African Journal of Agricultural Research Vol. 4 (11), pp. 1268-1275, November, 2009
Available online at http://www.academicjournals.org/AJAR
ISSN 1991-637X © 2009 Academic Journals

Full Length Research Paper

Vegetative compatibility groups and pathogenicity of *Verticillium dahliae* isolates from watermelon in Turkey

Sibel Dervis[1]*, Halit Yetisir[2], Fatih Mehmet Tok[1], Sener Kurt[1] and Fatih Karaca[2]

[1]Department of Plant Protection, Faculty of Agriculture, Mustafa Kemal University, Antakya/Hatay/Turkey.
[2]Department of Horticulture, Faculty of Agriculture, Mustafa Kemal University, Antakya/Hatay/Turkey.

Accepted 15 October, 2009

In this study, surveys were carried out for Verticillium wilt in watermelon fields (262 fields) in 13 provinces from five regions of Turkey. The proportion of fields having wilted plants was 40%. *Verticillium dahliae* was isolated from 15.2% of the fields showing wilted plants. At the end of surveys, 16 *V. dahliae* isolates (each from a different wilted field, collected from eight provinces of the Aegean, Central Anatolia, Marmara, Mediterranean and Southeastern Anatolia Regions) were obtained and used for vegetative compatibility analysis using nitrate non-utilizing mutants and reference isolates belonging to vegetative compatibility groups (VCGs) 1A, 2A, 2B, 3, 4A and 4B. Eleven *V. dahliae* isolates from Adana, Adıyaman, Balıkesir, Diyarbakır, Konya and Mersin provinces were assigned to VCG2B, two from Mersin province to VCG2A, one from Balıkesir province to VCG4B and two from Manisa and Aydın provinces to VCG1A whereas VCG3 and VCG4A were not defined among the isolates. To reveal a possible correlation between VCG and pathogenic group in *V. dahliae*, pathogenicity of all isolates representing the four VCGs were tested on three watermelon cultivars (*Citrullus lanatus* cultivars 'Crimson Sweet', 'Crimson Tide' and 'Crisby') and a susceptible cotton cultivar (*Gossypium hirsutum* cultivar 'Çukurova 1518') in a greenhouse. In watermelon cultivars, most VCG2B isolates caused significantly more severe symptoms than VCG4B, VCG2A and VCG1A. VCG4B isolate was more virulent on all watermelon cultivars than both VCG1A isolates. The isolates within VCG2A and VCG1A caused similar virulence patterns on 'Crimson Sweet' and 'Crimson Tide' cultivars but for 'Crisby' VCG1A did not cause any leaf symptom. Virulence to watermelon cultivars varied only among the isolates within VCG2B. Significant differences in virulence to cotton were observed between isolates from different VCGs except the similarity between VCG2A and VCG4B. The results expose that the population of *V. dahliae* from watermelon in Turkey is heterogeneous (four different VCGs among 16 isolates) but VCG2B seems to be a more specialized form for this host in Turkey.

Key words: *Citrullus lanatus*, *Verticillium wilt*, VCGs, virulence.

INTRODUCTION

Turkey is the world's second largest watermelon producing country after China, with 3.8 x 10^6 t per year (FAO, 2007). Watermelon is cultivated mostly under low tunnel for early production especially in southern Turkey. One of the most serious problems of watermelon production in this region of the country is a decrease in yield due to soil-borne diseases, in particular *Verticillium* and *Fusarium wilts*. *Verticillium* species, *V. dahliae* and *V. albo-atrum*, are of major economic importance because of vascular wilt diseases they cause on a broad range of host plants (McCain at al., 1981). *V. dahliae* is a soil-borne fungus which cause severe wilt of watermelon in all producing areas, and widely distributed in the agricultural soils affecting such diverse crops artichoke, cotton, pepper, eggplant, pistachio, olive, potato, strawberry, tomato, watermelon and a number of crucifer crops (Koike et al., 1994; Krikun and Bemier, 1987; Subbarao et al., 1997). Verticillium wilt symptoms in watermelon generally are not realized until fruit-set. Leaves become chlorotic and later develop necrotic sectors. The foliage of severely infected plants turns brown. The vascular root tissues turn tan to light brown (Gubler, 1996).

