table 1). Small pieces of discolored vascular tissue from the lower part of the stems of diseased plants were plated on acidified potato dextrose agar (APDA, 4.5 ml 25% lactic acid in 1 liter PDA, final pH 4.0) and incubated at $25 \pm 0.5^\circ\text{C}$ in the dark for 4 days. Isolates were identified as *F. oxysporum* by morphological criteria (3). A single microconidial culture was prepared from each isolate.

Pathogenicity tests. Pathogenicity of *F. oxysporum* isolates was tested as previously described (22,23) by root dip inoculation on seedlings at the two-true-leaf stage of four differential cucurbit species: cucumber cv. Knossos, melon (*Cucumis melo* L.) cv. Moulkeiko, sponge gourd (*Luffa aegyptiaca* Mill.), and pumpkin (*Cucurbita maxima* Duchesne $\times$ *C. moschata* Duch. ex Lam.). Final observation on disease development was made 30 days after inoculation. For each isolate and differential host, eight replicate plants were used. Tests were repeated once. Disease severity was assessed with a 0 to 3 visual scale, where 0 = no symptoms, 1 = light or moderate wilt, light vascular discoloration in the stem, 2 = severe wilt, vascular discoloration in the stem, and 3 = dead seedlings. To confirm infection of cucurbit plants by *F. oxysporum*, isolations were made from each plant with or without symptoms.

Vegetative compatibility tests. Tests were based on the ability of nitrate reductase (*nit*) mutants of *F. oxysporum* to form a heterokaryon and to grow on a nitrate-containing medium (17). Mutants from each Chinese *F. oxysporum* isolate from cucumber were generated on a minimal medium (FMM) amended with 1.5% potassium chlorate (FMMC) (17) and charac-

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terized as *nit1*, *nit3*, or NitM (5). Representative *nit* mutants (*nit1*, *nit3*, and NitM) from all isolates were paired with tester strains of *F. oxysporum* f. sp. *cucumerinum* and *F. oxysporum* f. sp. *radicis-cucumerinum*. These were as follows: *F. oxysporum* f. sp. *cucumerinum*: (a) tester strains of the four known VCGs 0180 (FOCU 56L/12 and FOCU 57N/18, both NitM), 0181 (NETH 10782A-2 and NETH

10782A-5, both NitM), 0182 (ATCC 36332B-1 and ATCC 36332B-2, NitM and *nit1*, respectively), and 0183 (FOC-1-9A-2 and FOC-1-9A-4, both NitM); (b) tester strains of the artificial VCG 018-, which consisted of the four single-member VCGs Cu:6-0 (Cu:6-0-6A-3 and Cu:6-0-6A-12, both NitM), Tf-213 (Tf-213-2A-3 and Tf-213-2A-6, *nit1* and NitM, respectively), Tf-304 (Tf-304-3A-2 and Tf-304-3A-4,

both NitM), and Tf-579 (Tf-579-4A-1 and Tf-579-4A-6, NitM and *nit1*, respectively) (23). *F. oxysporum* f. sp. *radicis-cucumerinum*: tester strains of the two known VCGs 0260 (AFU-15B-2 and AFU-14A-4, both NitM) and 0261 (AFu-4C-8 and AFu-4C-9, NitM and *nit1*, respectively) (23). The sources of these tester strains were the Mycology Laboratory of the Plant Protection Institute of Heraklio,

Table 1. Isolates of *Fusarium oxysporum* from cucumber obtained from various locations in China and used in this study, listed by origin, forma specialis, vegetative compatibility group (VCG), pathogenicity, and random amplified polymorphic DNA (RAPD) group

