Phytoparasitica (2015) 43:241–251
DOI 10.1007/s12600-014-0436-z

Recovery of *Verticillium dahliae* from commercially available potato seed lots planted in Turkey and characterization of isolates by vegetative compatibility and aggressiveness

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Received: 21 August 2014 / Accepted: 5 October 2014 / Published online: 11 November 2014

Abstract A total of 105 seed samples collected from commercially available potato seed lots in Turkey were assayed for *Verticillium dahliae*. *V. dahliae* was successfully isolated from 29 of the 105 seed lots tested. The frequency of infected lots was nearly 28%. Vegetative compatibility of the isolates was assessed through complementation tests using nitrate non-utilizing mutants. Of the 110 isolates obtained, 63 were assigned to VCG4A, 24 to VCG4B, 19 to VCG2A and one to VCG2B, while the three remaining isolates could not be assigned to any of the identified VCGs. All 36 of the isolates tested in the greenhouse on potato cv. 'Russet Burbank' were pathogenic to potato. As a group, AUDPC values were significantly higher ($P < 0.05$) for VCG4A than for VCG4B and VCG2 isolates. These data suggest that (i) commercial potato seed lots are commonly infected with *V. dahliae*, and that this is a primary method by which the pathogen can be introduced into production fields; (ii) potato isolates of *V. dahliae* belong to VCG4A, 4B and 2A and these isolates are widely distributed *via* seed lots; and (iii) VCG4A and VCG4B are distinct pathotypes of *V. dahliae* that vary in their aggressiveness to potato. The present study is the first report of natural infections of potato by VCG4A and VCG2A in Turkey.

Keywords Nit mutants · Seed health · *Solanum tuberosum* · Verticillium wilt

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Introduction

Damage caused by Verticillium wilt (*Verticillium dahliae* Kleb.) and its worldwide distribution, makes it one of the main diseases of potato (Dung *et al.* 2012; Rowe & Powelson 2002). Symptoms of Verticillium wilt of potato include unilateral wilting, chlorosis, and necrosis, which progress acropetally (Dung & Johnson 2012; Johnson & Dung 2010). Yield reductions of between 15% and 50% have been reported but the effects of Verticillium wilt on yield are also influenced by cultural and environmental factors (Cappaert *et al.* 1992; Johnson *et al.* 1986; Powelson & Rowe 1993). Since the first decade of the 2000's, Verticillium wilt has

progressively increased in many fields, and an unusually high incidence of a severe wilt disease of potato has been observed in Turkey. In 2011, epidemics of Verticillium wilt occurred in the Aegean region. The pathogen was isolated from the diseased plants in some areas of İzmir province and later found to be widespread throughout the region and later to the Black Sea, Central and Eastern Anatolia regions (Dane & Demirci 2012). It is not known for sure how fields newly brought into cultivation became infested with *V. dahliae*, but it is possible that long-distance spread of the pathogen could be a result of infected seed. In most cases, certification standards for potato seed in Turkey do not prescribe thresholds for infection by species of *Verticillium* nor is any widespread testing done of seed lots to assay for the presence of this pathogen. Results of our early studies on cotton showed that spread by infected seed was possible under greenhouse conditions that favor disease development (Göre *et al.* 2011). For this reason, understanding the population diversity within isolates of *V. dahliae* in commercially available potato seed lots is important for proper disease management programs. This information is a prerequisite for proper prevention of disease through seed treatment, setting tolerance levels for variety release, inspection of farmers' seed production schemes, quarantine, germplasm management, and exchange and optimization of storage conditions (Agarwal & Sinclair 1997).

Populations of the pathogen can be genetically characterized by means of vegetative or heterokaryon compatibility (Katan 2000; Korolev *et al.* 2000, 2008) and molecular markers (Dobinson *et al.* 2000; El-Bebany *et al.* 2013). Vegetative compatibility refers to the genetically controlled ability of individual fungal isolates to undergo hyphal anastomosis and form viable heterokaryons; compatible isolates are placed in the same vegetative compatibility group (VCG). For strictly asexually-reproducing fungi, such as *V. dahliae*, isolates in different VCGs are thought to be genetically isolated populations that may differ in many traits, including those related to pathogenicity and aggressiveness, adaptation to environments and sensitivity to fungicides (Katan 2000; Rowe 1995). Therefore, the characterization of local populations of *V. dahliae* into VCGs and phenotypic traits may help in the management of diseases they cause. The objectives of this study were to (i) examine and compare VCG diversity and composition among *V. dahliae* isolates obtained from commercial seed lots in Turkey and (ii) evaluate the aggressiveness of different VCGs to potato.

