Europ.J.Hort.Sci., **72** (5). S. 220–227, 2007, ISSN 1611-4426. © Verlag Eugen Ulmer KG, Stuttgart

Response of Turkish Melon Genotypes to *Fusarium oxysporum* f. sp. *melonis* Race 1 Determined by Inoculation Tests and RAPD Markers

S. Sensoy[1)], S. Demir[2)], S. Buyukalaca[3)] and K. Abak[3)]
([1)]Yüzüncü Yıl University, Agriculture Faculty, Department of Horticulture, Van, [2)]Yüzüncü Yıl University, Agriculture Faculty, Department of Plant Protection, Van, and [3)]Çukurova University, Agriculture Faculty, Department of Horticulture, Adana, Turkey)

Summary

The response to *Fusarium oxysporum* f. sp. *melonis* (*F.o.m.*) race 1 of 79 *Cucumis melo* L. genotypes - 56 accessions collected from different parts of Turkey, 5 local cultivars and 18 foreign genotypes - was determined by using pathogenicity tests and RAPD markers (E07 and G17). For pathogenicity tests, at least 15 individuals of each melon genotype were artificially inoculated by either conidia suspension culture or sand culture of *F.o.m.* race 1. Melon seedlings were evaluated on a 0–3 scale for the presence of vascular browning two weeks after inoculation with conidia suspension culture resp. four weeks after inoculation with sand culture. The disease incidence on the 79 melon genotypes ranged from 0 to 100 %. Seven melon genotypes were resistant (CU129, CU258, T8, Y9, Y10, Y15, and Y63), three melon genotypes (CU280, CU309, and T1) were heterogeneous, and all the others were susceptible to *F.o.m.* race 1 based on disease incidence and vascular browning. The success of the E07 marker in detecting susceptible genotypes was higher than that of G17; the mistmatch ratios of E07 and G17 markers were 5.06 and 58.23 %, respectively.

Key words. *Cucumis melo* – fusarium wilt – inoculation – local germplasm – susceptibility – vascular browning

Introduction

Fusarium oxysporum f. sp. *melonis* (*F.o.m*), an important soil-borne pathogen of melon, consists of four races (0, 1, 2, and 1,2) and is commonly found in Turkey as well as in many other parts of the world; In Turkey, the most prevalent are races 0 and 1 (Cohen et al. 1989a, 1996; Zink 1990; 1991; Jacobson and Gordon 1991; Windels 1993; Katan et al. 1994; Sari et al. 1994; Yucel et al. 1994; Erzurum et al. 1999; Schreuder et al. 2000; Kurt et al. 2002). The pathogen causes a reduction of the water and nutrient uptake of the infected plants, and results in yellowing of leaves, wilting of branches, and necrosis of stems, especially close to harvest time, so that the quality and quantity of melon production is reduced (Windels 1993; Martyn and Gordon 1996).

It is known that characteristics of environmental, plant, pathogenic factors and plant-pathogen-interaction are effective in the development of the soil-borne fusarium wilt (Cohen et al. 1996; Schreuder et al. 2000; Burger et al. 2003). Therefore, chemical treatments, which are effective against other soil-borned diseases, are ineffective against the fusarium wilt. The application of fungicides is also impractical and cost-intensive so the use of resistant cultivars or rootstocks has become more important in the control of fusarium wilt (Scherf and Mancab 1986; Martyn and Gordon 1996; Nisini et al. 2002). The resistance sources in melon germplasm are known: *Fom-1* and *Fom-3* genes confer resistance to races 0 and 2 while *Fom-2* gene confers resistance to races 0 and 1 (Zink and Thomas 1990; Zink 1991; Pitrat 1998).

Although pathogenicity tests have been employed to determine the susceptibility levels of melon genotypes to fusarium wilt (Turhan and Grosman 1987; Lecoq et al. 1991), large variations in the reaction of varieties have been observed even under controlled conditions. Burger et al. (2003) observed that light and heat intensity had a significant effect on the occurrence of *Fusarium* symptoms. Similarly, Cohen et al. (1989b) determined that disease incidence increased at low light intensity and in the presence of the herbicide acetochlor, both of which cause sugar accumulation. It was also noted that at least six weeks were required for reliable symptom expression (Wang et al. 2000). Because of these confounding factors, molecular DNA markers are an attractive aid in the quest to determine fusarium resistance in melon (Baudracco-Arnas and Pitrat 1996; Wetcher and Dean 1998; Zheng et al. 1999; Zheng and Wolff 2000; Wang et al. 1997, 2000; Burger et al. 2003; Joobeur et al. 2004).

Baudracco-Arnas and Pitrat (1996) found E07 and G17 RAPD markers linked to the *Fom-2* gene (1.6 and 4.5 cM, respectively) by constructing a genetic linkage map of 218 F_2 individuals from a cross between 'Vedrantais' and PI 161375. RAPD marker 596 was identified only in the multi-disease resistant breeding line MR-1 and produced a 1.6 kb band (Wetcher et al. 1995).

Zheng and Wolff (2000) studied the availability of RAPD markers for the marker-assisted selection of melon genotypes, which are resistant or susceptible to *Fusarium*

race 0 and 1. These researchers employed the RAPD markers E07, G17, and 596, the first two linked to a susceptible allele and the last linked to a resistance allele of the *Fom-2* gene, on 89 melon genotypes, 48 of which were resistant. They found that these markers correctly matched phenotypes in 88, 81, and 70 % of the genotypes. The combination and evaluation of both scores, E07 and G17, gave even higher correct matching (95 %).

Although some SCAR (Wetcher and Dean 1998), CAPS (Zheng et al. 1999), and SSR (Wang et al. 2000) markers developed in the determination of *Fom-2* resistance in melon, we employed RAPD markers in the present study due to the simplicity and cost efficiency of the method (McGregor 2000).