A better understanding of the genetics and virulence

*Corresponding author. E-mail: sibeldervis@gmail.com. Tel: + 903262455845, Fax: +903262455832

diversity in plant pathogen populations is a key for efficient plant disease management. For *V. dahliae*, the agent of *Verticillium wilt* diseases of hundreds of herbaceous and woody crops (Pegg and Brady, 2002), inability to recognize the genetic diversity within the pathogen has limited our understanding of disease development and efficacy of management practices (Rowe, 1995). During the last decade, usage of vegetative compatibility and molecular analysis have improved our understanding of *V. dahliae* and led to the realization that genetic diversity in this pathogen is higher than previously thought (Rowe, 1995; Barbara and Cleves, 2003; Katan, 2000). Vegetative compatibility is a useful marker for determining the genetic structure of some fungal populations (Morrell et al., 1997) and, in fungi, is controlled by multiple genetic loci. Hyphae of strains that have identical alleles present at all compatibility loci can anastomose to form a viable heterokaryon. Isolates that share compatibility loci and can anastomose with one another belong to a subpopulation designated as a vegetative compatibility group and are genetically isolated from other VCGs and each VCG is somewhat specific to a host plant or related group of hosts and may or may not be as virulent on other hosts (Morrell et al., 1997; Puhalla and Hummel, 1983). Four or more VCGs have been detected among *V. dahliae* isolates from various hosts (Puhalla and Hummel, 1983; Joaquim and Rowe, 1991; Chen, 1994; Daayf et al., 1995; Wakatabe et al., 1997; Bao et al., 1998; Elena, 2000). Several studies have correlated VCGs with pathogenicity on different host plants (Strausbaugh et al., 1992; Daayf et al., 1995).

Vegetative compatibility studies of *V. dahliae* populations infecting watermelon has not been studied in Turkey. Additionally, the variations of the virulence in *V. dahliae* populations associated with watermelon are not clear. A better understanding of host adaptation and virulence of *V. dahliae* isolates from watermelon would help to devise better disease management strategies and would be useful in developing wilt-resistant watermelon cultivars. The objectives of this study were to determine VCG diversity, pathogenicity and virulence of *V. dahliae* isolates obtained from naturally infected watermelon plants grown in major watermelon production areas of Turkey.

MATERIALS AND METHODS

Collection of isolates

Between 2005 and 2008, surveys for *V. wilt* were conducted in major watermelon producing areas in Adana, Adıyaman, Aydın, Batman, Balıkesir, Çanakkale, Diyarbakır, Gaziantep, İzmir, Konya, Manisa, Mersin and Şanlıurfa provinces in the Mediterranean, Aegean, Marmara, Central Anatolia and Southeastern Anatolia regions of Turkey during the period of May until August (Table 1 and Figure 1). Five samples of naturally infected watermelon plants showing typical wilt symptoms were taken from each field. For isolation, pieces of vascular tissue from watermelon stems were dipped in 70% ethanol, surface-disinfested with 1% NaOCl for 1 min, rinsed in sterile distilled water and dried on sterile filter paper. Each piece was then placed on potato dextrose agar (PDA, Merck, Damstradt, Germany) amended with 100 mg L^{-1} of streptomycin sulphate. Plates were incubated at 24°C for 5 - 7 days. For determination of primary fungi a unique colony developed around nearly all pieces of five samples from each field were transferred to PDA plates. The other samples showing variable isolation frequencies of different fungi were discarded. *V. dahliae* was identified on the basis of its morphological features according to the description of Smith (1965). Of the other soil-borne fungi recovered *Fusarium* sp., *Macrophomina* spp., *Rhizoctonia* spp. and *Pythium* spp. Identifications were based on Nelson et al. (1983), Holliday and Punithaligam (1970), Sneh et al. (1991) and Van der Plaats-Niterink (1981), respectively. All fungal cultures were stored on sterile Whatman filter papers at -20°C in sterile paper envelopes. Single spore isolates of *V. dahliae* were used for VCG characterization and pathogenicity tests.

Generation and characterization of *nit*rate-non-utilizing (*nit*) mutants

Nitrate non-utilizing mutants (*nit*) were generated using a modified version of Puhalla's technique (Puhalla and Hummel, 1983). Mycelial plugs cut from the edge of the monoconidial cultures were placed on water agar-chlorate medium (3%) amended with 0.02% glucose and 2.5 - 5% potassium chlorate (Korolev and Katan, 1997), and were then incubated at 25°C for up to 4 weeks. Conidia from chlorate-resistant sectors, which appear as thin fast-growing mycelial sectors or as fun-like sectors at the colony perimeter after 10 - 28 days, were streaked onto Minimal Medium (Czapek-Dox agar) with nitrate as the sole source of nitrogen. Nit mutants were identified by their thin, expansive, appressed growth. One to 10 *nit* mutants was obtained from each wild-type isolate. *Nit* mutants were separated into three phenotypic classes, *nit*1, *nit*3 and NitM, based on their growth on minimal medium amended with sodium nitrite and hypoxanthine (Correll et al., 1987). Mutants that grew profusely (similar to wild type) on CDA with nitrite or hypoxanthine were classified as *nit*I. Mutants that grew sparsely on CDA with hypoxanthine were classified as NitM. Mutants that grew profusely on hypoxanthine and sparsely on nitrite were classified as *nit*3. These partially phenotyped *nit* mutants were labeled and stored for future use.