Material and methods

Collection of seed lots and isolation of V. dahliae Samples from 105 commercial seed lots belonging to 68 different cultivars were obtained in January 2012 from commercial seed companies and research centers in Turkey (Table 1). These cultivars comprised approximately 90% of potato plantings in Turkey in 2012. Twenty-five randomly selected seeds from each seed lot were removed from 4°C storage and warmed to 23°C for 12 h, then washed, surface sterilized in 1.2% NaOCI for 5 min, rinsed thoroughly and dried. Four pieces of vascular tissue (5–10 mm diam) were excised aseptically from a cut surface 3–5 mm beneath the stem end of each tuber. These were placed on streptomycin sulfate alcohol agar (Nadakavukaren & Horner 1959). The petri dishes were incubated at 24°C with 12 h:12 h L:D cycle (near-UV and cool-white fluorescent light by day) for 14 days (du Toit *et al.* 2005). Agar sections containing *V. dahliae* grown from tissue were transferred to water agar for monoconidial isolation. The 110 *V. dahliae* isolates were subcultured to 1% potato dextrose agar (PDA) consisting of 10.0 g of PDA, 15.0 g of Bacto agar, and 0.1 g of streptomycin sulfate in 1 liter of distilled water, and incubated in the dark at 24°C for 7 days. Monoconidial isolates were obtained by micromanipulation of conidia streaked to Czapek Dox agar (CDA) as described by Nitzan *et al.* (2002).

Generation and characterization of nit mutants *nit* mutants were generated on water agar – chlorate (WAC) medium consisting of 2% agar, 0.02% glucose and 3% potassium chlorate (Korolev *et al.* 2008). Mycelial plugs were placed on WAC medium at five or six points in petri dishes (9-cm diam) and incubated at 24°C. Chlorate-resistant sectors, evident after 10 to 14 days, were transferred to CDA (5-cm diam plates). Sectors that grew on CDA as colonies with a thin, expansive mycelium were considered *nit* mutants. CDA amended with sodium nitrite (0.5 g l^{-1}) or hypoxanthine (0.2 g l^{-1}) was used for partial phenotyping of the *nit* mutants (Correll *et al.* 1987).

Vegetative compatibility grouping Complementation between phenotypically distinct *nit* mutants was tested

Table 1 Detection and vegetative compatibility characterization of *Verticillium dahliae* isolates from commercially available potato seed lots in Turkey

Source		Seed lots infected/lots assayed	VCG distribution of isolates				
Cultivar	Company[z]		VCG4A	VCG4B	VCG2A	VCG2B	ND[y]
Adora	5	0/1					
Agata	8	1/1	11 isolates; Agt/92 - Agt/102				
Agria	3, 4, 6, 8, 11	2/6		6 isolates; Ag/25 - Ag/29 and Ag/45			
Alegria	4, 8	1/2	Al/50	4 isolates; Al/46 - Al/49			
Amora	8	0/1					
Anais	9	1/1	2 isolates; Ans/22 and Ans/23	Ans/21	Ans/24		
Anna	7	1/1			Ann/91		
Banba	8	1/1			Bn/107		Bn/106
Blondine	9	1/1	Bl/20				Bl/19
Borwina	4	1/1	2 isolates; Bo/58 and Bo/59		Bo/57		
Capri	8	1/1		6 isolates; Ca/84 - Ca/89			
Carrera	5	0/1					
Casablanca	4, 8	0/2					
Challenger	3, 5	0/3					
Chicago	4, 8	0/2					
Cripscall	5	1/1	4 isolates; Cr/37 - Cr/40				
Dione	5	0/1					
Electra	7	0/1					
Elfe	2	0/2					
Elodie	9	0/1					
Estrella	9	0/1					
Felsina	3, 5	1/3	2 isolates; Fe/43 and Fe/44	Fe/41	Fe/42		
Fosuc	8	0/1					
Galata	4, 8	2/2	6 isolates; Ga/51, Ga/53, Ga/55, Ga/56, Ga/103 and Ga/104		2 isolates; Ga/52 and Ga/54		
Granola	8, 10, 11	0/3					
Hanna	4, 8	1/2	2 isolates; Ha/80 and Ha/83		2 isolates; Ha/81 and Ha/82		
Hermes	11	0/1					
Innovator	3, 5	0/3					
Jaerla	8	0/1					
Krone	8	0/1					
Laderla	4	0/1					
Lady Anna	8	0/1					
Lady Blanca	3	0/2					
Lady Claire	8	0/1					
Lady Lenora	8	0/1					
Lady Olympia	8	1/1			Lo/90		
Lady Rosetta	11	0/1					

