

## Physiologic Races of *Fusarium oxysporum* f.sp. *melonis* in the Southeastern Anatolia Region of Turkey and Varietal Reactions to Races of the Pathogen

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Thirty-four isolates of *Fusarium oxysporum* f.sp. *melonis* (*F.o.m.*) obtained from 205 fields in melon-producing areas in the southeastern Anatolia Region of Turkey were identified on the basis of colony morphology and pathogenicity by the root dip method. In this region the mean prevalence of wilt disease was 88.1% and the mean incidence of disease was 47.5%. Physiologic races 0, 1, 2, and 1,2 of the pathogen were determined by their reactions on differential melon cultivars 'Charentais T,' 'Isoblon', 'Isovac' and 'Margot' in the greenhouse. Race 1,2, representing 58.8% (20/34) of all isolates, was widely distributed. Of the other pathogenic isolates, eight were identified as race 0, five as race 1, and one as race 2. This is the first report of physiologic races of *F.o.m.* in Turkey. Of 44 melon cultivars tested in the greenhouse for resistance to *F.o.m.* races, 36 were found to be moderately resistant to race 0, 17 were susceptible to race 1,2, 34.1% were highly resistant to race 1, and 52.2% had moderate resistance to race 2.

KEY WORDS: Melon; *Fusarium oxysporum* f.sp. *melonis*; race; varietal reactions.

### INTRODUCTION

The southeastern Anatolia Region accounts for 10.9% of the total melon (*Cucumis melo* L.) production of 1,900,000 tons in Turkey. Diyarbakır, Mardin and Adıyaman provinces, the main melon-producing areas of the region, have the largest sowing area with *ca* 7,800 ha, producing 208 kg/da (2). *Fusarium* wilt of melon, caused by *Fusarium oxysporum* Schlechtend.: Fr. f.sp. *melonis* (Leach & Currence) W. C. Snyder & H. N. Hansen (*F.o.m.*), is one of the most prevalent and destructive diseases of melon in Turkey (9,17) and other areas of the world (16). On diseased plants, the veins of some leaves turn yellow on one side; later these leaves become completely yellow, wilt and die. A longitudinal brown necrotic streak and gummy exudates appear on the basal portion of vines (12).

In the control of *Fusarium* wilt of melon, studies indicated that no chemical had a significant effect on pathogen population level in the plant (8), and pretreatment of melon seedlings with the herbicide dinitramine induced resistance to subsequent inoculation with *F.o.m.* (7). The most effective and practical means of controlling *Fusarium* wilt of melon

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is by the use of resistant cultivars. As there are at least four races of the pathogen, it is necessary to know which race is present in a given area before a cultivar is chosen (12).

Physiologic races of *F.o.m.* were identified based on the nomenclature proposed by Risser *et al.* (15) using three differential cultivars. The races are designated as 0, 1, 2, and 1,2 for the resistance genes they overcome (10); race 0 attacks only cultivars that lack any genes for resistance to *F.o.m.* (e.g. cv. 'Charentais T'); race 1 overcomes the resistance gene *Fom1* in cv. 'Doublon'; race 2 overcomes the resistance gene *Fom2* in cv. 'CM17187'; and race 1,2 overcomes both resistance genes. Race 1,2 is divided into race 1,2 wilt and yellowing strains (16). Two genes for resistance, *Fom1* and *Fom2*, are currently used to identify races of the pathogen (11). Race studies revealed that prior to 1985, only race 2 was found in North America, and races 0 and 1 in Europe and Israel. Between 1985 and 1987, races 0 and 1 were reported in Maryland (USA), race 0 in the Rio Grande Valley in Texas (USA) and Mexico, and race 1 in Jalisco, Mexico (10). Races 2 and 1,2 were found in Israel (6), and races 0, 1 and 1,2 in Lebanon (1).

The main objective of this study was to determine the prevalence and distribution of *F.o.m.* races in the southeast Anatolia Region of Turkey.