Vegetative compatibility

The *nit* mutants of all isolate were paired with complementary *nit* mutants of the international tester isolates (Table 2). The *nit*1 and NitM mutants from each isolate were also paired with each other to test for heterokaryon self-compatibility. Complementation was tested on minimal medium. Generally, each 9 cm diameter Petri dish was inoculated with three mutants, 1 cm apart in a triangular pattern, and incubated for 28 days at 24°C in the dark. Pairings were then scored for prototrophic growth 7 - 28 days after inoculation. Complementation was indicated by the formation of a dense, aerial growth or black microsclerotia where mycelia of an unknown and a tester strain had met and formed a prototrophic heterokaryon (Correll at al., 1987).

Pathogenicity tests

Cotton (*G. hirsutum* cultivar 'Çukurova 1518') and watermelon (*Citrullus lanatus* cultivars 'Crimson Sweet', 'Crimson Tide' and 'Crisby') seeds were sown into non-sterilized potting mixture (peat:perlite 2:1, v/v) in plug trays. Plants were maintained on benches in a greenhouse at 22 - 26°C until inoculation. Relative hu-

Table 1. Presence of *V. dahliae* in surveyed watermelon areas of Turkey.

Province*	Location**	*V. dahliae* ± fields	Number of fields with wilt symptoms
Adana (60)	Karataş (1), Tuzla (1), Yumurtalık (1)	+	3
	Tuzla (11), Yemisli (2), Central (15), Karataş (7), Ceyhan (7), Yumurtalık (5)	–	47
Adıyaman (25)	Besni-Central (1)	+	1
	Besni-Central (3), Besni-Tekağaç Village (4), Central-Gölpınar Village (2)	–	9
Aydın (18)	Karpuzlu (1)	+	1
	Çine (1)	–	1
Balıkesir	Ayvalık (2), Bandırma-Çavuşköy (1)	+	3
(20)	Ayvalık (3), Bandırma- Çavuşköy (2)	–	5
Batman (11)	Central (2), Hasankeyf (1)	–	3
Çanakkale (9)	Biga (1)	–	1
Diyarbakır (33)	Çınar-Güzelşeyh Village (1)	+	1
	Bismil-Yenisalad (4), Ceylanpınar-Boğalı Village (1), Central (2)	–	7
Gaziantep (8)	Araban (1)	–	1
İzmir (13)	Kiraz Road (1), Tire-Belevi (1)	–	2
Konya (14)	Akşehir (1)	+	1
Manisa (10)	Akhisar (1)	+	1
	Kula-Uşak Road (1), Gölmarmara (1)	–	2
Mersin (25)	Tarsus (4), Yenice (1)	+	5
	Tarsus (5)	–	5
Şanlıurfa (16)	Bozova-Tutluca Village (1), Bozova-Kepireç Village (2), Bozova-Kesmetaş Village (1) Bozova-Sarıkaya Village (1), Central-Kurukazan Village (1)	–	6

* Numbers in parenthesis show the numbers of surveyed fields for corresponding provinces. ** Numbers in parenthesis show the numbers of surveyed fields having wilted plants (*V. dahliae* + / -) in corresponding location.

Figure 1. Surveyed provinces for watermelon wilt in Turkey.

Table 2. Summary information about the previously described international and Turkish tester isolates of *V. dahliae* used in this study.

Isolate	Origin	Mutant phenotype	VCG	Host plant	Reference
T9	USA	NitM	1A	Cotton	Joaquim and Rowe, 1990
CotVd19	Turkey	*nit*1 and NitM	1A	Cotton	Dervis et al, 2007
ep8	Israel	NitM	2A	Eggplant	Korolev et al., 2000
OVd211	Israel	*nit*1 and NitM	2A	Olive	Dervis et al, 2007
cot11	Israel	NitM	2B	Cotton	Korolev et al., 2000
Ch03	Turkey	*nit*1 and NitM	2B	Cotton	Dervis and Bicici, 2005
70-21	USA	NitM	3	Pepper	Joaquim and Rowe, 1990
171	USA	*nit*1	4A	Potato	Joaquim and Rowe, 1990
131M	USA	NitM	4A	Potato	Joaquim and Rowe, 1990
Pt15M	Israel	NitM	4B	Potato	Korolev et al., 2000
Pt9G	Israel	*nit*1	4B	Potato	Korolev et al., 2000