Table 1 (continued)

Source		Seed lots infected/lots assayed	VCG distribution of isolates				
Cultivar	Company[z]		VCG4A	VCG4B	VCG2A	VCG2B	ND[y]
Lanorma	10	0/2					
Latona	5	0/1					
Laura	8	0/1					
Lindita	8	1/1		Li/108			
Madeleine	8	0/1					
Marabel	8, 11	1/2					Mr/109
Marfona	6, 11	2/2	2 isolates; Ma/33 and Ma/34	2 isolates; Ma/31 and Ma/32		Ma/30	
Melody	1	1/2		Me/2			
Musica	1, 4, 8	0/4					
Natascha	1	1/1			Na/110		
Nectar	7	0/1					
Orchestra	1, 8	1/2	Or/3				
Orlo	7	0/1					
Pomqueen	4, 8	1/2	18 isolates; Po/60 - Po/74, Po/76, Po/78 and Po/79		2 isolates; Po/75 and Po/77		
Russet Burbank	3, 4, 11	0/4					
Safrane	9	1/1	3 isolates; Sf/4 - Sf/6				
Sagitta	5	0/1					
Sante	8, 11	1/3		Sn/105			
Savanna	8	0/1					
Shepody	8	0/2					
Sifra	5	0/1					
Sissi	8	0/1					
Slaney	7	0/1					
Soleia	9	1/1	7 isolates; So/8, So/9, So/11, So/12, So/14, So/16 and So/17		5 isolates; So/7, So/10, So/13, So/15 and So/18		
Spunta	8	0/1					
Surya	8	0/1					
Sylvana	5, 8	0/2					
Taurus	5	1/1	Ta/36		Ta/35		
Toscana	1	1/1		To/1			
Triomphe	9	0/1					
Vangogh	5, 8	0/2					
	Total	29/105	63	24	19	1	3

[z] Seed company identities are coded for proprietary purposes

[y] Not identified

on CDA. Each plate (5-cm diam) was inoculated with three mutants, 1 to 1.5 cm apart in a triangular pattern, and incubated at 24°C. Plates were scored for prototrophic growth 14 to 28 days after inoculation. Complementation was evident by the formation of a dense, aerial growth where mycelia from two mutants had met and formed a prototrophic heterokaryon. When mutants of two different isolates formed a heterokaryon, their parents were assigned to the same VCG. Each pairing was performed at least twice. Sixteen reference

isolates (Joaquim & Rowe 1990; Korolev *et al.* 2000, 2008) used in this study (Table 2) were kindly provided by R.C. Rowe & N. Korolev.