## MATERIALS AND METHODS

**Disease survey and isolation of *Fusarium oxysporum* f.sp. *melonis*** Systematic surveys for Fusarium wilt were carried out in the melon-growing areas of Diyarbakır, Adıyaman and Mardin provinces of the southeastern Anatolia Region of Turkey in July to September 1999 (Fig. 1). A total of 205 fields (ca 3255 ha), sown mostly to local cultivars, were inspected. The melon plants with typical Fusarium wilt symptoms were collected from fields in the major melon-producing areas of the region and taken to the laboratory. Incidence of plants showing foliar symptoms characteristic of Fusarium wilt was determined in a minimum of three representative groups of 30 consecutive plants (chosen arbitrarily) based on sizes of fields. In addition, prevalence of the disease was calculated using the percentage of fields with plants exhibiting disease symptoms.



Fig. 1. Major melon-producing areas in the southeastern Anatolia Region of Turkey.

For isolation, root and stem tissues of plants affected with wilt symptoms were washed under running tap water. Plant pieces taken from the lower hypocotyls and upper taproot

TABLE 1. Classification of *Fusarium oxysporum* f.sp. *melonis* races based on Risser *et al.* (15)

<i>Fusarium oxysporum</i> f.sp. <i>melonis</i> races	Differential Cultivars and Resistance Genes			
	Charentais T	Isoblon ( <i>Fom1</i> )	Isovac ( <i>Fom2</i> )	Margot
0	S	R	R	R
1	S	S	R	R
2	S	R	S	R
1,2	S	S	S	S

S= susceptible (yellowing and death); R= resistant (no symptoms, healthy plants).

were surface-sterilized in 1% NaOCl solution for 1 to 2 min, rinsed twice in sterile distilled water, and dried between sterile filter papers. Pieces of disinfested tissues were plated on potato dextrose agar (PDA) amended with 50  $\mu\text{g ml}^{-1}$  streptomycin sulfate. The plates were incubated at 27°C for 7–10 days (4). Colonies exhibiting the taxonomic features of *F. oxysporum* were identified according to Nelson *et al.* (13), and single-spore or hyphal tip subcultures of these isolates were made on PDA.

**Pathogenicity tests and race determination** Field isolates of *F.o.m.* were used for the pathogenicity tests. To confirm identity of the fungus, pathogenicity tests were conducted using the susceptible cv. ‘Kirkagac’. Melon seedlings were inoculated by the root-dip method using a spore suspension of  $10^6$  conidia  $\text{ml}^{-1}$  (18). Inoculated seedlings were transplanted to plastic pots filled with sterilized potting soil and placed in a greenhouse (18–20°C). Symptoms were evaluated 10 to 15 days after inoculation.

Four differential melon cultivars, ‘Charentais T’, ‘Isoblon’, ‘Isovac’ and ‘Margot’ (Table 1), were used in virulence tests for race identification according to the systems suggested by Risser *et al.* (15). Charentais T and Margot lack specific resistance genes, whereas Isoblon and Isovac carry the resistance genes *Fom1* and *Fom2*, respectively (14). Melon seeds treated with 7.5%  $\text{H}_2\text{O}_2$  solution for 10 min were planted in sterilized soil. After approximately 10 to 14 days, plants in the cotyledon to first-true-leaf stage were removed from the seedling pots and the roots were washed in tap water, pruned to ~2.5 cm and dipped for 1 min in the inoculum suspension ( $10^6$  conidia  $\text{ml}^{-1}$ ) prepared from 7- to 10-day-old cultures. The roots of control plants were pruned to ~2.5 cm and dipped only in tap water. The inoculated and control seedlings were transplanted into cell-type plastic growing trays filled with a sterilized mixture of soil, sand and manure (1:1:1), and placed in a greenhouse at 20–27°C by day and 18–22°C by night, with 12 h fluorescent light. Race determination was made by observing symptom development (yellowing, necrotic, wilted or dead seedlings) of the differential cultivars over a 4-week period (20). Final assessments of the wilt reaction were made 30 days after inoculation. Plants free of external wilt symptoms were considered resistant (19).