Isolate	Origin (province/location)	Year of isolation	Disease index ^a				RAPD group ^b
			Cucumber	Melon	Sponge gourd	Pumpkin	
<i>Fusarium oxysporum</i> f. sp. <i>cucumerinum</i>							
VCG 0183 ^c							
0014	Beijing/Haidian	2000	2.8 (100)	0.0 (0)	0.0 (0)	0.0 (0)	III
0018	Xingjiang/Kuitun	2000	3.0 (100)	0.0 (0)	0.0 (0)	0.0 (0)	III
0019	Xingjiang/Kuitun	2000	2.8 (100)	0.0 (0)	0.0 (0)	0.0 (0)	III
VCG 0184 (new)							
Tf-579 ^d	Japan	1996	2.8 (92)	0.0 (0)	0.0 (0)	0.0 (0)	III
9906-1	Zhejiang/Zhoushan	1999	2.1 (73)	0.0 (0)	0.0 (0)	0.0 (0)	III
9906-2	Zhejiang/Zhoushan	1999	2.3 (75)	0.0 (0)	0.0 (0)	0.0 (0)	III
9906-3	Zhejiang/Zhoushan	1999	1.5 (67)	0.0 (0)	0.0 (0)	0.0 (0)	III
VCG 0185 (new)							
Tf-213 ^d	Japan	1985	2.8 (92)	0.0 (0)	0.0 (0)	0.0 (0)	III
9909-2	Heilongjiang/Shangzhi	1999	1.2 (42)	0.0 (0)	0.0 (0)	0.0 (0)	III
VCG 0186 (new)							
9901-1	Gansu/Langzhou	1999	1.5 (67)	0.0 (0)	0.0 (0)	0.0 (0)	XXI
9901-2	Gansu/Langzhou	1999	0.8 (58)	0.0 (0)	0.0 (0)	0.0 (0)	XXI
9902-1	Gansu/Langzhou	1999	1.5 (67)	0.0 (0)	0.0 (0)	0.0 (0)	XXI
9902-2	Gansu/Langzhou	1999	1.5 (50)	0.0 (0)	0.0 (0)	0.0 (0)	XXI
9903-1	Gansu/Langzhou	1999	1.1 (42)	0.0 (0)	0.0 (0)	0.0 (0)	XXI
9903-2	Gansu/Langzhou	1999	0.8 (58)	0.0 (0)	0.0 (0)	0.0 (0)	XXI
9904-1	Gansu/Langzhou	1999	2.3 (75)	0.0 (0)	0.0 (0)	0.0 (0)	XXI
9904-2	Gansu/Langzhou	1999	1.8 (58)	0.0 (0)	0.0 (0)	0.0 (0)	XXI
9904-3	Gansu/Langzhou	1999	2.0 (67)	0.0 (0)	0.0 (0)	0.0 (0)	XXI
9905-0	Gansu/Langzhou	1999	1.3 (58)	0.0 (0)	0.0 (0)	0.0 (0)	XXI
9909-3	Heilongjiang/Shangzhi	1999	2.0 (67)	0.0 (0)	0.0 (0)	0.0 (0)	nt ^e
VCG 0187 (new)							
0016	Zhejiang/Hangzhou	2000	2.8 (100)	0.0 (0)	0.0 (0)	0.0 (0)	III
0017	Zhejiang/Hangzhou	2000	3.0 (100)	0.0 (0)	0.0 (0)	0.0 (0)	III
0020	Zhejiang/Hangzhou	2000	2.1 (82)	0.0 (0)	0.0 (0)	0.0 (0)	III
VCG 018-							
Tf-304 ^d	Japan	1985	2.7 (100)	0.0 (0)	0.0 (0)	0.0 (0)	III
Cu:6-0 ^d	Japan	—	2.6 (100)	0.0 (0)	0.0 (0)	0.0 (0)	III
9806	Jiangsu/Nanjing	1998	1.0 (33)	0.0 (0)	0.0 (0)	0.0 (0)	III
018-HSI							
FHG 9801	Zhejiang/Ningbo	1998	0.8 (20)	0.0 (0)	0.0 (0)	0.0 (0)	III
<i>F. oxysporum</i> f. sp. <i>radicis-cucumerinum</i>							
VCG 0260/0261 (bridging group)							
9909-1	Heilongjiang/Shangzhi	1999	1.8 (58)	1.8 (83)	1.7 (70)	0.0 (0)	I
Fisher's LSD (<i>P</i> = 0.05)			0.62				
Avirulent isolates of <i>F. oxysporum</i> from China obtained from cucumber							
9802	Zhejiang/Hangzhou	1998	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	XXII
9803	Zhejiang/Hangzhou	1998	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	XXIII
9804	Zhejiang/Hangzhou	1998	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	XXIV
9805	Jiangsu/Zhenjiang	1998	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	XXV
9807	Jiangsu/Zhenjiang	1998	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	XXVI
9907-3	Jiangsu/Zhenjiang	1998	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	XXVII
0011	Zhejiang/Daxuemiao	2000	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	XXVIII
0012	Guangxi/Qingzhou	2000	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	XXIX
0013	Guangxi/Qingzhou	2000	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	XXX
0015	Zhejiang/Daxueningchang	2000	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	XXXI

^a Disease index was the mean disease severity value on artificially inoculated plants, which was calculated as described in Materials and Methods. Numbers in parentheses indicate percentage of diseased seedlings. Pathogenicity tests were carried out for each isolate.

^b As determined by RAPD-PCR using eight arbitrary primers.