Tests for pathogenicity and aggressiveness Thirty-six *V. dahliae* isolates representing the multimember four VCGs were tested in two independent experiments (I and II) on potato with the dipping method (Omer *et al.* 2000). Twenty of these isolates belonged to VCG4A, eight to VCG4B, seven to VCG2A and one to VCG2B. Pathogenicity of *V. dahliae* isolates was evaluated using cv. 'Russet Burbank', which is moderately susceptible to Verticillium wilt (Azad *et al.* 1987). For the dipping inoculation, disinfested (1% NaOCl for 2.5 min) seeds were planted in plastic trays filled with a sterilized potting mixture (sand : clay loam : peat, 1:1:1, vol:vol). Plants were grown in a growth chamber under fluorescent illumination of 216–270 μE $m^{-2}s^{-1}$, 14h:10h L:D. Temperature and relative humidity, respectively, were 22–24°C and 50–70% during the light period, and 18–20°C and 60–80% during the dark period. Plants were watered as required and fertilized every 2 weeks with a water-soluble fertilizer (20-10-20, N:P:K). Three-week-old plants were gently removed from the potting mixture, roots were washed and then were immersed in a spore suspension (approximately10^6 conidia per ml) of each isolate for 10 min. Roots of control plants were treated similarly with sterile distilled water (Omer *et al.* 2000). Seedlings were then transplanted into 15-cm-diam plastic pots filled with sterilized potting mixture.

Disease severity in individual plants was rated weekly on a scale of 1–6 according to the percentage of foliage affected by chlorotic, necrotic and wilt symptoms, in an acropetal progression (1 = no visible symptoms; 2 = slight chlorosis of the lower leaves; 3 = extensive chlorosis of the lower leaves; 4 = extensive chlorosis and some necrosis of the lower and upper leaves; 5 = severe stunting with chlorosis and necrosis of the entire plant; 6 = dead or nearly dead plants). To determine aggressiveness for each isolate, area under the disease progress curve (AUDPC) was calculated for each individual plant based on foliar symptom ratings during the 1–7-week period following inoculation (Campbell & Madden 1990; Omer *et al.* 2000).

Statistical analysis Each experiment was conducted in a randomized complete block design with eight replicates, each one consisting of a single potted plant. The experiments were repeated once. Recorded values were averaged across plants within each experimental unit for further data analysis. Results across replicated experiments were consistent. Therefore, disease severity data from all experiments were pooled and the combined

Table 2 *nit* mutant tester strains of *Verticillium dahliae* previously assigned to vegetative compatibility groups

Isolate	VCG and Reference	Mutant phenotype	Host of origin	Geographical origin
T9	1A Joaquim & Rowe 1990, 1991	*nit*1 and NitM	Cotton	USA
cot200	1A Korolev *et al.* 2008	*nit*1 and NitM	Cotton	Israel
9.6	1B Chen 1994	*nit*1and NitM	Yellowwood	USA
1990.1	1B Chen 1994	NitM	Japanese maple	USA
PH	2A Joaquim & Rowe 1990, 1991	*nit*1 and NitM	Pistachio	USA
pt72	2A Korolev *et al.* 2000	*nit*1	Potato	Israel
ep8	2A Korolev *et al.* 2000	NitM	Eggplant	Israel
cot11	2B Korolev *et al.* 2000	NitM	Cotton	Israel
cot274	2B Korolev *et al.* 2008	*nit*1	Cotton	Israel
115	2B Joaquim & Rowe 1990, 1991	*nit*1 and NitM	Cotton	Syria
70-21	3 Joaquim & Rowe 1991	*nit*1 and NitM	Pepper	USA
PCW	3 Joaquim & Rowe 1990, 1991	*nit*1 and NitM	Pepper	USA
BB	4A Joaquim & Rowe 1990, 1991	*nit*1 and NitM	Potato	USA
S39	4B Joaquim & Rowe 1991	*nit*1 and NitM	Potato	USA
pn4	4B Korolev *et al.* 2000	NitM	Peanut	Israel
Tom53	4B Korolev *et al.* 2000	*nit1*	Tomato	Israel

data set was analyzed as a one-way analysis of variance using the PROC Mixed procedure in SAS (Brunner *et al.* 2002; Shah & Madden 2004). Linear single-degree-of-freedom contrasts were computed to test the effect of selected experimental treatment combinations (Gómez & Gómez 1984).