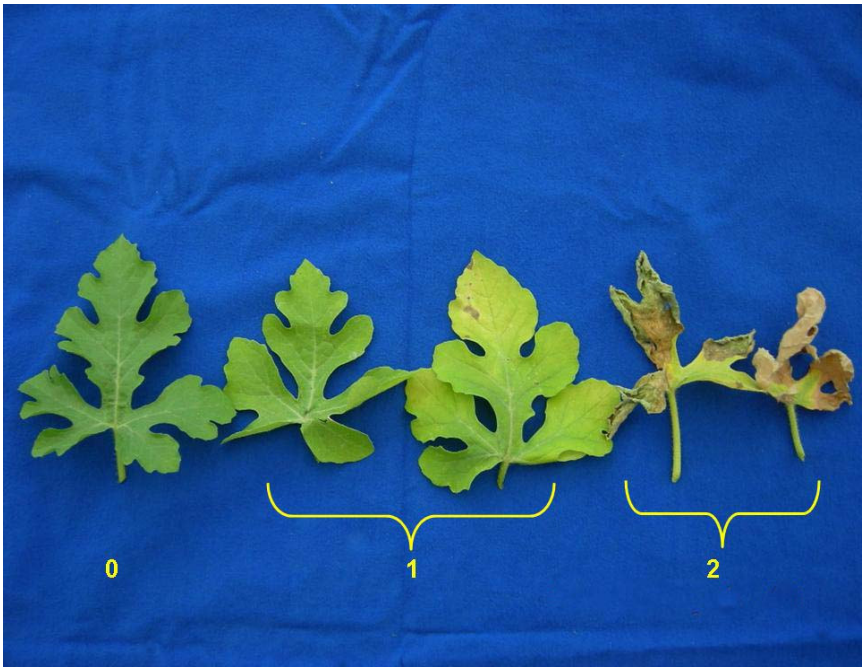

Figure 2. 0 - 2 disease severity scale for *Verticillium wilt* on watermelon (0 = no sign of wilting, 1 = leaves with wilt, 2 = leaves with necrosis).

midity in greenhouse ranged from 60 - 90%. Inoculum was prepared by growing isolates on PDA plates at 25°C for 1 month. Spore suspensions were prepared by adding 15 ml of sterile distilled water to each plate and scraping the cultures with a spatula. The conidial density of each isolate was adjusted to approximately 10^6 conidia ml^{-1}. Plants were removed from the seedling trays 2 - 4 days after emergence (first true leaf stage) and 2 cm of root was trimmed just before inoculation, since trimmed or intact roots do not affect the plant reaction (Koike et al., 1994). Fifteen plants of both cotton and watermelon were inoculated per isolate by dipping the roots in a spore suspension for at least 3 min and planting them individually into 12 cm diameter plastic pots filled with potting mixture. Roots of 15 plants of each host were trimmed, dipped in sterile distilled water, and maintained as non-inoculated controls. Experiments were arranged in a randomized block design on the greenhouse benches. All plants were watered as needed and fertilized every 2 weeks with a water-soluble fertilizer (20-10-20, N-P-K). Disease symptoms were evaluated five weeks after inoculation. Mean disease severity index (DSI) was calculated from each treatment by summing the scores of 30 plants (three replicates of five plants per isolate, two experiments) using the scale of 0 to 4 for cotton plants (Bejarano-Alcazar et al., 1995) and 0 to 2 (0 = no sign of wilting, 1 = leaves with wilt, 2 = leaves with necrosis) (Figure. 2) for watermelon plants, expressing the value as a percent. Statistical analysis of the data on DSI was carried out using SPSS software (version 13.0; SPSS Inc., Chicago, IL, USA). Arcsine transformation was performed on data before statistical analysis. Analysis of variance was followed by mean separation using the Duncan's Multiple Range Test ($p < 0.05$). The percentage of watermelon plants with vascular discoloration was also calculated for

each treatment.

RESULTS

The distribution of *Verticillum dahliae* in watermelon areas of Turkey

In the present study, a total of 262 fields in 13 provinces where watermelon is an important crop were surveyed. The proportion of fields having wilted plants was 40%. *V. dahliae, Fusarium oxysporum, V. albo-atrum, Pythium* spp., *Macrophomina* spp*, Rhizoctonia* spp. and *Fusarium solani* were isolated from different provinces during the growing seasons of 2005 through 2008 (Table 1). For each field, the presence of the major fungi varied by provinces. *V. dahliae* was isolated from 15.2% of the fields having wilted plants in eight provinces (Adana, Adıyaman, Aydın, Balıkesir, Diyarbakır, Konya, Manisa and Mersin provinces) (Table 1). *F. oxysporum* was the most frequently isolated fungus from 64.8% of fields having wilted plants in all 13 provinces except Konya suggesting its potential importance in the wilt appearance in watermelon producing areas of Turkey. The other fungi predominantly isolated included *F. solani* from Adana, Adıyaman and Mersin*, Rhizoctonia* spp. from Adana, Aydın, Balıkesir and Diyarbakır, *Pythium* spp. from Adıyaman and Batman, *V. albo-atrum* from Balıkesir and *Macrophomina* spp. from Batman with the frequency of 9.5, 6.7, 1.9, 1.0 and 1.0%, respectively.