Asia, from the Mediterranean Sea to Japan, is the secondary centre of diversification of melon, and Turkey resp. Anatolia have a great genetic variability in this species (Pitrat et al. 1998; Kucuk et al. 2002). The collection studies of melon genotypes have been carried out in Turkey since the 1960's and good collections of melon germplasm have been formed (Kucuk et al. 2002). One of the rich Anatolian melon collections is in University of Cukurova, Adana, Turkey. The genetic relatedness among the core collection of this germplasm has been evaluated by phenotypically and morphologically (Gomez-Guillamon et al. 2004). Moreover, the investigations about the genetic potential of this germplasm to biotic and abiotic stress conditions have been started. The present study is the one of first attempts for evaluation of this broad Turkish melon germplasm to one of the most important pathogens of melon, *Fusarium*. This study aimed to find whether there were fusarium wilt resistant genotypes in Turkish melon germplasm and to see whether the already known RAPD markers were efficient for selection of susceptible ones.

Material and Methods

Plant material

Turkey's melon genotypes were obtained from the melon germplasm of the Cukurova University in Adana, Turkey. This melon germplasm constitutes more than three hundred genotypes. The detailed genetic studies have been launched by choosing a core collection among this germplasm. The core collection with fifty-six Turkish melon (*Cucumis melo* L.) accessions collected from different parts of Turkey together with 5 local and 18 foreign melon genotypes (Σ= 23) constitute the plant material of the present study (Table 1). Among the reference accessions are five genotypes with known resistance reaction to *F.o.m.* race 1 including the resistant 'Isabelle' (75=Y15) and 'Shiro Uri Okoyama' (69=Y9)) and the susceptible 'Topmark' (61=Y1), 'Vedrantais' (63=Y3), and 'Honeydew Green Flesh' (65=Y5) (Baudracco-Arnas and Pitrat 1996; Zheng and Wolff 2000).

Fungal culture, host inoculation, and disease scoring for fusarium wilt

At least 15 individuals of each melon genotypes were artificially inoculated two times by either conidia suspension (Lecoq et al. 1991) or sand culture (Turhan and Grosman 1987) of *F.o.m.* race 1 (A-49 isolate of *F.o.m.* was obtained from Ankara University Plant Protection Department). The mycelia were cultured on potato dextrose agar (PDA) plates at 25±2 °C and used in the preparation of cultures. To check the pathogenicity level of this isolate, the control genotype, 'Kirkagac', was inoculated with it and a very high pathogenicity level (100 %) was observed

In suspension cultures, surface-disinfected melon seeds submerged in 1.5 % sodium hypochlorite for 5 min and rinsed in water were sown in 15x10x5 cm pots containing autoclaved sand. The two-week-old seedlings were uprooted, their roots were slightly trimmed and dipped in a conidial aqueous suspension of 10^6 conidia ml^{-1} for two minutes before transplanting them to plastic containers (20x10x10 cm) filled with autoclaved sand.

In the sand culture method, corn meal (20 g), agarose (20 g), and washed coarse river sand (120 g) were combined in 350 ml glass bottles, and were watered and then sterilized by autoclaving. Seven to ten-day-old *F.o.m.* isolates grown on PDA cultures were mixed to the prepared sterilized medium at the rate of one-fourth petri dish to each bottle. Final mixture was incubated at 25±2 °C for 4 weeks. These four-week-old cultures were mixed with autoclaved sand at the rate of 5 % (v/v). The final mixture was divided into plastic containers (20x10x10 cm), which received surface-disinfected melon seeds (in 1.5 % sodium hypochlorite for 5 min and rinsed in water).

All plastic containers were placed in a growth chamber at 25±1 °C by day and 18±1 °C by night, with 12 h fluorescent light. The intensity of illumination was 5.000 lux. Symptoms were determined after two weeks for suspension cultures and after four weeks for sand cultures with a 0–3 scale (Demir et al. 2006) and disease incidence was calculated according to the following equation;

Disease incidence (intensity) (%)

$= ((0*_{n0}) + (1*n_1) + (2*n_2) + (3*n_3))*100 / n*3$

where n_0 was the number of plants with no symptoms, n_1 was the number of plants having one third necrosis at the stem-root-neck, n_2 was the number of plants having two third necrosis at the stem-root-neck, n_3 was the number of dead plants, n was the total plant number, and 3 was the highest value at the scale.

Susceptibility or resistance reactions of melon genotypes to *F.o.m.* race 1 were established by a modified scale from Hassan Dar et al. (1997) and five different reaction classes were formed as: highly resistant (0–10 %), resistant (11–25 %), slightly resistant (or slightly susceptible) (26–50 %), susceptible (51–75 %), highly susceptible (76–100 %).

In addition, the vascular systems of all seedlings were examined for presence of internal browning or discoloration in the stem-root-neck of seedlings.

DNA extraction

About 20 seeds of each genotype were germinated in a greenhouse at the University of Yuzuncu Yıl, Van. Genomic DNA was extracted from leaf tissue sampled in equal amounts from at least 15 plants at the two-to-tree leaf stages, employing the CTAB procedure (Doyle and Doyle 1987).

RAPD amplification

Two 10-mer primers from Operon Technologies (E07 and G17) and one 10-mer primer from the University of

Table 1. Origins, donors, and names of melon accessions, their reactions to A-49 (*F.o m.* race 1) – disease incidence, reaction level and vascular discoloration – and the presence of RAPD markers.