Results

Collection of V. dahliae isolates and assignment to VCG *Verticillium dahliae* was isolated from 29 of 105 seed lots belonging to 68 cultivars grown in Turkey. The frequency of infected lots was nearly 28% (Table 1). With regard to cultivars in tested seed lots, 'Pomqueen' was the cultivar from which *V. dahliae* was predominantly isolated (18.2% of the total number of isolates) (Table 1). Cultivar 'Soleia' was second in importance regarding the number of *V. dahliae* isolates collected (11% of the total). Cultivar 'Agata' was the third in the ranking from which pathogen isolates were sampled (10%).

When grown on chlorate-amended medium, mycelial growth of all isolates was restricted due to chlorate sensitivity. Sectoring frequency on chlorate-containing medium and phenotype ratio of *nit* mutants varied among isolates. Four to eight *nit* mutants were obtained from each isolate of *V. dahliae*. A total of 681 *nit* mutants were obtained from the 110 isolates. The *nit*1 phenotype was recovered most frequently (87%), followed by NitM (13%). Several *nit*1 and NitM mutants from each isolate were selected for complementation tests. No self incompatibility was observed between complementary *nit* mutants recovered from the same isolate of *V. dahliae.* Based on positive complementation reactions with reference testers, four VCGs were found: 63 of 110 isolates were assigned to VCG4A, 24 isolates to VCG4B, 19 isolates to VCG2A, and one to VCG4B (Table 1).

Frequency of VCG4A and disease incidence caused by isolates of this group were higher than those found for VCG4B and VCG2A in most of the cultivars inspected (Table 1). Only in 'Agria', 'Alegria', 'Anna', 'Banba', 'Capri', 'Lady Olympia', 'Lindita', 'Melody', 'Natascha', 'Sante' and 'Toscana' cultivars was the frequency of VCG4A lower than that of VCG4B or VCG2A. VCG4B was detected in nine cultivars, with the highest pathogen frequency and disease incidence in 'Capri'. VCG2A representatives were found in 12 cultivars, with the highest incidence in 'Soleia'. Isolates of VCG2B showed the lowest frequency value and were detected only in the cultivar 'Marfona'. In three of 68 cultivars, both VCG4A and VCG4B isolates were recovered from the same seed lot. Simultaneous presence of VCG4A, VCG4B and VCG2A was detected only in 'Anais' (Table 1).

Pathogenicity and aggressiveness Aggressiveness levels on potato cv. 'Russet Burbank' varied among the *V. dahliae* isolates that were tested in this study. AUDPC values indicated that as a sub-population, VCG4A isolates caused more (P <0.05) disease than VCG4B or VCG2A. One isolate designated as VCG2B tested in each experiment had AUDPC values that did not differ significantly (P <0.05) from isolates assigned to VCG2A. Some exceptions to this trend were observed across experiments. For instance, some isolates within a VCG were significantly (P <0.05) different from one another, and some isolates in VCG4B and VCG2A were as virulent as some isolates in VCG4A. This pattern of greater aggressiveness among isolates in VCG4A was especially evident when mean AUPDC values were calculated for each VCG and contrasts were used for mean comparisons (Table 3). Mean AUDPC was significantly highest in VCG4A (341.77) followed by VCG4B (298.09) and VCG2A (255.25), whereas coefficient of variation was low in VCG4A (15.03) followed by VCG4B (20.40) and VCG2A (25.40).

Collectively, isolates in VCG4A were significantly (P <0.05) more virulent than isolates assigned to other VCGs. Median, AUDPC, mean rankings and estimated relative effects for the severity of symptoms caused by isolates on the 'Russet Burbank' in experiments, as well as results of linear single-degree-of-freedom contrasts computed to test the effect of selected treatment combinations, are shown in Table 3. Significant differences (P <0.05) in mean symptom severity rankings were observed between cultivar and VCGs of isolates. Examination of the distribution of individual isolates of each VCG within selected ranges of AUPDC values illustrated that the majority of isolates in VCG4A were more virulent (Table 3). In experiments I and II, nine of 20 and 13 of 20 isolates, respectively, assigned to VCG4A resulted in AUDPC values above 350. Of isolates assigned to VCG4B, only two of eight in experiment I and three of eight in experiment II had AUDPC values >350. Only two of seven and none of seven isolates assigned to