**Reactions of melon cultivars to races of wilt pathogen** Forty-four cultivars of melon obtained from Antalya Citrus Research Institute, Menemen Agricultural Research Institute, and seed companies (Seminis, Agromar and Altun Seed Co.) were evaluated for resistance to the wilt pathogen by the root-dip test. Procedures for sowing and inoculum preparation, concentration, inoculation technique, and evaluation were the same as described above. The experiment was conducted in a controlled environment chamber set at 23°C by day and 18°C by night, with 14 h fluorescent light. As a result of this trial, the cultivars were placed in four reaction classes (3): highly resistant (0–10% wilt), moderately resistant (11–40% wilt), slightly resistant (41–70% wilt), and susceptible (71–100% wilt).

## RESULTS

**Disease survey and isolation of *Fusarium oxysporum* f.sp. *melonis*** In the surveys, in which 602 plants obtained from 205 fields (~3255 ha) were sampled, *Fusarium* wilt of melon was observed early in the growing season as sudden wilting without yellowing. Plants infected early in the season often produced poor quality fruits. On diseased plants, symptoms resembling root rot or damping off were observed. Necrosis, gum exudates, stunting, vine death, and light to dark brown vascular discoloration were recorded on diseased plants late in the season. The *Fusarium* wilt surveys are summarized in Table 2, and results show that *Fusarium* wilt of melon was widespread in Diyarbakır, Adıyaman and Mardin provinces, with disease incidence being 53.3%, 42.5% and 46.6% (average, 47.5%), respectively; the prevalence of wilt was 96.0%, 86.8% and 81.5% (average, 88.1%), respectively.

TABLE 2. Results of survey on *Fusarium* wilt of melon carried out in 1999 in the southeastern Anatolia Region of Turkey

Area	Field location	No. of fields evaluated	Disease prevalence (%) <sup>z</sup>	Disease incidence (% foliar symptoms) <sup>y</sup>
Diyarbakır	Central	13	100.0	65.7
	Cinar	40	97.5	37.3
	Ergani	15	86.6	36.5
	Bismil	17	100.0	73.7
	Avg.	21	96.0	53.3
Adıyaman	Central	16	79.4	62.5
	Besni	33	93.6	34.8
	Golbası	9	88.6	15.4
	Kahta	14	85.6	57.4
	Avg.	18	86.8	42.5
Mardin	Central	10	90.0	54.6
	Nusaybin	17	65.0	38.7
	Midyat	10	80.0	41.7
	Savur	11	91.0	51.4
	Avg.	12	81.5	46.6
	General avg.	17	88.1	47.5

<sup>z</sup>Fields with melon plants showing foliar symptoms (%).

<sup>y</sup> Assessed late in the melon growing season in a minimum of three groups of 30 adjacent plants that were chosen arbitrarily in each field.

Typical cultures of *F.o.m.* were isolated from representative diseased plants with external symptoms. Pathogenicity was verified in melon cv. Kirkagac, which is susceptible to this wilt pathogen. Of the 34 pathogen isolates of *F.o.m.*, 15 isolates were identified from Diyarbakır, ten from Mardin, and nine from Adıyaman (Table 3). The pathogenicity tests revealed that these isolates caused 20–85% wilt. *F.o.m.* was re-isolated from inoculated melon plants.