	Geno-type	Origin	Original/ Seed Donor*	Donor ID	Disease incidence (%)			Reaction level**			Vascular discoloration %			Final Reac-tion ***	RAPD-Marker	
					Susp.	Sand	Mean	Susp.	Sand	Mean	Susp.	Sand	Mean		E 07	G 17
1	CU-1	Van	YYU/CU	A01	100.0	100.0	100.0	5	5	5.0	100.0	100.0	100.0	S	+	+
2	CU-2	Banaz	YYU/CU	A02	26.7	77.8	52.2	3	5	4.0	80.0	77.8	78.9	S	+	–
3	CU-4		CU	Ananas selection	86.7	100.0	93.3	5	5	5.0	86.7	100.0	93.4	S	+	+
4	CU-15	Midyat	YYU/CU	A22	93.3	62.50	77.9	5	4	4.5	100.0	62.5	81.3	S	+	–
5	CU-20	Yalova	YYU/CU	A32	80.0	71.43	75.7	4	5	4.5	80.0	100.0	90.0	S	+	–
6	CU–23	Yalova	YYU/CU	A35	55.6	95.56	75.6	4	5	4.5	66.7	100.0	83.3	S	+	+
7	CU–33	Bilecik	YYU/CU	B13	46.7	86.7	66.7	3	5	4.0	70.0	100.0	85.0	S	+	+
8	CU–37		AU/CU		100.0	100.0	100.0	5	5	5.0	100.0	100.0	100.0	S	+	–
9	CU–38		AU/CU		46.7	100.0	73.3	3	5	4.0	100.0	100.0	100.0	S	+	–
10	CU–45	Turkmen	Turkmen/ CU	Turkmen 2	0.0	85.71	42.9	1	5	3.0	0.0	85.7	42.9	S	+	–
11	CU–56	Kirklareli	YYU/CU	B42	33.3	100.0	66.7	3	5	4.0	75.0	100.0	87.5	S	+	–
12	CU–57	Elazig	YYU/CU	B43	100.0	66.7	83.3	5	4	4.5	100.0	100.0	100.0	S	+	+
13	CU–63	Mardin	YYU/CU	C05	3.3	97.78	50.6	1	5	3.0	10.0	100.0	55.0	S	+	–
14	CU–65	Erzincan	YYU/CU	C07	100.0	100.0	100.0	5	5	5.0	100.0	100.0	100.0	S	+	+
15	CU–69	Adiyaman	YYU/CU	C12	54.2	100.0	77.1	4	5	4.5	100.0	100.0	100.0	S	+	+
16	CU–73	Yalova	YYU/CU	C16	83.3	72.2	77.8	5	4	4.5	100.0	83.3	91.7	S	+	–
17	CU–78	Van	YYU/CU	D01	66.7	100.0	83.3	4	5	4.5	100.0	100.0	100.0	S	+	–
18	CU–101	Turkmen	Turkmen/ CU		83.3	100.0	91.7	5	5	5.0	100.0	100.0	100.0	S	+	–
19	CU–104		Agromar/ CU	Kis kavunu	25.0	88.9	56.9	2	5	3.5	25.0	100.0	62.5	S	+	–
20	CU–106		Agromar/ CU	Hasanbey	33.3	92.3	62.8	3	5	4.0	75.0	100.0	87.5	S	+	–
21	CU–116		Adiya-man/ CU	Saf turuncu	25.0	90.5	57.7	2	5	3.5	25.0	100.0	62.5	S	+	+
22	CU–120	Polatli	YYU/CU	A14	0.0	100.0	50.0	1	5	3.0	0.0	100.0	50.0	S	+	–
23	**CU–129**	**Azerbai-jan**	**Azer-baijan/ CU**	**Nahcivan 3**	**0.0**	**0.0**	**0.0**	**1**	**1**	**1.0**	**0.0**	**0.0**	**0.0**	**R**	**+**	**–**
24	CU–131	Ankara	Kazan/CU	Yuva	44.4	80.0	62.2	3	5	4.0	75.0	86.7	80.8	S	+	–
25	CU–135		EARI/CU	Cinikiz	25.0	82.2	53.6	2	5	3.5	50.0	80.0	65.0	S	+	–
26	CU–139		EARI/CU	Cesme 1/8	50.0	0.0	25.0	3	1	2.0	50.0	100.0	75.0	S	+	–
27	CU–151	Van	S. Sensoy/ CU	2000–3 (Mezmeze)	30.0	51.1	40.6	3	4	3.5	50.0	66.7	58.3	S	+	+
28	CU–175	Viransehir	CU	Salengo 5	55.6	75.6	65.6	4	5	4.5	83.3	66.7	75.0	S	+	+
29	CU–192	Viransehir	CU	Sekerpare	63.3	100.0	81.7	4	5	4.5	77.8	100.0	88.9	S	+	–
30	CU–196	Midyat	CU		44.4	66.7	55.6	3	4	3.5	60.0	100.0	80.0	S	+	–
31	CU–198	Midyat	CU		0.0	100.0	50.0	1	5	3.0	37.5	100.0	68.8	S	+	–
32	CU–203	Silvan	CU	Haci Haso	14.3	100.0	57.2	2	5	3.5	42.9	100.0	71.4	S	+	+
33	CU–206	Batman	CU	Azizo 2	70.0	100.0	85.0	4	5	4.5	100.0	100.0	100.0	S	+	–
34	CU–208	Diyarbakir	CU		61.9	66.7	64.3	4	4	4.0	57.1	66.7	61.9	S	+	+
35	CU–229	Balikesir	CU	Hirsizcal-maz	3.3	93.3	48.3	1	5	3.0	10.0	93.3	51.7	S	+	–
36	CU–234	Susurluk	CU	Acur Kavunu	21.4	80.0	50.7	2	5	3.5	35.7	60.0	47.9	S	+	+
37	CU–236	Gonen	CU	Gonen Beyazi	22.2	44.44	33.3	2	3	2.5	33.3	50.0	41.7	S	+	–