Table 3 Median (M), mean area under the disease progress curve (AUDPC), mean rank (*R*), relative treatment effects (*REs*) and 95% confidence interval (CI) calculated for the severity of Verticillium wilt symptoms on 'Russet Burbank' caused by isolates of *Verticillium dahliae* from commercially available potato seed lots

VCG	Isolate	M[1]	AUDPC	*R*	*REs*[2]	95% CI for *REs*	
						Lower limit	Upper limit
4A	Sf/5	5.00	288.08	27.13	0.52	0.49	0.55
	So/8	5.25	323.56	28.00	0.60	0.49	0.69
	So/10	5.00	463.43	43.63	0.55	0.35	0.74
	So/12	6.00	442.55	43.62	0.83	0.81	0.85
	So/13	4.75	384.10	44.50	0.47	0.31	0.64
	Bl/20	4.00	263.03	27.56	0.27	0.24	0.30
	Cr/40	3.75	281.81	39.81	0.28	0.12	0.56
	Al/49	5.00	325.65	41.44	0.52	0.49	0.55
	Al/50	5.00	296.43	30.38	0.52	0.49	0.55
	Ga/56	4.00	254.68	29.63	0.27	0.24	0.30
	Po/63	4.50	308.95	41.44	0.40	0.31	0.49
	Po/65	5.50	384.10	41.44	0.68	0.55	0.78
	Po/67	5.75	361.14	31.25	0.75	0.64	0.84
	Po/68	5.50	404.98	42.75	0.68	0.55	0.78
	Po/71	6.00	338.18	39.38	0.83	0.81	0.85
	Po/72	6.00	342.35	23.88	0.83	0.81	0.85
	Lo/90	6.00	375.75	41.44	0.83	0.81	0.85
	Agt/92	6.00	367.40	29.63	0.83	0.81	0.85
	Agt/94	5.75	331.91	39.38	0.75	0.64	0.84
	Ga/103	5.50	296.43	39.38	0.68	0.55	0.78
4B	To/1	5.25	311.04	19.75	0.60	0.49	0.69
	Ag/29	5.00	384.10	32.12	0.63	0.36	0.83
	Ma/31	3.25	277.64	31.25	0.14	0.10	0.20
	Fe/41	3.75	290.16	31.25	0.30	0.13	0.57
	Ag/45	5.00	288.08	34.50	0.52	0.49	0.55
	Al/46	5.00	254.68	34.50	0.52	0.49	0.55
	Ha/81	3.75	198.31	25.94	0.23	0.18	0.29
	Ca/87	5.50	379.93	39.38	0.68	0.55	0.78
2A	So/17	3.00	125.25	19.75	0.16	0.07	0.37
	Ag/25	5.25	356.96	29.63	0.61	0.43	0.76
	Ta/35	3.00	192.05	25.94	0.12	0.07	0.20
	Fe/42	3.75	269.29	28.00	0.28	0.12	0.56
	Bo/57	4.00	317.30	32.12	0.27	0.24	0.30
	Ha/82	4.00	258.85	25.94	0.31	0.18	0.48
	Na/110	4.00	267.20	29.63	0.28	0.24	0.30
2B	Ma/30	3.75	302.69	23.87	0.28	0.12	0.56
Contrast (*P*)[3]		F value	Pr > F		VCG	Mean AUDPC	CV[1]
VCG4A *vs* VCG4B		15.73	0.0003		4A	341.77	15.03
VCG4A *vs* VCG2A		19.67	<.0001		4B	298.09	20.40

Table 3 (continued)

VCG	Isolate	M[1]	AUDPC	*R*	*REs*[2]	95% CI for *REs*	
						Lower limit	Upper limit
VCG4A *vs* VCG2B		22.60	<.0001		2A	255.25	25.40
VCG4B *vs* VCG2A		-	ns		2B	302.75	-
VCG4B *vs* VCG2B		-	ns				
VCG2A *vs* VCG2B		-	ns				

[1] For median disease rating (M), severity of Verticillium wilt symptoms was assessed visually according to the percentage of foliar tissue affected using an ordinal 1-to-6 rating scale in which 1 = no visible symptoms; 2 = slight chlorosis of the lower leaves; 3 = extensive chlorosis of the lower leaves; 4 = extensive chlorosis and some necrosis of the lower and upper leaves; 5 = severe stunting with chlorosis and necrosis of the entire plant; 6 = dead or nearly dead plants, and CV = coefficient of variation