^c Vegetative compatibility groups (VCGs) as determined in this study and numbered according to the system adopted by Puhalla (17) and further standardized by Kistler et al. (11). VCG numbering system, in which 026# = VCG code for *F. oxysporum* f. sp. *radicis-cucumerinum* as suggested by Vakalounakis and Fragkiadakis (23); 018# = VCG code for *F. oxysporum* f. sp. *cucumerinum* as suggested by Katan et al. (9); 018- = an artificial group containing single-member VCGs of *F. oxysporum* f. sp. *cucumerinum*; 018-HIS = an artificial group containing heterokaryon self-incompatible isolates that did not form heterokaryons upon pairing with themselves or with any of the other isolates.

^d Isolates included in previous publications: Tf-213 (22), Tf-304 (22), Tf-579 (22).

^e Not tested.

Crete, Greece, and the American Type Culture Collection (ATCC). Complementation tests were made on FMM at 23°C in the dark (17). Heterokaryon formation was evident after 7 to 20 days by the formation of a wild-type mycelium at the contact zone between two *nit* mutants (17). 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 (9), whereas 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 (9). An isolate was assigned to a given VCG if it was capable of producing robust heterokaryon with at least one other isolate of the same VCG.

RAPD-PCR analysis. DNA preparation and RAPD-PCR were carried out as previously described (23) using the primers OPA-01, OPB-01, OPB-4, OPB-7, OPB-8, OPB-14, OPB-20, and OPF-05 (Operon Technologies, Inc., Alameda, CA) (Table 2), which had been found (23) to generate banding patterns capable of differentiating *F. oxysporum* f. sp. *cucumerinum* and *F. oxysporum* f. sp. *radicis-cucumerinum* isolates. Amplified DNA was electrophoresed in 1.2% agarose gels, stained with ethidium bromide, and photographed under UV light (302 nm) (23). The PCR reactions were carried out at least three times, using different DNA extracts from each of the isolates tested.

Data analysis. Pathogenicity tests were conducted in a completely randomized design. Disease index data of all virulent Chinese isolates of *F. oxysporum* on cucumber seedlings were analyzed using Fisher's LSD ($P = 0.05$), although there is no implied linearity in the disease scales used (20,25). Replicate experiments gave similar results.

RESULTS

Pathogenicity tests. A total of 23 of the 34 isolates of *F. oxysporum* from China were proved virulent under artificial inoculation conditions on cucumber cv. Knos-sos, but not on melon cv. Moulkeiko, sponge gourd, or pumpkin (Table 1), and caused symptoms resembling those incited by *F. oxysporum* f. sp. *cucumerinum* (22). The aggressiveness of these isolates ranged from low to high (Table 1). Based on disease symptoms after natural and artificial infection as well as on their virulence on cucumber (but not on melon, sponge gourd, and pumpkin), these 22 isolates were identified as *F. oxysporum* f. sp. *cucumerinum*. One isolate (9909-1) of *F. oxysporum* from the province of Heilongjiang (northeastern China), obtained in 1999 from a field cucumber crop with several diseased plants presenting root and stem rot symptoms, was virulent under artificial

inoculation conditions on cucumber, melon, and sponge gourd but not on pumpkin (Table 1), causing symptoms resembling those incited by *F. oxysporum* f. sp. *radicis-cucumerinum* (22). The aggressiveness of this isolate was moderate (Table 1). Based on disease symptoms under natural infection and artificial inoculation conditions, and its virulence on cucumber, melon, and sponge gourd but not on pumpkin, the 9909-1 isolate was identified as *F. oxysporum* f. sp. *radicis-cucumerinum*. The remaining 10 isolates, 9802, 9803, 9804, 9805, 9807, 9907-3, 0011, 0012, 0013, and 0015 (Table 1), were avirulent on cucumber, melon, sponge gourd, or pumpkin.