Table 1. Continue

	Genotype	Origin	Original/ Seed Donor*	Donor ID	Disease incidence (%)			Reaction level**			Vascular discoloration %			Final Reaction ***	RAPD-Marker	
					Susp.	Sand	Mean	Susp.	Sand	Mean	Susp.	Sand	Mean		E07	G17
38	CU–237	Balıkesir	CU	Gonen Beyazi	8.3	83.3	45.8	1	5	3.0	33.3	92.9	63.1	S	+	–
39	CU–238	Gonen	CU	Gonen Sarisi	100.0	100.0	100.0	5	5	5.0	100.0	100.0	100.0	S	+	–
40	CU–240	Biga–Erdek	CU		20.0	60.0	40.0	2	4	3.0	40.0	80.0	60.0	S	+	–
41	CU–252	Kayadibi	CU	Kirkagaç	100.0	93.3	96.7	5	5	5.0	100.0	100.0	100.0	S	+	–
42	**CU–258**	**Van**	**YYU**	**65ER05**	**0.0**	**0.0**	**0.0**	**1**	**1**	**1.0**	**0.0**	**0.0**	**0.0**	**R**	**–**	**–**
43	CU–269	Van	YYU	65ERC02–Semame	33.3	87.9	60.6	3	5	4.0	60.0	100.0	80.0	S	+	–
44	CU–272	Van	YYU	65ERC05	33.3	88.9	61.1	3	5	4.0	66.7	100.0	83.3	S	+	–
45	**CU–280**	**Van**	**YYU**	**65ERC13**	**0.0**	**23.8**	**11.9**	**1**	**2**	**1.5**	**0.0**	**21.4**	**10.7**	**H**	**+**	**–**
46	CU–286	Van	YYU	65ERC19	66.7	100.0	83.3	4	5	4.5	86.7	100.0	93.3	S	+	–
47	CU–305	Adana	CU	Yabani kavun	16.7	0.0	8.3	2	1	1.5	10.0	0.0	5.0	S	+	+
48	CU–307	Tekirdag	CU	Topatan	33.3	95.6	64.5	3	5	4.0	33.3	100.0	66.7	S	+	+
49	CU–308	Tekirdag	CU	Suluklu	40.0	95.6	67.8	3	5	4.0	80.0	100.0	90.0	S	+	+
50	**CU–309**	**Tekirdag**	**CU**	**Ziraat–Beyaz**	**0.0**	**44.4**	**22.2**	**1**	**3**	**2.0**	**20.0**	**33.3**	**26.7**	**H**	**+**	**–**
51	CU–310	Kirklareli	CU	Bagributun	20.0	70.4	45.2	2	4	3.0	80.0	33.3	56.7	S	+	–
52	CU–311	Kirklareli	CU	Sari Kislik	64.4	100.0	82.2	4	5	4.5	86.7	100.0	93.3	S	+	–
53	CU–315	Edirne	CU	Cobanaldatan	48.7	100.0	74.4	3	5	4.0	84.6	100.0	92.3	S	+	+
54	CU–323	Ankara	CU	Karakavun	0.0	64.4	32.2	1	5	3.0	0.0	60.0	30.0	S	+	–
55	CU–326	Ankara	CU		66.7	85.7	76.2	4	5	4.5	100.0	85.7	92.9	S	+	+
56	CU–327	Ankara	CU		33.3	75.6	54.5	3	5	4.0	33.3	86.7	60.0	S	+	–
57	**T1**	**Turkey**	**Pinaper Seed**	**Kırkagaç 637 (Altınbaş) (Ino)**	**0.0**	**7.4**	**3.7**	**1**	**1**	**1.0**	**33.3**	**11.1**	**22.2**	**H**	**–**	**–**
58	T5	Turkey	İstanbul Toh.	Ananas (Can)	–	100.0	100.0	–	5	5.0	–	100.0	100.0	S	+	+
59	T6	Turkey	Bursa Toh.	Acur (Flex)	31.1	100.0	65.6	3	5	4.0	60.0	100.0	80.0	S	+	+
60	**T8**	**Turkey**	**Anadolu Toh.**	**Galia (Can)**	**0.0**	**0.0**	**0.0**	**1**	**1**	**1.0**	**0.0**	**0.0**	**0.0**	**R**	**+**	**–**
61	Y1	USA	Pitrat–France	Topmark (Can)	44.4	100.0	72.2	3	5	4.0	66.7	100.0	83.3	S	+	+
62	Y2	USA	Pitrat–France	Hale's Best (Can)	100.0	100.0	100.0	5	5	5.0	100.0	100.0	100.0	S	+	+
63	Y3	France	Pitrat–France	Vedrantais TG–94 (Can)	–	83.3	83.3	–	5	5.0	–	87.5	87.5	S	+	+
64	Y4	Israel	Pitrat–France	Ogen 2 (Can)	22.2	83.3	52.8	2	5	3.5	33.3	75.0	54.2	S	+	–
65	Y5	USA	Pitrat–France	Honeydew Green Flesh (Ino)	33.3	100.0	66.7	3	5	4.0	50.0	100.0	75.0	S	+	+
66	Y6	Spain	Pitrat–France	Piel de Sapo 134 (Ino)	–	66.7	66.7	–	4	4.0	–	100.0	100.0	S	+	–

