

Physiologic Races and Vegetative Compatibility Groups of *Fusarium oxysporum* f. sp. *melonis* in Israel

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ABSTRACT

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Two vegetative compatibility groups (VCGs) and four physiologic races (0, 1, 2, and 1-2) were identified among 122 isolates of *Fusarium oxysporum* f. sp. *melonis* in Israel. One VCG corresponded to the previously described VCG 0135, whereas the other, VCG 0138, is new and was divided into two subgroups. VCG 0135 contained isolates of races 0 and 2, and

VCG 0138 contained isolates of races 0, 1, and 1-2. In one region with a long history of cucurbit cropping, all races and VCGs were found within 17 km of each other, suggesting a high diversity of the pathogen within a relatively small area. Based on vegetative compatibility, it is suggested that in Israel race 2 could have evolved from race 0 of VCG 0135, and races 1 and 1-2 from race 0 of VCG 0138.

Additional keywords: Fusarium wilt, muskmelon.

Resistant cultivars are one of the most promising options for effective control of Fusarium wilt of muskmelon caused by *Fusarium oxysporum* Schlechtend. f. sp. *melonis* W.C. Snyder & H. N. Hans. However, this approach has been hampered by the appearance of physiologic races (2,11-13,16). The three common physiologic races are designated races 0, 1, and 2 according to the system suggested by Risser et al (16). Reports on a fourth race, designated race 1-2, indicate its limited occurrence to date (1,2,5,7,11,16). Race 1-2 is virulent to muskmelon cultivars possessing resistance genes *Fom-1*, *Fom-2* and *Fom-3* (6,16). In Israel, all four races of *F. o. melonis* exist within a relatively small area (2). It is not known whether the races in Israel evolved locally because of frequent cropping of muskmelon or originated from foreign sources. The genetic relatedness and possible evolutionary linkages among the Israeli strains and between Israeli strains and those from other countries are not known.

In addition to physiologic races, diversity in *F. o. melonis* is manifested by differences in vegetative compatibility; seven vegetative compatibility groups (VCGs) have been identified among 187 isolates of a worldwide collection (6,7). The relative genetic distances among these VCGs have been estimated by comparison

of their mitochondrial DNA (8).

In an attempt to understand their evolution, the prevalence of physiologic races of *F. o. melonis* in Israel and their possible relatedness to strains from other countries in terms of vegetative compatibility was determined.

MATERIALS AND METHODS

Media. Potato-dextrose agar (PDA) was used to isolate *F. oxysporum* from diseased plants. The Fusarium-minimal medium (FMM) was Puhalla's minimal nitrate agar (MM), a sucrose-salt medium that contains nitrate as the nitrogen source (15). Nitrite, hypoxanthine, and ammonium media were used for partial phenotypic characterization of *nit* mutants such as *nit1*, *nit3*, or *NitM* (4). FMM was used to recognize *nit* mutants and for complementation (heterokaryon) tests. Chlorate media, based on FMM or PDA amended with 15 g/L KClO₃, were used to generate *nit* mutants (4,15). GYP (2% glucose, 0.5% yeast extract, 0.5% peptone, 2% agar) was used to grow inoculum for virulence tests.

Isolates. A collection of 122 isolates of *F. o. melonis* was assembled during 1984-1992 from 19 sites in Israel (Fig. 1; Table 1). About 80 of the isolates originated from diseased plants in commercial fields. The remainder were obtained from young

wilting plants of the susceptible *Cucumis melo* var. *reticulatus* 'Ananas-Ein-Dor', grown in the greenhouse in soil from sites 1, 11, 12, 17, and 18. Although the largest distance between sampling sites did not exceed 200 km, these sites covered a wide range of environmental conditions, including altitude (e.g., -350 m at site 1 near the Dead Sea, +50 m at sites 4 and 9, and +900 m at site 2), temperature, precipitation, and soil type (including artificial soilless media). The various agricultural practices employed at the different sites included greenhouses as well as open fields with or without irrigation. *F. oxysporum* was isolated from diseased plants by placing plant tissues from the lower part of the stem, after surface disinfection with 1% sodium hypochlorite for 2 min, on PDA and incubating at 27 C for 5 to 7 days. Monoclonal cultures were prepared from most of the isolates. Isolates were maintained on dried Whatman no. 54 filter paper (4) without apparent loss of pathogenicity.