Vegetative compatibility tests. Based on the formation of strong complementary heterokaryons with the testers of VCG 0183 of *F. oxysporum* f. sp. *cucumerinum*, the Chinese isolates 0014, 0018, and 0019 were assigned to this VCG (Table 1), which also includes the Japanese isolates FOC-1 and Cu:5-0 (23). The Chinese isolates 9906-1, 9906-2, and 9906-3 were compatible with the testers of the Japanese isolate Tf-579, and the Chinese isolate 9909-2 was compatible with the testers of the Japanese isolate Tf-213 of VCG 018- of *F. oxysporum* f. sp. *cucumerinum* (Table 1). According to the numbering system adopted by Puhalla (17) and further standardized by Kistler et al. (11), it is suggested that these VCGs should be assigned as 0184 and 0185, respectively. A total of 14 Chinese isolates of *F. oxysporum* f. sp. *cucumerinum* belonged to two new VCGs designated 0186 (11 isolates) and 0187 (three isolates). The isolate 9806 (2161-1) formed a single-member VCG, which was grouped into the artificial VCG 018-, and the isolate FHG 9801 was HSI (Table 1). The complementary *nit* mutants chosen as testers of VCG 0186 of *F. oxysporum* f. sp. *cucumerinum* were 9904-3-1 (*nit1*, parental isolate 9904-3) and 9904-3-2 (*NitM*, parental isolate 9904-3), and of VCG 0187 were 0020-2 (*nit1*, parental isolate 0020) and 0020-6 (*NitM*, parental isolate 0020). The isolate 9909-1 of *F. oxysporum* f. sp. *radicis-cucumerinum* formed a strong heterokaryon with the tester AFu-15B-2 of VCG 0260 and the tester AFu-4C-8 of VCG 0261 *F. ox-*

ysporum f. sp. *radicis-cucumerinum* and was assigned to the VCG 0260/0261 (bridging group) (Table 1).

No heterokaryon formation was observed, either among avirulent Chinese isolates of *F. oxysporum* from cucumber, or between them and the tester strains of all VCGs of either *F. oxysporum* f. sp. *cucumerinum* or *F. oxysporum* f. sp. *radicis-cucumerinum* studied.

RAPD-PCR analysis. The quantitative data from RAPD-PCR experiments are presented in Table 2. All 10 Chinese isolates of *F. oxysporum* f. sp. *cucumerinum* belonging to VCGs 0183, 0184, 1085, 0187, and 018-, as well as the HSI isolate FHG 9801, were classified into the RAPD group III as established in a previous study (23), which also contained the reference isolate ATCC 36332 (race 3) from Japan (Table 1, Fig. 1). In contrast, all 10 Chinese isolates of *F. oxysporum* f. sp. *cucumerinum* assigned to VCG 0186 were classified into a new (23) RAPD group (XXI) (Table 1, Fig. 1). The isolate 9909-1 of *F. oxysporum* f. sp. *radicis-cucumerinum* was classified into the RAPD group I (Fig. 1), also previously established (23); this group also contained the reference isolates AFu-4 and AF1-a from Crete (23). The avirulent isolates of *F. oxysporum* could be distinguished from the pathogenic isolates, because each isolate group belonged to a separate RAPD group (Table 1). The Chinese virulent isolates of both *F. oxysporum* f. sp. *cucumerinum* and *F. oxysporum* f. sp. *radicis-cucumerinum* could be distinguished from avirulent isolates of *F. oxysporum* from cucumber by both VCG and RAPD (Table 1).

DISCUSSION

The observed agreement in the identification of the 34 isolates of *F. oxysporum* from China obtained from diseased cucumber plants as *F. oxysporum* f. sp. *cucumerinum* (23 isolates), *F. oxysporum* f. sp. *radicis-cucumerinum* (one isolate), and nonpathogenic *F. oxysporum* (10 isolates) by pathogenicity, VCG, and RAPD indicates the reliability and usefulness of these methods as diagnostic tools in population studies of *F. oxysporum* from cucumber.

The genetic relationships among the isolates of *F. oxysporum* f. sp. *cucumerinum*

Table 2. Primers used in this study and major random amplified polymorphic DNA (RAPD) fragments generated from the *Fusarium oxysporum* isolates tested^a

Primer code	Primer sequence	Amplified fragments	Fragments present in RAPD group			
			I	II	III	XXI
OPA-01	5'-CAGGCCCTTC-3'	9	5	8	7	7
OPB-01	5'-GTTTCGCTCC-3'	5	5	3	4	5
OPB-04	5'-GGACTGGAGT-3'	6	4	6	6	6
OPB-07	5'-GGTGACGCAG-3'	9	9	7	7	7
OPB-08	5'-GTCCACACGG-3'	8	7	4	3	5
OPB-14	5'-TCCGCTCTGG-3'	10	2	3	3	6
OPB-20	5'-GGACCCTTAC-3'	8	3	5	5	5
OPF-05	5'-CCGAATCCCC-3'	7	4	6	6	6

^a In all experiments, only intense and reproducible bands were recorded.