[2] Estimated relative effects (*REs*) in experiments based on the analysis of variance-type statistics of ranked data using the PROC Mixed procedure in SAS for the severity of Verticillium wilt symptoms on the cultivar 'Russet Burbank' caused by *Verticillium dahliae* isolates of vegetative compatibility groups (VCGs), 95% confidence intervals

[3] Linear single-degree-of-freedom contrast computed to test the effect of selected treatment combinations. Probability for the *t* statistic of linear single-degree-of-freedom contrasts, significance level $P < 0.05$, ns = not significant

VCG2A in experiments I and II, respectively, had a value >350. In both experiments, no isolates assigned to VCG2B had AUDPC values >350.

Discussion

Diversity of *V. dahliae* isolates originating from potato seed lots into VCGs in Turkey is reported for the first time. Twenty-nine out of 105 seed lots (approx. 28%) that were tested were infested with *V. dahliae*. *V. dahliae* was detected in 26 of 68 cultivars tested (38%) and was most abundant in the cultivars 'Pomqueen', 'Agata' and 'Soleia'. Considering that only 25 seed-tubers were tested from each lot, it is likely that additional potato seed lots were infested at lower rates that were not detected due to the small sample size used. Nevertheless, the findings reported here indicated that commercial potato seed lots that are grown in Turkey are subjected to infestation by *V. dahliae*, which is a primary means of the pathogen's dispersion.

In the present study, two phenotypic classes of Nit mutants were identified among 681 mutants: 87% of these isolates were identified as *nit*1/*nit*3, and 13% as NitM. Although others have reported similar results (Brooker *et al.* 1991; Göre 2007; Korolev *et al.* 2008), the ratio of *nit*1 to NitM mutants varies widely among studies (as high as 49:51, and as low as 6:94). This could be attributed to a number of factors, *e.g.* source, condition, and age of isolates, as well as media type and components. *nit*3 mutants were rarely produced in this study and the vast majority of mutants were of the *nit*1 phenotype mutants. Some *nit*3 mutants could not be distinguished from *nit*1 because they did not grow on nitrite medium. Similar results were also reported by several researchers (Daayf *et al.* 1995; Korolev *et al.* 2000; Strausbaugh 1993). In these studies, such mutants in *V. dahliae* were phenotyped as *nit*1, because they complemented NitM mutants but did not complement *nit*1. Therefore, we also considered this kind of mutant as nit1. Overall, four multimember VCGs (VCG4A, VCG4B, VCG2A, VCG2B) were identified among the 110 isolates. Remarkably, VCG4A was the most prevalent (57.2%) VCG in seed lots, followed by VCG4B (21.8%), VCG2A (17.3%) and VCG4B (0.9%). In previous studies, similar VCG diversity in *V. dahliae* populations obtained from potato was identified. In the first study of the diversity of *V. dahliae* isolates from potato in Turkey, Dane & Demirci (2012) determined VCGs of 111 isolates from Erzurum province. Based on complementarity of *nit* mutants, 69.4% of the isolates were assigned to VCG4B and 30.6% to VCG2B. Demirci & Genç (2009) used 21 isolates of *V. dahliae* from weeds in potato fields in Erzurum province to determine VCGs; 52.4% of the isolates were identified as VCG2B, 47.6% as VCG4B. The frequency of VCGs of *V. dahliae* from potato differed in some other countries. In