Table 1. Continue

	Geno-type	Origin	Original/ Seed Donor*	Donor ID	Disease incidence (%)			Reaction level**			Vascular discoloration %			Final-Reac-tion ***	RAPD-Marker	
					Susp.	Sand	Mean	Susp.	Sand	Mean	Susp.	Sand	Mean		E 07	G 17
67	Y7	Spain	Pitrat–France	Rochet 2 (Ino)	66.7	25.0	45.8	3	2	2.5	100.0	25.0	62.5	S	+	–
68	Y8	Spain	Pitrat–France	Amarillo 140 (Ino)	22.2	100.0	61.1	2	5	3.5	66.7	100.0	83.3	S	+	–
69	**Y9**	**Japan**	**Pitrat–France**	**Shiro Uri Okoyama (Con)**	**0.0**	**0.0**	**0.0**	**1**	**1**	**1.0**	**0.0**	**0.0**	**0.0**	**R**	**–**	**–**
70	**Y10**	**Cuba**	**Pitrat–France**	**Cuba 3 (Chi)**	**0.0**	**0.0**	**0.0**	**1**	**1**	**1.0**	**0.0**	**0.0**	**0.0**	**R**	**–**	**–**
71	Y11	Turkey	Pitrat–France	PI 177362 (Dud)	6.7	84.4	45.6	1	5	3.0	6.7	86.7	46.7	S	+	–
72	Y12	India	Pitrat–France	PI 414723 TG95 (Mom)	50.0	100.0	75.0	3	5	4.0	50.0	0.0	25.0	S	+	–
73	Y13	Sudan	Pitrat–France	Humaid 95–1 (Agr)	100.0	100.0	100.0	5	5	5.0	100.0	100.0	100.0	S	+	+
74	Y14	Sudan	Pitrat–France	HSD 192 (Fle)	100.0	100.0	100.0	5	5	5.0	100.0	100.0	100.0	S	+	–
75	**Y15**	**France**	**Pitrat–France**	**Isabelle (Can)**	**0.0**	**0.0**	**0.0**	**1**	**1**	**1.0**	**0.0**	**0.0**	**0.0**	**R**	**–**	**–**
76	Y17	Leen de Mos	Staub–USA	Ref no: 23849 (Can)	100.0	100.0	100.0	5	5	5.0	100.0	100.0	100.0	S	+	+
77	Y31	Israel	Perl–Treves–Israil	Arava F #165 (Can)	20.0	100.0	60.0	2	5	3.5	40.0	100.0	70.0	S	+	–
78	Y62	India	Pitrat–France	PI124112 (Mom)	44.4	100.0	72.2	3	5	4.0	66.7	100.0	83.3	S	+	–
79	**Y63**	**Korea**	**Pitrat–France**	**PI161375 (Con)**	**11.1**	**0.0**	**5.6**	**2**	**1**	**1.5**	**0.0**	**0.0**	**0.0**	**R**	**–**	**–**
				Mean	40.3	75.3	59.4	2.8	4.3	3.6	55.0	78.0	68.3			

*AU: Ankara University; CU: Cukurova University; EARI: Agean Agricultural Research Institute; YYU: Yuzuncu Yil University; Turkmen: Turkmenistan;
Cucumis melo: Can: Cantalupensis Grp.; Chi: Chito Grp.; Con: Conomon Grp. Dud: Dudaim Grp; Ino: Inodorus Grp. Fle: Flexuosus Grp; Agr: subsp. *agrestis*; Mom: subsp. *agrestis* var. *momordica*.
Susp.: Suspensions culture; Sand: Sand culture
**: 1: Highly resistant (0–10 %); 2: Resistant (11–25 %); 3: Slightly resistant or susceptible (26–50 %); 4: Susceptible (51–75 %); 5: Highly susceptible (76–100 %)
***: S: susceptible; R: resistant; H: heterogenous

British Columbia (BC596), the first two linked to a susceptible allele and the last linked to a resistance allele of *Fom-2* gene, were examined based on the study of Zheng and Wolff (2000). The optimised reaction contained 30 ng DNA, 0.2 μM primer, 100 μM dNTPs, 1 U Taq DNA Polymerase (Fermentas), 100 mM TRIS-HCl, 1.5 mM $MgCl_2$, and 50 mM KCl, pH 8.8, in a 15-μl final volume. DNA reactions were performed in a Model 212-1CE thermal cycler (Lab-Line Instruments Inc.). After 5 min of heating at 94 °C, amplifications were performed under the following regime: 40 cycles of 60 s at 94 °C, 63 s 36 °C, 59 s ramps, 120 s 72 °C, a final extension reaction of 10 min at 72 °C. Reactions were replicated at least twice to control reproducibility of patterns. After amplification, PCR products were analysed in 1.5 % agarose gels in 1* TAE at 90 V using a Model 192 horizontal gel electrophoresis system (BIO-RAD) for 3 h and stained with ethidium bromide and photographed by the gel documentation analysis system (Syngene, UK).

Results

Reaction of melon genotypes to F.o.m. *race 1 based on inoculation tests*

The isolate A-49 had higher pathogenicity on melon genotypes and typical symptoms of the disease were observed and re-isolation of this isolate was obtained from the plants having symptoms in the trial. Disease incidence and reaction levels of melon genotypes to A-49 isolate of *F.o.m.* race 1 based on 0–3 scaling are presented in Table 1. Disease incidence of A-49 isolate on 79 melon genotypes ranged between 0 % and 100 %. Sand culture caused 34.95 % higher disease incidence than the suspension culture. Final decision of the reaction types of the melon genotypes was done according to the discoloration at vascular tissue (Table 1). As a result, it was found that 7 melon genotypes (CU129, CU258, T8, Y9, Y10, Y15, and Y63) were resistant, 3 melon genotypes (CU280, CU309, and T1) were heterogeneous and all the others were susceptible to race 1 of fusarium wilt based on the disease incidence, susceptibility rate and vascular browning.

Assessment of melon genotypes by RAPD markers linked to Fom-2

Melon genotypes were assessed by three RAPD markers (E07, G17 and 596) linked to *Fom-2*. Primer 596 formed some unrepeatable indistinct 1.55 kb bands; therefore, it was not evaluated. Markers obtained from the primers E07 and G17 formed reproducible and strong 1.25 kb and 1.0 kb bands, respectively (Fig. 1 and Fig. 2). When compared with inoculation test results, the mistmatch ratios of these two markers in melon genotypes were found to be 5.06 % and 58.23 %, respectively (Table 1).