Virulence tests. Virulence tests were carried out using the root-dip technique, essentially as described (3). Inoculum consisting of conidia and mycelial fragments was prepared by growing the isolates on GYP medium. After a 7-day incubation in the dark at 27 C, the content of each plate was macerated in 100 ml of water for 1 min (resulting in suspensions of $1-3 \times 10^6$ conidia per milliliter) and used for inoculation. One day after emergence, seedlings were removed and washed, and their roots were dipped in an inoculum suspension. The inoculated seedlings were planted and kept in the greenhouse at diurnal temperatures of 22-30 C. Each isolate was used to inoculate two replicates of each of seven seedlings from each cultivar. Wilt symptoms were first observed after 6-8 days, and the number of diseased plants was recorded

for an additional 14 days. Similarly treated, noninoculated seedlings served as controls. All isolates were first inoculated on the susceptible cultivar. Isolates that did not induce wilt were tested twice more, using a conidial concentration of 5×10^6 /ml in four replicates of seven seedlings. If no wilt occurred, such isolates were categorized as nonpathogenic to muskmelon.

Physiologic races of *F. o. melonis* were identified according to Risser et al (16). The local muskmelon cultivars Ananas-Ein-Dor (susceptible to all races), Hemed (resistant to races 0 and 2) and Makdimon-I (resistant to races 0 and 1) had been compared previously to the Charentais series of Risser et al (16) and were found to differentiate races 0, 1, 2 and 1-2 of *F. o. melonis* (2). These cultivars were used along with the new cv. Emek 2, which is resistant to races 0, 1, and 2 but susceptible to race 1-2 (17). All isolates were tested at least twice; some isolates were tested up to five times in the framework of breeding programs.

Isolation of *nit* mutants and complementation tests. Plates 9-cm diameter) of chlorate media were inoculated at four points with small mycelial plugs (one isolate per plate) and incubated at 27 C. Fast-growing sectors that emerged from the restricted colonies were transferred to FMM plates (6-cm diameter) and examined after 3 days. Colonies with a thin expanding mycelium were considered *nit* mutants. All *nit* mutants showed wild-type growth on PDA. Complementation between *nit* mutants was tested on FMM plates (6-cm diameter). Three mutants were usually inoculated on each plate, in triangular pattern, and the plates were incubated at 27 C. Complementation was evident by the formation of a dense aerial wild-type mycelium where two mutants had met and formed a heterokaryon (9). Absence of wild-type growth at the contact zone between two *nit* mutants of the same parent isolate indicated allelic, overlapping, or otherwise noncomplementary mutations, or vegetative self-incompatibility (6). On the other hand, absence of wild-type growth at the contact zone of *nit* mutants from different parent isolates indicated either noncomplementarity or inability to form heterokaryons due to a lack of vegetative compatibility. Heterokaryons were usually evident within 5 to 14 days. When mutants of two different isolates formed a heterokaryon, their parent isolates were assigned to the same VCG.

RESULTS

Virulence. Variation in aggressiveness among the wilt-inducing isolates was evident as manifested by the number of seedlings affected and the rate of symptom development when each was tested against a universal susceptible cultivar. Percentage of wilted seedlings ranged from 40 to 100 for different isolates. About 20% of the isolates induced only 40-60% wilt and therefore were tested twice more. Although all of them were pathogenic, most were confirmed as less aggressive. Only isolates that were consistently pathogenic on muskmelon were identified as *F. o. melonis*. Twenty-three isolates were nonpathogenic on muskmelon.

When tested for pathogenicity on the four race-differential muskmelon cultivars, all four previously described physiologic races (2,16) were identified among 122 isolates. The regional distribution and frequency varied among races (Table 1): Race 0 was found in 12 out of 19 sites, scattered throughout the country; race 1 was found in seven widely distributed sites; and races 2 and 1-2 were found only in a northern region, delimited by sites 15-19 (Fig. 1).

Vegetative compatibility groups. A group of 15 isolates, representing each race, was chosen for the initial tests of vegetative compatibility. Seven of these isolates had been described previously (2). Complementary *nit* mutants were generated from each isolate and paired on FMM in various combinations. The pattern of heterokaryon formation between the mutants of the different isolates revealed two distinct VCGs comprising six and eight of the 15 isolates involved (Table 2); the remaining isolate (FOM-1451) was self-incompatible (6). Complementary mutants from each VCG were paired with tester strains of the seven known VCGs (0130-0136) of *F. o. melonis* (7). In these tests, one group of isolates corresponded to VCG 0135 (Table 2), previously known