**Race determination** The race identification of 34 isolates of *F.o.m.* obtained from melon-growing areas of the southeastern Anatolia Region was done according to the nomenclature proposed by Risser *et al.* (15) using the differential melon cultivars. Race

TABLE 3. Regional distribution of isolates belonging to physiologic races of *Fusarium oxysporum* f.sp. *melonis* obtained in surveyed melon fields

Area	Field location	No. of samples infected with <i>F.o.m.</i>	No. of pathogen isolates	Races of <i>F.o.m.</i>			
				0	1	2	1,2
Diyarbakır	Central	21	9	2	-	1	6
	Cınar	10	3	1	-	-	2
	Bismil	15	3	1	-	-	2
Mardin	Central	11	4	1	-	-	3
	Kızıltepe	8	2	1	-	-	1
	Nusaybin	6	3	1	-	-	2
Adıyaman	Central	9	3	1	1	-	1
	Besni	17	6	-	4	-	2
	Golbası	11	1	-	-	-	1
Total		108	34	8	5	1	20

0 isolates were pathogenic only to cv. Charentais T, race 1 isolates were pathogenic only to cvs. Charentais T and Isovac, and race 1,2 isolates were pathogenic to all differential cultivars. On the basis of the reaction of these four cultivars, the distribution of *F.o.m.* races was determined (Table 3).

Based on these findings, eight isolates (23.5%) were designated as race 0, five isolates (14.7%) as race 1, one isolate (2.9%) as race 2, and 20 isolates (58.8%) as race 1,2. Of the isolates recovered from Diyarbakır, 26.6% were assigned to race 0, 66.7% to race 1,2, and 6.7% to race 2; race 1 was not detected in this province. In Mardin, 33.3% of the isolates obtained were identified as race 0 and 66.7% as race 1,2; in this province races 1 and 2 were not found. In Adıyaman, 10% of the isolates obtained were classified as race 0, 50% as race 1, and 40% as race 1,2; race 2 was not detected in this province.

**Reactions of melon cultivars to races of wilt pathogen** Of the local and commercial cultivars of *C. melo* tested in the greenhouse for resistance to *F.o.m.* races 0, 1, 2, and 1,2 of *F.o.m.*, 81.8% proved moderately resistant to race 0, but no susceptible cultivar to this race was detected (Table 4). While 38.6% of the cultivars were susceptible to race 1,2, no cultivar was identified as highly resistant to races 0, 2, and 1,2. However, while 34.1% of the cultivars were classified as highly resistant to race 1, 52.2% of the cultivars proved moderately resistant to race 2.

## DISCUSSION

*Fusarium* wilt of melon may affect plants at all stages of the growing season. Systematic surveys in the melon-growing areas of the southeastern Anatolia Region of Turkey indicated that this wilt is a potentially serious threat to melon production in the region. In particular, the disease was determined to be very prevalent under dry conditions. During the surveys, sudden wilting, necrosis, gum exudates, stunting, vine death, and light to dark brown vascular discoloration were observed on diseased melon plants in the growing season. It was found that *F.o.m.* is widespread in Diyarbakır, Adıyaman and Mardin provinces. The figures on disease incidence and prevalence in these provinces (Table 2) were similar to those reported for the melon-growing areas of the Mediterranean Region of Turkey in 1991 and 1992: disease incidence and prevalence of 66.6% and 72.2% in

TABLE 4. Reaction of melon (*Cucumis melo*) cultivars to artificial inoculation with different races of *Fusarium oxysporum* f.sp. *melonis* (*F.o.m.*) under greenhouse conditions