Generation and characterization of nitrate-non-utilizing (*nit*) mutants

One hundred and ten (110) chlorate-resistant sectors were obtained from 16 isolates of *V. dahliae*; in 10 - 15 replications, each isolate produced 1 - 30 chlorate-resistant sectors. For each isolate of *V. dahliae,* 1 - 20 of sectors was phenotyped as *nit* mutants. The number of *nit* mutants recovered from apparently resistant sectors was 90 out of 110 (81.8%). Most mutants (85%) grew profusely on CDA with nitrite or hypoxanthine and were classified accordingly as *nit*1. About 12.8% of the *V. dahliae* grew sparsely on CDA with hypoxanthine and was classified as NitM. A low proportion (2.2%) of the *V. dahliae* mutants, which grew profusely on hypoxanthine and sparsely on nitrite, were classified as *nit*3. All *nit* mutants showed wild-type growth on PDA.

Vegetative compatibility

All isolates belonging to a distinct VCG complemented strongly with at least one of the tester strains of that group. Based on positive complementation reactions with reference testers, four VCGs were found: 11 isolates (68.75%) were assigned to VCG2B, 2 isolates (12.50%) to VCG2A, and 2 (12.50%) to VCG1A, and one (6.25%) to VCG4B (Table 3). VCG2B isolates were recovered from all provinces except Aydın and Manisa. Only VCG2B isolates were recovered from Adana province of the Mediterranean Region, Konya province of the Central Anatolia Region and Diyarbakır and Adıyaman provinces of the Southeastern Anatolian Region. Of three isolates from Balıkesir province of the Marmara Region two belonged to VCG2B and one to VCG4B. Five *V. dahliae* isolates from Mersin province of the Mediterranean Region were assigned to VCG2B (three isolates) and VCG2A (two isolates). Only VCG1A isolates were recovered from Aydın and Manisa provinces of the Aegean Region.

Pathogenicity tests

The majority of VCG2B isolates from watermelon were more virulent on watermelon cultivars than VCG1A isolates both causing higher disease indices and higher percents of plants showing vascular discolorations but less virulent on cotton cultivar ‘Çukurova 1518’ (Table 3). VCG4B isolate WVd7 was more virulent on all watermelon cultivars than both VCG1A isolates (Table 3). Furthermore, this isolate was in the most virulent group on watermelon cv. ‘Crimson Sweet’. The isolates within VCG2A and VCG1A caused similar virulence patterns on ‘Crimson Sweet’ and ‘Crimson Tide’ but for ‘Crisby’ VCG1A did not cause any leaf symptom but caused vascular discoloration (Table 3). Isolates belonging to different VCGs were clearly differentiated into different pathogenic groups on cotton except the similarity between VCG2A and VCG4B, in which the isolates are in the same pathogenic group. Cotton plants inoculated with VCG1A isolates showed defoliation and often death in greenhouse trials. All VCG2B isolates caused severe foliar symptoms, stunting, and partial defoliation on cotton. On cotton plants inoculated with VCG1A and VCG2B isolates, disease symptoms developed earlier and were more severe than those with VCG4B and VCG2A.

DISCUSSION

Turkey is the second most important watermelon producing country (FAO, 2007). Watermelon cultivation has been carried out intensively for many years in those regions where surveys were conducted. One of the most serious problems of watermelon production was a decrease in yield due to soil-borne diseases, in particular Fusarium and Verticillium wilts, and successive cropping. *V. dahliae* was detected in eight provinces (Adana, Adıyaman, Aydın, Balıkesir, Diyarbakır, Konya, Manisa and Mersin provinces) and from 15.2% of all fields showing wilted plants. Isolation from wilted watermelon samples has also demonstrated the presence of some other fungi including *F. oxysporum, F. solani, Rhizoctonia* spp., *Pythium* spp., *V. albo-atrum,* and *Macrophomina* spp*,* being *F. oxysporum* the most common one (64.8%). These fungal species were the primary fungi from the

Table 3. Pathogenicity tests conducted using watermelon cultivars 'Crimson Sweet', 'Crimson Tide' and 'Crisby' and cotton cultivar 'Çukurova 1518' inoculated with *Verticillium dahliae* isolates belonging to different vegetative compatibility groups (VCGs).