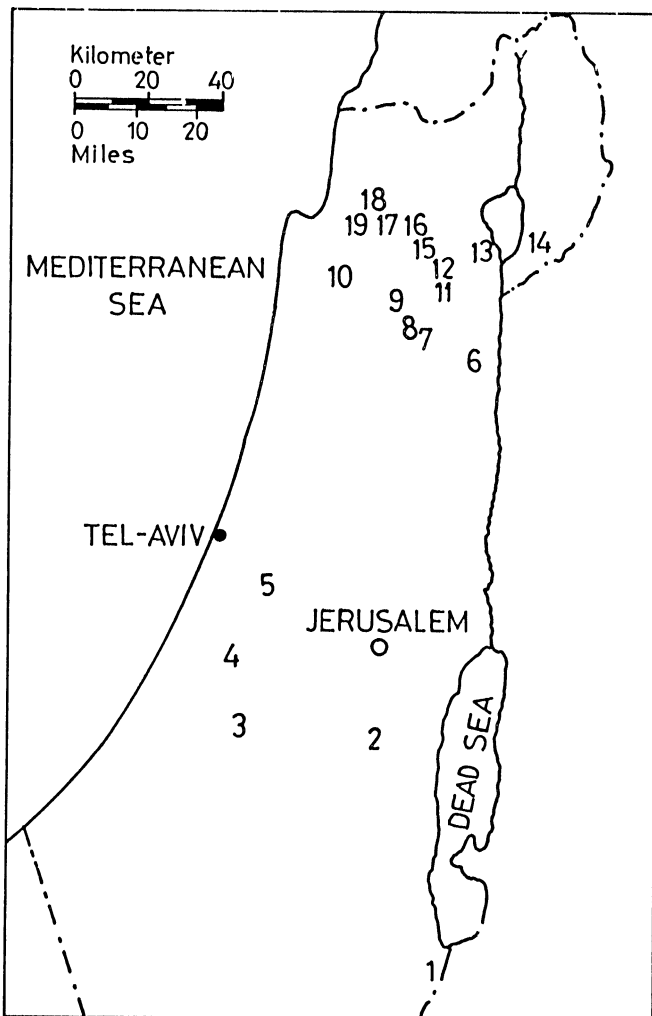


Fig. 1. Sites in Israel from which isolates of *Fusarium oxysporum* f. sp. *melonis* were obtained.

TABLE 1. Regional distribution and vegetative compatibility of isolates belonging to four physiologic races of *Fusarium oxysporum* f. sp. *melonis*

Site ^a	Number of Isolates														
	Total	Race 0			Race 1				Race 2			Race 1-2			
		VCG ^b 0135	VCG 0138		VCG 0135	VCG 0138		SI ^c	VCG 0135	VCG 0138		VCG 0135	VCG 0138		
			I	II		I	II			I	II		I	II	
1	5	4	1	
2	2	...	2	
3	1	1	
4	1	1	
5	1	1	
6	1	1	
7 ^d	1	1	...	
8	1	1	
9	4	4	
10	1	1	
11	14	12	2	
12	6	5	1	
13	1	1	
14	1	1	
15	22	2	1	19	
16	4	1	3	...	
17	23	16	2	5	...	
18	28	4	6	15	3	
19	1	1	...	
15-19 ^d	1	1	
Unknown	3	3	
Total	122	54	2	0	0	13	0	1	4	0	0	0	26	22	
No. of sites	19	12	1	6	...	1	2	6	2	

^a Sampling site numbers correspond to those in Fig. 1.

^b Vegetative compatibility group.

^c Vegetatively self-incompatible.

^d Sampling site uncertain.

TABLE 2. Isolates of *Fusarium oxysporum* f. sp. *melonis* from Israel used to distinguish vegetative compatibility groups

Isolate	Physiologic race	Site ^a
VCG 0135		
FOM-318 (ATCC 66052) ^b	0	5
FOM-637	0	13
FOM-714	0	8
FOM-ED2	0	11
FOM-RM2 ^b	2	15-19
FOM-UMI	2	16
VCG 0138I		
FOM-331/1	0	2
FOM-313A	1	1
FOM-SNIa ^b	1	11
FOM-SNII	1	11
FOM-373 ^b	1-2	17
FOM-374 ^b	1-2	17
VCG 0138II		
FOM-KNH ^b	1-2	15
FOM-KEI	1-2	15
Self-incompatible		
FOM-1451 ^b	1	3

^a Sampling site numbers correspond to those in Fig. 1.

^b Described in Cohen et al (2).

to comprise two race 0 strains from Israel and France (7). The other group did not correspond to any of the known VCGs and was therefore designated VCG 0138 (Table 2) in accordance with Puhalla's (15) numbering system.