Melon cultivars	Varietal reactions to races of <i>F.o.m.</i>			
	Race 0	Race 1	Race 2	Race 1,2
8 F1 Galia type	MR	HR	MR	MR
34 F1 Ananas type	MR	MR	SR	MR
33 F1 Ananas type	MR	SR	MR	SR
Amilara	MR	MR	S	SR
Peteg (Hasocerko)	MR	S	S	S
Azizo	MR	SR	S	S
Diyarbakır Yuvarlak	MR	S	S	S
Diyarbakır Uzun	MR	S	S	SR
Adıyaman	SR	MR	MR	S
Diyarbakır Dilimli	MR	MR	SR	S
Adıyaman Cumra	SR	S	S	S
Altınbas	MR	MR	S	MR
Kırkgaç	MR	S	S	MR
Cesme melon 7/8	SR	HR	SR	SR
Cesme melon 1/3	SR	MR	SR	SR
Cesme melon 1/1	MR	MR	SR	MR
Cesme melon 5/11	MR	HR	MR	MR
Cinikız-98	MR	MR	S	MR
Hasanbey	SR	MR	MR	SR
Kırkgaç-589	SR	MR	MR	MR
Kırkgaç-637	MR	HR	MR	MR
Babieca RZ 929	MR	SR	MR	MR
Makdimon	MR	HR	SR	S
Aıkıdo F1 hibrit	MR	MR	MR	S
1051 Rito	SR	MR	MR	S
Bergpass F1	MR	MR	MR	SR
Altın 2 F <sub>1</sub>	SR	MR	MR	S
Altın 4 F <sub>1</sub>	MR	MR	MR	MR
Altın 1 F <sub>1</sub>	MR	MR	MR	S
Altın 3 F <sub>1</sub>	MR	MR	MR	SR
Royal Sluis 4100878	MR	HR	SR	SR
Royal Sluis RS 455	MR	HR	SR	S
Royal Sluis RS 605	MR	HR	MR	S
Royal Sluis RS 296	MR	HR	MR	S
Royal Sluis RS 692	MR	HR	MR	S
Royal Sluis RS 894	MR	HR	MR	S
Royal Sluis RS 895	MR	HR	MR	S
Royal Sluis RS 537	MR	HR	SR	SR
Royal Sluis RS 160	MR	HR	SR	SR
Royal Sluis RS 095	MR	HR	SR	MR
Agromar Winter melon	MR	MR	MR	MR
Agromar Kırkgaç	MR	MR	MR	SR
Agromar Altınbas	MR	MR	SR	MR
Agromar Hasanbey	MR	MR	MR	SR

HR, highly resistant; MR, moderately resistant; SR, slightly resistant; S, susceptible.

Adana, and 66.6% and 92.8% in Hatay, respectively (17). The disease is reported to be long established in the central Anatolia Region of Turkey (9).

Identification of *F.o.m.* on differential melon cultivars revealed four physiologic races in the region, with race 1,2 being the most prevalent. In ecological terms, this point can be expressed as the parasite being capable of diversifying its pathogenic potential in the

absence of the host, and the host plant playing a dual role in the process of the appearance of new races (5). All isolates were highly virulent on susceptible cv. Charentais. Eight isolates obtained from three provinces of the region surveyed were virulent only to cv. Charentais, and therefore identified as race 0. Of the race 1 isolates, five were recovered only from melon fields of Adıyaman. These isolates were avirulent on cvs. Isovac (*Fom2*) and Margot, and virulent on cvs. Charentais T and Isoblon (*Fom1*). Only one isolate from Diyarbakır was assigned to race 2; this isolate was virulent on cvs. Charentais T and Isovac, and avirulent on cvs. Isoblon and Margot. Of the race 1,2 isolates, ten were recovered from Diyarbakır, six from Mardin, and four from Adıyaman; all differential cultivars were highly susceptible to these races. races 1 and 2 were not found in two of the three counties surveyed. Race 1,2, the most prevalent race in the region, was reported in previous studies carried out in other regions of Turkey (9,17).

Resistance to *F.o.m.* is expressed very clearly at the seedling stage and the mechanism of action of this resistance is believed to be controlled by genes *Fom1* and *Fom2* using the phenomenon of host–parasite interaction recognition. This study demonstrated that local cultivars such as ‘Peteg’ (Hasocerko) and ‘Azizo’, which are commonly grown in the region, lacked resistance to *Fusarium* wilt, in particular to race 1,2. In Diyarbakır, where the disease is the most prevalent, local cultivars have been grown repeatedly in the same fields. Currently, race 1,2 is responsible for heavy losses in the fields where this race was first recorded. In conclusion, because *Fusarium* strains vary widely with respect to their pathogenicity, the resistance of different melon cultivars to Turkish strains of *F.o.m.* should be evaluated every year in an attempt to develop resistant cultivars carrying genes *Fom1* and *Fom2*.

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