Discussion

Reaction of melon genotypes to F.o.m. *race 1 based on inoculation tests*

Reactions of melon genotypes to *F.o.m.* race 1 could have been determined by two inoculation tests of isolate (A-49) having high pathogenicity on susceptible cultivars. Typical symptoms of the disease were observed and re-isolation of these isolates was obtained from the plants having symptoms. Burger et al. (2003) reported that melon genotypes in *Fusarium* inoculation tests were affected by the environment and that *Fusarium* preferred low light intensity in the environment and low sugar content in the plants. The light intensity was low and the pathogenicity level of isolate was high; therefore, the relatively healthier results were obtained from the inoculation tests.

Disease incidence and reaction levels of melon genotypes to *F.o.m.* race 1 were determined by the concordance from the sand and suspension culture results backed with discoloration at vascular tissue because some susceptible plants may occasionally escape detection due to the late development of external symptoms such as wilting, yellowing or death of the plants (Karsies et al. 2000; Wang et al. 2000). It was finally decided that 7 melon genotypes (CU129, CU258, T8, Y9, Y10, Y15 and Y63) were resistant, 3 melon genotypes (CU280, CU309, and T1) were heterogeneous and all the other

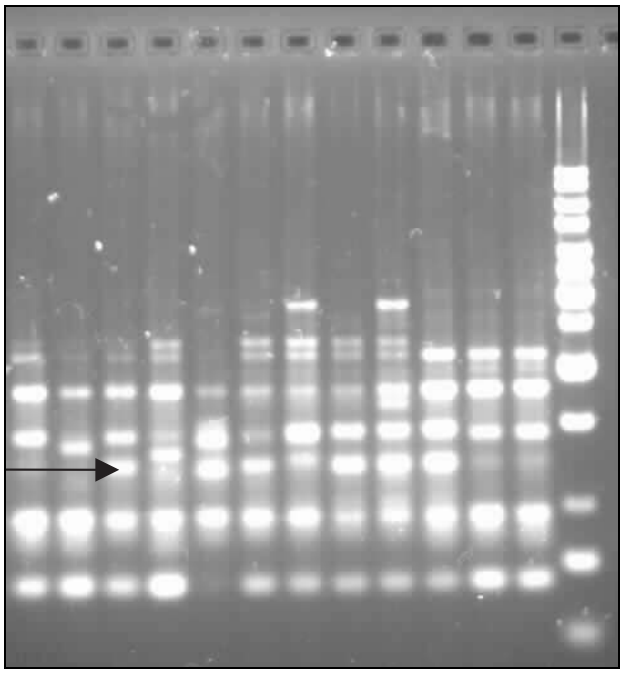


Fig. 1. Ethidium bromide-stained gel of PCR amplified products of some melon genotypes using decamer primer E07 (arrow shows the 1.25 kb polymorphic band and DNA ladder at the end of the row).

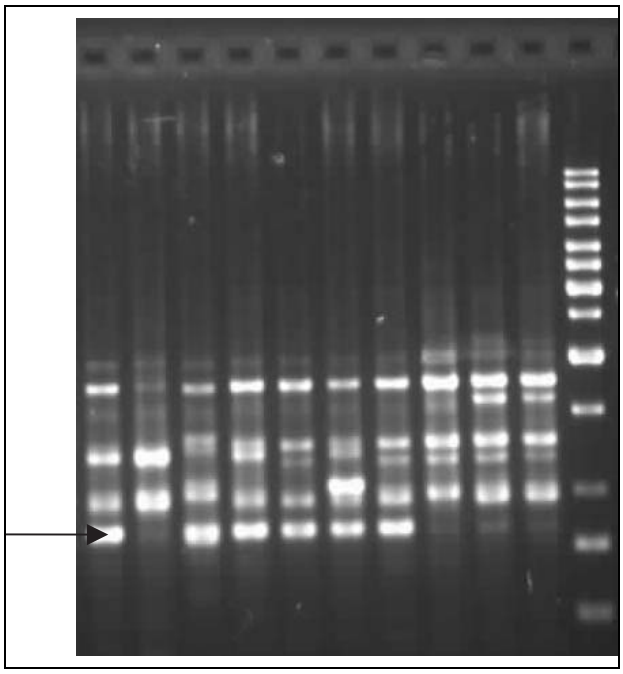


Fig. 2. Ethidium bromide-stained gel of PCR amplified products of some melon genotypes using decamer primer G17 (arrow shows the 1.0 kb polymorphic band and DNA ladder at the end of the row).

69 genotypes were susceptible to race 1 of fusarium wilt, controlled by a dominant gene: *Fom-2* (PITRAT 1998), based on the disease incidence, susceptibility rate and vascular browning. Because Turkey is one of the secondary gene centres for melon, it was expected and that there might be some resistant cantaloupe (such as CU258 and CU280) and inodorous (such as CU129, CU390 and T1) genotypes among this germplasm; and so it was. The all resistant ('Isabelle' (75=Y15) and 'Shiro Uri Okoyama' (69=Y9)) and susceptible ('Topmark' (61=Y1), 'Vedrantais' (63=Y3), and 'Honeydew Green Flesh' (65=Y5)) reference genotypes of the previous studies (BAUDRACCO-ARNAS and PITRAT 1996; ZHENG and WOLFF 2000) gave similar results in the present study. Moreover, similar results were met in some studies done in Turkey with some local genotypes (SARI et al. 1994; YUCEL et al. 1994; KURT et al. 2002); only few melon genotypes ('Kirkagac 637' and 'Galia') possessed some degree of resistance to *F.o.m.* race 1 and most genotypes (especially South Anatolian genotypes and the cultivar 'Yuva') were lack of the resistance. DEMIR et al. (2006) determined only five resistant genotypes among 53 melon landraces grown in the Lake Van Basin located in the eastern part of Turkey.

Assessment of melon genotypes by RAPD markers linked to Fom-2

Of the three RAPD markers (E07, G17 and 596) linked to *Fom-2*, the last one did not work in the present study evaluating Turkish melon genotypes beside reference accessions. In fact, ZHENG and WOLFF (2000) also reported that the marker 596 was not effective especially in some resistant melon genotypes.