Based on their ability to form strong heterokaryons with many mutants, two complementary *nit* mutants were chosen as testers of VCG 0135: FOM-RM2B/6 (NitM, parental isolate FOM-RM2, race 2) and FOM-U (*nit*1, parental isolate FOM-318, race 0). Similarly, complementary NitM tester strains were chosen for VCG 0138: FOM-SNIa/8 (parental isolate FOM-SNIa, race 1) and FOM-V (parental isolate FOM-374, race 1-2). However, in the experiments with VCG 0138, mutants of isolates FOM-KNH

and FOM-KEI, which formed complementary heterokaryons among themselves, often reacted weakly, slowly, or not at all with the testers of VCG 0138 and with additional mutants of this VCG. It was therefore suspected that VCG 0138 might consist of two subgroups, as described in other formae speciales of *F. oxysporum* (10,14). Therefore, two additional NitM testers were chosen for this VCG: FOM-KNH/7 and FOM-KEI/8 (parental isolates FOM-KNH and FOM-KEI, respectively, both race 1-2) (Table 2).

The *nit* mutants were generated from the remaining 107 isolates of *F. o. melonis* and from 23 isolates of *F. oxysporum* non-pathogenic to muskmelon. The isolates were assigned to VCGs according to the ability of their mutants to form stable complementary heterokaryons when paired with the three pairs of testers. Of 121 self-compatible isolates, 58 were assigned to VCG 0135, 41 to VCG 0138I, and 22 to VCG 0138II. Thus, only two VCGs were found among the *F. o. melonis* isolates of this collection, and no compatibility was found between isolates of the two VCGs. None of the 23 nonpathogenic self-compatible isolates were compatible with the *F. o. melonis* testers.

Assignment to VCG 0135 was usually based on a single mutant that formed visible heterokaryons with one or both testers within 5 to 8 days. A small number of isolates gave rise to mutants that formed weak or slow heterokaryons both among themselves (intrastrain) and with the testers. In such cases, several mutants were examined, and mutants of additional isolates were included as testers to verify the VCG assignment.

Assignment of isolates to the presumptive subgroups of VCG 0138 was also based at first on single mutants. Whereas all the mutants formed strong heterokaryons with one or both testers of one subgroup within 5 to 8 days, only a few of them exhibited a positive but weak reaction with the testers of the other subgroup, even after prolonged incubation. The morphology of those weak heterokaryons was similar to that observed between subgroups of *F. o. radialis-lycopersici* (10), characterized by slow growth, frequently discontinuous or as patches or dots of mycelial tufts. The greater tendency of an isolate to form heterokaryons with

the testers of one subgroup was verified using additional, complementary mutants. Of all the pairings attempted between mutants and testers of VCG 0138, the testers of subgroup I were able to recognize 40–50% of the isolates of subgroup II, the testers of subgroup II were able to recognize 55–70% of the subgroup I isolates, and each pair of testers was able to recognize 100% of the isolates in its respective subgroup. In addition to heterokaryon tests with tester strains, the ability of complementary mutants to form interstrain heterokaryons between subgroups of VCG 0138 was compared to heterokaryosis within the subgroups. Each pairing involved a NitM mutant of one isolate and a *nit1* or *nit3* mutant of another. Heterokaryon formation was recorded for up to 20 days (Table 3). The reactions between mutants within each subgroup were clearly positive in 89–96% of the pairings. In contrast, positive reactions between mutants of different subgroups were observed in only 19% of the pairings. Furthermore, many of the pairings between subgroups showed inconsistent or uncertain results, even after prolonged incubation (Table 3). These results indicate that the relationships between the testers of subgroups I and II are representative of the relationships between subgroups of VCG 0138.

VCGs and physiologic races. Race composition of the two VCGs showed the following trends (Table 4): most race 0 isolates (54 of 56, from 12 sites) were in VCG 0135, and only two (from one site) were in VCG 0138I (Tables 1 and 4). VCG 0135 also included all four race 2 isolates. On the other hand, all 13 self-compatible race 1 isolates (from six sites) were in VCG 0138I, which also included 26 race 1-2 isolates (from six sites), while the remaining 22 race 1-2 isolates were the only constituents of VCG 0138II. Thus, in this collection, races 1 and 1-2 were grouped together and were vegetatively distinct from race 2 (Table 4).