Isolate	VCG	Province	Location	Crimson Sweet			Crimson Tide			Crisby			Çukurova 1518	
				DSI [x]		PVD	DSI [x]		PVD	DSI [x]		PVD	DSI [x]	
WVd01	2B	Adana	Karataş	63.9	ab	60	50.9	c	80	39.1	b	80	68.9	cd
WVd02	2B	Adana	Tuzla	82.0	a	100	90.0	a	100	57.0	a	100	71.4	c
WVd03	2B	Mersin	Tarsus	90.0	a	100	90.0	a	100	57.0	a	100	67.4	cde
WVd04	2A	Mersin	Yenice	13.1	de	20	33.0	de	40	26.1	c	60	55.8	de
WVd05	1A	Manisa	Akhisar	19.9	de	20	33.0	de	40	0.0	d	40	90.0	a
WVd06	2B	Konya	Akşehir	11.1	de	20	0.0	f	20	0.0	d	20	66.1	cde
WVd07	4B	Balıkesir	Ayvalık	84.4	a	100	57.0	bc	20	26.1	c	80	59.2	cde
WVd08	2B	Balıkesir	Bandırma-Çavuşköy	90.0	a	100	90.0	a	80	57.0	a	100	65.9	cde
WVd09	1A	Aydın	Karpuzlu	27.3	cde	40	31.0	de	40	0.0	d	80	85.7	ab
WVd10	2B	Diyarbakır	Çınar-Güzelşeyh Village	63.4	ab	100	63.9	b	40	57.0	a	100	73.9	bc
WVd11	2B	Adana	Yumurtalık	0.0	e	20	0.0	f	60	0.0	d	60	69.6	cd
WVd12	2B	Adıyaman	Besni	50.8	bc	60	26.1	e	60	26.1	c	60	70.1	cd
WVd13	2B	Balıkesir	Ayvalık	90.0	a	100	90.0	a	100	57.0	a	100	71.1	c
WVd14	2A	Mersin	Tarsus	33.2	cd	60	39.1	d	80	33.0	bc	60	53.8	e
WVd15	2B	Mersin	Tarsus	63.4	ab	100	90.0	a	100	57.0	a	100	69.2	cd
WVd16	2B	Mersin	Tarsus	26.6	cde	20	50.9	c	100	50.9	a	100	69.2	cd

DSI- Mean disease severity index (%). Arcsine transformation was performed prior to statistical analysis. Data are presented as the mean of two independent experiments. [x]Means not followed by the same letter within a column are significantly different ($P < 0.05$) according to Duncan's multiple Range Test. PVD-Plants showing vascular discoloration (%)

fields which they were isolated but their occurrence with wilt appearance remained unclear since the pathogenicity experiments were not conducted with these fungi except *V. dahliae* because the present study was focused on *V. wilt.* Relative importance of the frequently isolated soil-borne pathogens in this study and possible relationships with wilt appearance should be studied further.

Most *V. dahliae* isolates from watermelon were assigned to VCG2 (A and B) (%81.25), in accordance with the previous study of Elena (2000). Some other VCG studies of *V. dahliae* included only one (Korolev et al., 2000) or two *V. dahliae* isolates from watermelon (Dervis and Bicici, 2005). This is the first detailed study of vegetative compatibility of *V. dahliae* isolates from watermelon in Turkey. The sample of 16 isolates is quite small but the isolates were obtained from different sites in Turkey. The results expose that the population of *V. dahliae* from watermelon in Turkey is heterogeneous, because four VCGs (2B, 2A, 1A and 4B) were found among only 16 isolates. Nevertheless, the majority of *V. dahliae* isolates from watermelon growing areas of Turkey is belonged to VCG2B which seems to be a specialized form for this host in Turkey. Therefore, VCG's seem to be of importance for watermelon growers in practice.

In order to determine if there is a correlation between VCG and pathogenic group in *V. dahliae*, we compared virulence of the isolates on susceptible watermelon cultivars. Most VCG2B isolates were observed as the most aggressive and gave rise to the highest percents of plants showing vascular discoloration. Isolates of VCG4B isolate were moderately aggressive, and VCG2A and VCG1A were the least aggressive ones with the exception of the occasional VCG2B isolates in general. These results are in agreement with results from previous studies on *V. dahliae*, which have demonstrated some correlations between VCGs and virulence on certain hosts (Tsror et al., 2001; Joaquim and Rowe, 1991; Daayf et al., 1995; Korolev, 1998). The relationships between VCGs and virulence of the D, ND and PD isolates to cotton from current study are also in consistence with the previous reports from cotton and olive since VCG1A induced D, VCG2B induced PD, and VCG2A and VCG4B induced ND symptoms (Korolev et al., 2000; Dervis et al., 2008). These results expose *V. dahliae* isolates assigned in different VCGs from watermelon would cause similar virulence patterns on cotton to those of the isolates from cotton and olive. We suggest that consistency in virulence patterns of the isolates from watermelon belonging to the same VCGs on both watermelon and cotton plants except virulence of VCG2B isolates to watermelon plants points out that watermelon-wilt causal agent is often isolated genetically, in contrast to generalized forms.

Combined breeding programs could be applied to control *V. wilts* (McCreight et al., 1993), including: use of resistant or tolerant cultivars or rootstocks, reducing soil inoculum, limiting disease spread and manipulating to advantage factors which influence disease severity (Hiemstra,