When compared with inoculation test results, the mistmatch ratios of the E07 and G17 RAPD markers in melon genotypes of the present study were found to be 5.06 and 58.23 %, respectively. ZHENG and WOLFF (2000) reported in their study dealing 89 melon genotypes that mistmatch ratios of these markers were 12 and 19 %, respectively and combination and evaluation of E07 and G17 scores together gave even higher correct matching (95 %). When compared this two studies, it was seen that E07 marker was more effective in detecting susceptible genotypes in the Turkey's melon germplasm than G17 marker. On the other hand, combination and evaluation of E07 and G17 scores together (at least one matching) gave even higher correct matching (97 %) in the present study. Besides, when both markers were present, there was no mismatch in the present study.

Conclusion

According to the overall results obtained in the present study, it was determined that there were some resistant melon genotypes such as 'Nahcivan-3', 65ER05, 65ERC13, 'Ziraat-Beyaz', and 'Kirkagac 637' in the Turkey's germplasm, though few in number, to *F.o.m.* race 1 and molecular evaluation of resistance level of these genotypes were also possible. Although the sole applications of RAPD markers 596 and G17 were not recommended, the sole applications of RAPD markers E07 and/or the combined evaluation of E07 and G17 RAPD markers were definitely useful in detecting *F.o.m.* race 0 and 1 susceptible melon genotypes in germplasms.

Acknowledgments

The authors express gratitude to Dr. Kudret Erzurumlu and Dr. Seral Yucel for providing *Fusarium* isolates; to Dr. Maria L. Gomez-Guillamon, Dr. Rafael Perl-Treves, Dr. Michel Pitrat, Dr. Nebahat Sari, and Dr. Jack E. Staub for providing some seed samples and for their advices; to Dr. Bunyamin Sogut and Dr. Murat Sipahioglu for providing their labs' facilities. This research was partly supported by Project no. TUBITAK/TOGTAG-3055 from the Scientific and Technical Research Council of Turkey, Ankara and by projects KAP-2002-ZF-49 and 2003-YYU-ZF-009 from the Scientific Projects Council of Yuzuncu Yıl University, Van-Turkey and Cukurova University, Adana-Turkey, respectively.

References

BAUDRACCO-ARNAS, S. and M. PITRAT 1996: A genetic map of melon (*Cucumis melo* L.) with RFLP, RAPD, isozyme, disease resistance and morphological markers. Theor. Appl. Genet. **96**, 57–64.

BURGER, Y., N. KATZIR, G. TZURI, V. PORTNOY, U. SAAR, S. SHRIBER, R. PERL-TREVES and R. COHEN 2003: Variation in the response of melon genotypes to *Fusarium oxysporum* f. sp. *melonis* race 1 determined by inoculation tests and molecular markers. Plant Pathol. **52**, 204–211.

COHEN, R., T. KATAN and R. COHEN 1989a: Occurrence of *Fusarium oxysporum* f. sp. *melonis* race 1,2 on muskmelon in Israel. Phytoparasitica. **17**, 319–322.

COHEN, R., B. BLAIER, A.A. SCHAFFER and J. KATAN 1989b: Effect of acetochlor treatment on fusarium wilt and sugar content in melon seedling. Eur. J. Plant Path. **102**, 45–50.

COHEN, R., Y. ELKIND, Y. BURGER, R. OFFENBACH and H. NERSON 1996: Variation in the response of melon genotypes to sudden wilt. Euphytica **87**, 91–95.

DEMIR, S., O. TURKMEN, S. SENSOY, A. AKKOPRU, C. ERDINC, M. YILDIZ and T. KABAY 2006: Reactions of melon landraces grown in the Lake Van Basin to the physiological races (Race 1 and Race 2) of *Fusarium oxysporum* f. sp. *melonis*. Europ. J. Hort. Sci. **71**, 91–95.

DOYLE, J.J. and J.L. DOYLE 1987: A rapid DNA isolation procedure from small quantities of fresh leaf tissues. Phytochem. Bull. **19**, 11–15.

ERZURUM, K., Y. TANER, E. SECER, R. YANMAZ and S. MADEN 1999: Occurence of races of *Fusarium oxysporum* f. sp. *melonis* causing wilt on melon in Central Anotolia. J. Turk. Phytopath. **28**(3), 87–97.

GÓMEZ-GUILLAMON, M.L., J.M. ALVAREZ, A. BÖRNER, K. ABAK, N. SARI, E. MORIONES and M. LUIS 1999: Management, convervation, and valorization on genetic resources of *C. melo* and wild relatives. European Union Project No: GEN RES-CT99-108.

HASSAN DAR, G., M.Y. ZARGAR and G.M. BEIGH 1997: Biocontrol of fusarium root rot in the common bean (*Phaseolus vulgaris* L.) by using symbiotic *Glomus mosseae* and *Rhizobium leguminosarum*. Microbial Ecology. **34**, 74–80.

JACOBSON, D.J. and T.R. GORDON 1991: *Fusarium oxysporum* f. sp. *melonis*: A case study of diversity within a forma specialis. Phytopath. **81**, 1064–1067.

JOOBEUR, T., J.J. KING, S.J. NOLIN, C.E. THOMAS and R.A. DEAN 2004: The fusarium wilt resistance locus *Fom-2* of melon contains a single resistance gene with complex features. Plant J. **39**, 283–297.

KARSIES, T., R. DEAN and C. THOMAS 2000: Toward the development of molecular markers linked to race 2 fusarium wilt resistance in melon (*Cucumis melo* L.). Acta Hort. **510**, 415–419.

KATAN, T., J. KATAN, T.R. GORDON and D. POZNIAK 1994: Physiologic races and vegetative compatibility groups of *Fusarium oxysporum* f. sp. *melonis* in Israel. Phytopath. **84**, 153–157.