from China were complex, as reflected by the multiple VCGs and the two RAPD groups. A large number of VCGs is generally recorded in fungi that reproduce sexually (13), such as *F. moniliforme* (teleomorph *Gibberella fujikuroi*) (6). In contrast, fungi that reproduce only asexually, such as many of the formae speciales of *F. oxysporum*, generally have large VCGs that indicate a high degree of clonality (11). In *F. oxysporum* f. sp. *cucumerinum*, including the VCGs recently found (23), 11 VCGs have been identified so far. Considering the number of VCGs in 37 formae speciales of *F. oxysporum*, *F. oxysporum* f. sp. *cucumerinum* ranks third after *F. oxysporum* f. sp. *cubense* (24 VCGs) and *F. oxysporum* f. sp. *vasinfectum* (12 VCGs) (8). This demonstrates a high level of genetic diversity in *F. oxysporum* f. sp. *cucumerinum*, which suggests that clonality is limited. This observation is in agreement with the results of Kim et al. (10), who reported that *F. oxysporum* f. sp. *cucumerinum* is the most diverse among the formae speciales of *F. oxysporum* infecting cucurbits.

Aggressiveness of isolates of *F. oxysporum* f. sp. *cucumerinum* in VCG 0186 was significantly lower than that of other VCGs (Table 1). These results suggest that VCG is associated with aggressiveness in this pathogen. A similar relation has also been reported for *F. oxysporum* f. sp. *cucumerinum* in Korea (1).

About one-third of the Chinese isolates of *F. oxysporum* f. sp. *cucumerinum* belonged to VCGs 0183, 0184, 0185, 0187, and 018-, which as previously found, included all the Japanese isolates examined.

Such a genetic similarity between Chinese and Japanese populations of *F. oxysporum* f. sp. *cucumerinum* is expected given the close proximity of China and Japan and the frequent exchanges of agricultural products that could allow distribution of genetically similar isolates. The occurrence of common VCGs could be explained by the spread of the pathogen from one country to the other through seeds, seedlings, diseased debris present in composts, and human activities.

Data suggesting that the isolate 9909-1 of *F. oxysporum* obtained from a diseased cucumber crop in the Shangzhi area of the province of Heilongjiang, in northeastern China, is *F. oxysporum* f. sp. *radicis-cucumerinum* were obtained by isolation of the pathogen from naturally infected plants, the completion of Koch's postulates, and determination of the pathogen's host range. Its identification by pathogenicity was also supported by the VCG and RAPD data. Since its first record in Greece in 1989, *F. oxysporum* f. sp. *radicis-cucumerinum* has also been reported in several other countries in Europe and North America, causing significant yield losses (23,24). To the best of our knowledge, this is the first report of this pathogen in China, and it appears to be the first record in Asia. Although the characterization and documentation of appearance of *F. oxysporum* f. sp. *radicis-cucumerinum* in China was based on only one isolate, this result reflects the sampling density of the present study. Our samples were collected over a huge geographic area representing the entire country of China. In the future, increasing sampling density,

especially in the vicinity of the expected dissemination of the pathogen, is anticipated to verify the presence of *F. oxysporum* f. sp. *radicis-cucumerinum* and to increase the number of reported incidents.

The isolate 9909-1 was assigned to RAPD group I and bridging VCG 0260/0261 of *F. oxysporum* f. sp. *radicis-cucumerinum*. Earlier phylogenetic analysis of exclusively RAPD data from strains of cucurbitaceous hosts supported the monophyletic nature of this pathogen (23). Consequently, the cross-compatibility between *nit* mutants from strains of VCGs 0260 and 0261, which have the same RAPD pattern, indicates that *F. oxysporum* f. sp. *radicis-cucumerinum* may represent a single and recently diverged clonal lineage.

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Fig. 1. Random amplified polymorphic DNA (RAPD) analysis from *Fusarium oxysporum* isolates with primer OPB-14. The products were separated on a 1.2% agarose gel stained with ethidium bromide and photographed under UV light. Lanes 1, 2, 3, and 6 represent RAPD group II isolates of *F. oxysporum* f. sp. *cucumerinum*, namely ATCC 16416, ATCC 36330, FOCU 56, and FOCU 57. Lane 4 represents RAPD group I isolates *F. oxysporum* f. sp. *radicis-cucumerinum*, namely 9909-1. Lane 5 represents RAPD group III isolates of *F. oxysporum* f. sp. *cucumerinum*, namely ATCC 36332. Lanes 7, 8, 9, 10, 11, and 12 represent RAPD group XXI isolates of *F. oxysporum* f. sp. *cucumerinum*, namely 9902-1, 9902-2, 9903-2, 9904-1, 9904-2, and 9905-0. Note the differences in the main bands between RAPD groups II and III, and the newly established group XXI. In lane M, a DNA marker was separated. The DNA fragments consist of 125 bp (bottom), 2,027 bp, 2,322 bp, 4,361 bp, 6,557 bp, and higher (top).

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