1998). One of the most effective means controlling soil-borne diseases is crop rotation, suggesting that watermelon should not be cultivated at least for 5 years in the same field infested with the Fusarium wilt pathogen (Messiaen, 1974). However, *V. dahliae* can survive for many years in soil, making effective crop rotation difficult**.** Grafting onto resistant rootstocks may enable the control of soil-borne diseases and have positive impact on yield and quality (Lee and Oda, 2003). Grafting was used widely in watermelon to control soil-borne disease such as *F. wilt* (Lopez-Galarza et al., 2004; Miguel et al., 2004). The use of grafted seedlings in vegetables, particularly in watermelon production, has recently increased dramatically in Turkey (Atasayar, 2006). Since *F. oxysporum* f. sp. *niveum*, wilting agent of watermelon, is specific for watermelon, it is easy to control by grafting watermelon onto gourd species (Yetisir et al., 2007). On the other hand, *V. dahliae* can cause wilts on a broad range of host plants (McCain at al., 1981) and some watermelon rootstocks currently used in our country against Fusarium wilt showed susceptibility to *V. dahliae* (Dervis et al., 2009). Testing the currently used rootstocks against *V. dahliae* is our further project and the present results should be taken into consideration in future rootstocks usage and breeding programs. Finally, current study illuminating the characterization of the *V. dahliae* on watermelon plantations in Turkey would be of much interest for implementing potential disease management strategies for Verticillium wilt.

ACKNOWLEDGEMENT

This study was supported in part by The Scientific and Technological Research Council of Turkey (TUBİTAK Project TOVAG 104-O-578).

REFERENCES

Atasayar A (2006). Türkiye'de aşılı karpuz fidesi kullanımı. Hasad, 21: 87-91.

Bao JR, Katan J, Shabi E, Katan T (1998). Vegetative-compatibility groups in *Verticillium dahliae* from Israel. Eur. J. Plant Pathol., 104: 263-269.

Barbara DJ, Clewes E (2003). Plant-pathogenic *Verticillium* species: How many of them are there? Mol. Plant Pathol., 4: 297-305.

Bejarano-Alcazar J, Melero-Vara JM, Blanco-Lopez MA, Jimenez-Diaz RM (1995). Influence of inoculum density of defoliating and non-defoliating pathotypes of *Verticillium dahliae* on epidemic of *Verticillium wilt* of cotton in southern Spain. Phytopathology, 85: 1474-1481.

Chen W (1994). Vegetative compatibility groups of *Verticillium dahliae* from ornamental woody plants. Phytopathology, 84: 214-219.

Correll, JC, Klittich CJR, Leslie JF (1987). Nitrate non-utilizing mutants of *Fusarium oxysporum* and their use in vegetative compatibility tests. Phytopathology 77: 1640-1646.

Daayf F, Nicole M, Geiger JP (1995). Differentiation of *Verticillium dahliae* populations on the basis of vegetative compatibility and pathogenicity on cotton. Eur. J. Plant. Pathol. 101: 69-79.

Dervis S, M Bicici (2005). Vegetative compatibility groups in *Verticillium dahliae* isolates from cotton in Turkey. Phytoparasitica 33: 157-168.

Dervis S, Yetisir H, Karaca F (2009). Effectiveness of Gourd Rootstocks against *Verticillium wilt* in Watermelon Production. Turkish III Plant Protection Congr. (Van, Turkey), p. 226.

Dervis S, Erten L, Soylu S, Tok FM, Kurt S, Yıldız M, Soylu EM (2007). Vegetative compatıbility groups in *Verticillium dahliae* isolates from olive in Western Turkey. Eur. J. Plant. Pathol. 119: 437–447.

Dervis S, Kurt S, Soylu S, Erten L, Soylu EM, Yıldız M, Tok FM (2008). Vegetative Compatibility Groups of *Verticillium dahliae* from Cotton in the Southeastern Anatolia Region of Turkey. Phytoparasitica 36: 74–83.

Elena K (2000). Vegetative compatibility among *Verticillium dahliae* isolates from watermelon in Greece**.** Phytoparasitica 28: 115-120.

FAO (2007). FAO Statistical Database, www.fao.org.

Gubler WD (1996). Verticillium wilt. *in:* Zitter, T.A., Hopkins, L.D. and Thomas, C.E. [Eds.] Compendium of Cucurbit Diseases. APS Press, St. Paul, MN, USA.

Hiemstra JA (1998). Some general features of *Verticillium wilt* in tress. In: Compendium of *Verticillium wilt* in Tree Species. Ponsen & Looijen, Wageningen, Netherlands pp. 5–110.

Holliday P, Punithaligam E (1970). *Macrophomina phaseolina.* Commonwealth Mycological Institute Descriptions of Pathogenic Fungi and Bacteria, Nº 275. Kew, UK: Commonwealth Mycological Institute.

Joaquim TR, Rowe RC (1991). Vegetative compatibility and virulence of strains of *Verticillium dahliae* from soil and potato plants. Phytopathology 81: 552-558.

Joaquim TR, Rowe RC (1990). Reassessment of vegetative compatibility relationships among strains of *Verticillium dahliae* using nitrate-nonutilizing mutants. Phytopathology 80: 1160-1166.

Katan T (2000). Vegetative compatibility in populations of Verticillium-an overview. In: Advances in *Verticillium* Research and Disease Management (Eds.: E. Tjamos, R.C. Rowe, J.B. Heale, and D. Fravel). The American Phytopathological Society, St. Paul, MN, USA, pp. 77-94.