KUCUK, A., K. ABAK and N. SARI 2002: Cucurbit genetic resources collections in Turkey. First AD HOC Meeting on Cucurbit Genetic Resources. 19 January 2002, Adana, Turkey. 46–51.

KURT, S., B. BARAN, N. SARI and H. YETISIR 2002: Physiologic races of *Fusarium oxysporum* f. sp. *melonis* in the South-

eastern Anatolia Region of Turkey and varietal reactions to races of the pathogen. Phytoparasitica **30**, 395–402.
LECOQ, H., D. BLANCARD, F. BERTNARD, A. NICOT, A. GLANDART, P.M. MOLOT and P. MAS 1991: Techniques d'inoculation artificielle du melon avec differents agents pathogénes pour la séléction de variétés résistances. INRA, Domaine Saint Maurice BP 94, 84143 Montfavet Cedex.
MCGREGOR, C.E., C.A. LAMBERT, M.M. GREYLING, J.H. LOUW and L. WARNICH 2000: A comparative assessment of DNA fingerprinting techniques (RAPD, ISSR, AFLP and SSR) in tetraploid potato (*Solanum tuberosum* L.) germplasm. Euphytica **113**,135–144.
MARTYN, R.D. and T.R. GORDON 1996: Fusarium Wilt of Melon. In: ZITTER, T.A., D.L. HOPKINS and C.D. THOMAS (eds): Compendium of cucurbit diseases. American Phytopathological Society, St. Paul, MN, USA. pp. 14–15.
NISINI, P.T., G. COLLA, E. GRANATI, O. TEMPERINI, P. CRINÒ and F. SACCARDO 2002: Rootstock resistance to fusarium wilt and effect on fruit yield and quality of two muskmelon cultivars. Sci. Hort. **93**, 281–288.
PITRAT, M. 1998: Gene list for melon. Cucurbit Genet. Coop. Rep. **21**, 69–81.
SARI, N., M. PITRAT, K. ABAK and S. YUCEL 1994: The reactions of some watermelon and melon cultivars widely grown in Turkey to some fungal and viral diseases. Special edition for the 25th Anniversary of J. of Cukuruva University Agriculture Faculty. 35–50. (in Turkish)
SCHERF, A.F. and A. MANCAB 1986: Vegetable diseases and their control. Second Edition. A Wiley-Intercience Publication. ISBN 0471-05860-2 USA (1986), 728 p.
SCHREUDER, W., S.C. LAMPRECHT and G. HOLZ 2000: Race determination and vegetative compatibility grouping of *Fusarium oxysporum* f. sp. *melonis* from South Africa. Plant Dis. **84**, 231–234.
TURHAN, G. and F. GROSSMAN 1987: Antagonistic activity of *N. vasinfecta var. africans* (von Arx.) Cannon and Hawkworth against soilborne fungi. J. Phytopath. **123**,199–206.
WANG, Y.H., C.E. THOMAS and R.A. DEAN 1997: A genetic map of melon (*Cucumis melo* L.) based on amplified fragment length polymorphism (AFLP) markers. Theor. Appl. Genet. **95**, 791–798.
WANG, Y.H., C.E. THOMAS and R.A. DEAN 2000: Genetic mapping of a fusarium wilt resistance gene (*Fom-2*) in melon (*Cucumis melo* L.). Mol. Breed. **6**,379–389.
WETCHER, W.P., M.P. WHITEHEAD, C.E. THOMAS and R.A. DEAN 1995: Identification of a randomly amplified polymorphic DNA marker linked to the *Fom-2* fusarium wilt resistance gene in muskmelon Mr-1. Phytopathology **85**, 1245–1249.
WETCHER, W.P. and R.A. DEAN 1998: Development of sequence-specific primers that amplify a 1.5-kb DNA marker for race 1 fusarium wilt resistance in *Cucumis melo* L. HortScience **33**, 291–292.
WINDELS, C.E. 1993: *Fusarium*. In: SINGLETON, L.L., J.D. MIHAIL and C.M. RUSH (eds): Methods for research on soilborn phytopathogenic fungi. APS Press. St. Paul. Minnesota. pp. 115–128.
YUCEL, S., H. PALA, N. SARI and K. ABAK 1994: Determination of *Fusarium oxysporum* f. sp. *melonis* races in the East Mediterranean Region of Türkiye and response of some melon genotypes the disease. 9th Congress of the Mediterranean Phytopathological Union. Kusadası. Aydın. Türkiye, 87–89.
ZHENG, X.Y. and D.W. WOLFF 2000: Randomly amplified polymorphic DNA markers linked to fusarium wilt resistance. HortScience **35**, 716–721.
ZHENG, X.Y., D.W. WOLFF, S. BAUDRACCO-ARNAS and M. PITRAT 1999: Development and utility of cleaved amplified polymorphic sequences (CAPS) and restriction length polymormphisms (RFLPs) linked to the *Fom-2* fusarium wilt resistance gene in melon (*Cucumis melo* L.). Theor. Appl. Genet. **99**, 453–463.
ZINK, F.W. and C.E. THOMAS 1990: Genetics of resistance to *Fusarium oxysporum* f. sp. *melonis* races 0, 1, and 2 in muskmelon line MR-1. Phytopath. **80**, 1230–1232.
ZINK, F.W. 1991: Origin of fusarium wilt resistance in Texas AES muskmelon cultivars. Plant Disease **75**(1), 24–26.

Received April 17, 2006 / Accepted October 30, 2006

Addresses of authors: Suat Sensoy (corresponding author), Yüzüncü Yıl University, Agriculture Faculty, Department of Horticulture, 65080 Van, Turkey, Semra Demir, Yüzüncü Yıl University, Agriculture Faculty, Department of Plant Protection, 65080 Van, Turkey, and Saadet Buyukalaca and Kazim Abak, Çukurova University, Agriculture Faculty, Department of Horticulture, 01330 Adana, Turkey, e-mail: suatsensoy@yyu.edu.tr.