DISCUSSION

Two VCGs and four physiologic races were found among 122 isolates of *F. o. melonis* in Israel. One VCG corresponded to VCG 0135, which was previously known to include two isolates, one from France and one from Israel (6). The other VCG, 0138,

was new and could be divided into two subgroups, as has been reported for other formae speciales of *F. oxysporum* (10,14).

Previous studies reported VCG 0135 to be associated only with race 0. Our collection of VCG 0135 was predominantly race 0 (54/58 isolates) but also included four race 2 isolates. Although race 2 has been reported from North America, Japan, and Italy (7), no isolates have been found in VCG 0135. This suggests that race 2 in Israel was not an introduction from other areas known to be infested with *F. o. melonis*. Rather, race 2 in Israel may be of local origin. If so, it presumably was derived from race 0, the predominant virulence phenotype associated with VCG 0135. The limited distribution of race 2 (Table 1, Fig. 1) would be consistent with a recent derivation of this race from race 0.

All three races (0, 1, and 1-2) that are associated with VCG 0138 may have originated in Israel, as this VCG has not been identified anywhere else in the world. If so, the progenitor race in VCG 0138 could be race 0 although, unlike race 0/VCG 0135, race 0 isolates associated with VCG 0138 are not common. This may be an artifact of sampling, or it may be indication that race 0 is not the aboriginal pathotype in VCG 0138. The limited distribution of race 0/VCG 0138 may reflect low fitness of this strain or simply a chance lack of dispersal. It must be emphasized that because the collection did not result from a systematic survey, its race composition was probably affected by the sampling procedure and might also reflect the cultivars from which isolates were obtained.

Race 1-2 (VCG 0138), like race 2, is restricted to a small northern region of Israel, delimited by sites 15–19, which has a long history of cucurbit cropping. The presence of race 1-2 may reflect selection for mutant derivatives of local race 1 strains, owing to the widespread use of cultivars with the *Fom-2* gene. In other countries, either race 1 or race 2 (of VCG 0134) (7) could have served as intermediates in the evolution of race 1-2; however, race 2 in Israel cannot be considered such an intermediate because it is associated with a different VCG than race 1-2. Although selection based on the use of resistant cultivars is certainly plausible, Bouhot (1) reported that race 1-2 existed in France for at least 12 yr before resistant cultivars were introduced. This finding suggests that the presence of a particular race may not require selection pressure resulting from host resistance. A race's widespread occurrence can thus be explained.

Considerable diversity was evident in *F. o. melonis*, in respect to virulence phenotypes, in a small area of Northern Israel (region 15-19). However, all four races were included in two VCGs. Although race and VCG diversity already has been documented in a worldwide collection of *F. o. melonis* (6), levels of race diversity comparable to what we describe for a limited area have not been reported. As discussed above, inoculum introduced from other countries is unlikely to be a major contributor to this diversity. Major factors considered likely to be important, both in inoculum buildup and diversity, are frequent or even continuous cropping of muskmelons and regular opportunities for movement of inoculum by agricultural machinery, passing vehicles, and flooding of large areas almost every winter.

Our understanding of the genetic structure of *F. o. melonis* is based on the host resistance genes that define physiologic races, vegetative compatibility, and other pathogen traits. Additional information on the occurrence of races and VCGs should shed light on the evolution and worldwide spread of this important plant pathogen.

TABLE 3. Formation of heterokaryons between complementary *nit* mutants of the same or different subgroups of VCG 0138 of *Fusarium oxysporum* f. sp. *melonis*

Subgroup combination ^a	Heterokaryons ^b			Total ^c
	+	–	±	
I + I	24	0	1	25
II + II	17	1	1	19
I + II	7	20	10	37

^a Each pairing was between a NitM mutant and a *nit1* or *nit3* mutant, from different isolates. The mutants represented seven isolates of subgroup I and eight isolates of subgroup II. Tester strains not included.

^b Growth at the contact zone between *nit* mutants on nitrate minimal medium: + = complementation (including strong, discontinuous, weak, and slow types of heterokaryotic growth); – = no complementation; ± = uncertain.

^c Total number of isolate combinations examined (not all possible combination were tested).

TABLE 4. Occurrence of physiologic races in two vegetative compatibility groups (VCG) of *Fusarium oxysporum* f. sp. *melonis* in Israel

Race	VCG 0135	VCG 0138		SI ^a	Total
		I	II		
0	54	2	ND ^b	ND	56
1	ND	13	ND	1	14
2	4	ND	ND	ND	4
1-2	ND	26	22	ND	48
Total	58	41	22	1	122
No. of sites	13	11	2	1	19

^a Self-incompatible.

^b None detected.

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