Koike ST, Subbarao KV, Davis RM, Gordon TR, Hubbard JC (1994). *Verticillium wilt* of cauliflower in California. Plant Dis. 78: 1116-1121.

Korolev N, Katan T (1997). Improved medium for selecting *nit*rate non-utilizing (*nit*) mutants of *Verticillium dahliae*. Phytopathology 87: 1067-1070.

Korolev N (1998). Virulence and VCG diversity in Verticillium spp. from plant tissue and soil. Phytoparasitica 26: 266-267.

Korolev N, Katan J, Katan T (2000). Vegetative compatibility groups of *Verticillium dahliae* in Israel: Their distribution and association with pathogenicity. Phytopathology 90: 529-536.

Krikun J, Bernier CC (1987). Infection of several crop species by two isolates of *Verticillium dahliae*. Can. J. Plant Pathol., 9: 241-245.

Lee JM, Oda M (2003). Grafting of herbaceous vegetable and ornamental crops. Horticult. Rev. 28: 61-124.

Lopez-Galarza SA, San Bautista DM, Perez DM, Miguel A, Baixauli C, Pascual B, Maroto JV Guardiola JL (2004). Effect of grafting and cytokinin induced fruit setting on color and sugar content traits in glasshouse-grown triploid watermelon. J. Hort. Sci. Biotechnol. 79: 971-976.

McCain AH, Raabe RD, Wilhelm S (1981). Plants resistant or susceptible to *Verticillium wilt*. Cooperative extension, US Depart-ment of Agriculture, University of California, Berkeley.

McCreight JD, Nerson H, Grumet R (1993). Melon (*Cucumis melo* L.). In: Kallo, G., Bergh B.O. (Eds), Genetic Improvement of Vegetable Crops. Pergamon Press, Ltd., pp. 267-283.

Messiaen CM (1974). Le Potager Tropical (1- généralités). Agence de Coop., Culturelle et Technique Publ., Paris – France.

Miguel A, Maroto JV, San Bautista A, Baixauli C, Cebolla V, Pascual B, Lopez S, Guardiola JL (2004). The grafting of triploid watermelon is an advantageous alternative to soil fumigation by methyl bromide for control of *Fusarium wilt*. Sci. Hortic. 103: 9-17.

Morrell A, Schreiber A, Lundy R (1997). Pests, pest control, and fertilizer use in Washington mint. Washington State University Cooperative Extension, MISC0188, Pullman.

Nelson PE, Toussoun TA, Marasas WFO (1983). Fusarium Species: An Illustrated Manual for Identification. State College, PA, USA: Pennsylvania State University Press.

Pegg GF, Brady BL (2002). *Verticillium wilts.* CAB International, Oxford.

Puhalla JE, Hummel H (1983). Vegetative compatibility groups within *Verticillium dahliae*. Phytopathology 73: 1305-1308.

Rowe RC (1995). Recent progress in understanding relationships between *Verticillium* species and subspecific groups. Phytoparasitica 23: 31-38.

Smith HC (1965). The morphology of *Verticillium albo-atrum*, *V. dahliae*, and *V. tricopus*. New Zealand. J. Agric. Res. 8: 450-478.

Sneh B, Burpee L, Ogoshi A (1991). Identification of *Rhizoctonia* Species. APS Press, St. Paul, Minnesota, USA.

Strausbaugh CA, Schroth MN, Weinhold AR, Hancock JG (1992). Assessment of vegetative compatibility of *Verticillium dahliae* tester strains and isolates from California potatoes. Phytopathology 82: 61-68.

Subbarao KV, Hubbard JC, Greathead AS, Spencer GA (1997). Verticillium wilt. In: Compendium of Lettuce Diseases (Eds.: R.M. Davis, K.V. Subbarao, R.N. Raid and E.A. Kurtz). The American Phytopathological Society, St. Paul, MN, USA, pp. 26-27.

Tsror (Lahkim) L, Hazanovsky M, Mordechi-Lebiush S, Sivan S (2001). Aggressiveness of *Verticillium dahliae* isolates from different vegetative compatibility groups to potato and tomato. Plant. Pathol. 50: 477–482.

Van der Plaats-Niterink AJ (1981). Monograph of the genus *Pythium*. Stud. Mycol. 21: 1-242.

Wakatabe D, Nagao H, Hiroaki A, Shiraishi T, Koike M, Iijima T (1997). Vegetative compatibility groups of Japanese isolates of *Verticillium dahliae*. Mycoscience 38: 17-23.

Yetisir H, Kurt S, Sari N, Tok MF (2007). Rootstock potential of Turkish *Lagenaria siceraria* germplasm for watermelon: plant growth, graft compatibility, and resistance to *Fusarium*. Turk. J. Agric. For. 31(6): 381-388.