America, Joaquim & Rowe (1990) reported that out of 187 isolates of *V. dahliae* recovered from potato plants and soil in Ohio, two were assigned to VCG1, 53 to VCG2, 32 to VCG4A, 90 to VCG4B and six to VCG4AB. The remaining four isolates could not be tested for vegetative compatibility because of their inability to yield *nit* mutants. When 47 additional isolates from potato plants grown in nine U.S. states were tested, two were assigned to VCG2, 36 to VCG4A, five to VCG4B and four to VCG4AB. In another study of 33 isolates of *V. dahliae* obtained from potato plants grown in southern Idaho, 87.9% were classified as VCG4A, 9.1% as VCG4B, and 3% as VCG4AB (Strausbaugh 1993). Omer *et al.* (2000) reported that 162 isolates of *V. dahliae* recovered from certified seed potatoes in North America, 64% were classified as VCG4A, 33% as VCG4B and 3% as VCG4AB. In Canada, most potato isolates showed strong compatibility with VCG4A (91.7%) and 8.3% of the isolates were identified as VCG4B (El-Bebany *et al.* 2013). In Israel, VCG4B was the largest group and included 235 isolates (96.7% of all the isolates) and the remaining 3.3% of the isolates were identified as VCG2A (Korolev *et al.* 2000). In our study, *V. dahliae* isolates were obtained from a wide range of potato cultivars grown in Turkey. More than half of the isolates were VCG4A; the rest were VCG4B, VCG2A and a few were VCG2B. To the best of our knowledge, the present study is the first report of natural infections of potato by VCG4A and VCG2A.

Overall, aggressiveness of *V. dahliae* isolates on cv. 'Russet Burbank' correlated with their VCG: isolates of VCG4A and VCG2A were the most and least virulent, respectively. The first symptoms developed 2 to 3 weeks after inoculation. The plants inoculated with isolates of VCG4A exhibited extensive stunting, chlorosis, and necrosis in both lower and upper leaves, which generally remained attached to the stem after death. Most plants were dead or nearly dead 7 weeks after inoculation. In contrast, most potato plants inoculated with isolates in VCG4B and VCG2 showed milder symptoms, usually restricted to chlorosis in the lower and upper leaves. Stunting and death of plants were rarely observed in inoculations with these isolates. Inoculations with isolates in VCG4B resulted in mildly virulent reactions similar to those with VCG2, although some isolates in VCG4B were as virulent as isolates in VCG4A (Joaquim & Rowe 1991; Strausbaugh 1993). These findings are in agreement with results from previous studies on *V. dahliae*, which have demonstrated some correlations between VCGs and aggressiveness on certain hosts (Bhat & Subbarao 1999; Daayf *et al.* 1995; Göre 2009; Tsror *et al.* 2001). So far, the defoliating (D) and non-defoliating (ND) pathotypes from cotton have been reported from several locations in the Americas (Schnathorst & Mathre 1966), China (Zhengjun *et al.* 1998), Central Asia (Daayf *et al.* 1995), Spain (Bejarano-Alcázar *et al.* 1995; Korolev *et al.* 2008), Turkey (Göre 2007) and Israel (Korolev *et al.* 2008). Epidemics caused by the D pathotype (VCG1A) develop earlier, more rapidly, and result in a greater reduction of cotton yield compared with the losses caused by the ND pathotype (VCG1B, VCG2A, VCG2B, VCG4B) (Bejarano-Alcázar *et al.* 1995, 1997). In North America, VCG4A isolates were more aggressive on potato than VCG4B, 4AB, and 2B isolates (Joaquim & Rowe 1991; Omer *et al.* 2000; Strausbaugh 1993). Moreover, VCG4A isolates interacted synergistically with the root lesion nematode *Pratylenchus penetrans*, causing reduced tuber yield (Botseas & Rowe 1994). In Israel, VCG2A isolates were the most aggressive to tomato; VCG2B isolates were the least aggressive to both potato and tomato (Tsror *et al.* 2001). In Turkey, VCG1A isolates were the most aggressive to both chrysanthemum and olive, and VCG2B isolates were the most aggressive to eggplant (Derviş *et al.* 2009, 2010; Göre 2009). Differences in aggressiveness among isolates within a VCG have been observed also in *Fusarium oxysporum* (Correll *et al.* 1987; Elmer & Stephens 1989), *Colletotrichum* spp. (Brooker *et al.* 1991; Nitzan *et al.* 2006), *Aspergillus* spp. (Wicklow & Horn 2007) and *Neurospora crassa* (Tomsett & Garrett 1980). Variation in aggressiveness among isolates in the same VCG is viewed by some workers as an indication of the presence of a continuum of aggressiveness rather than distinct pathotypes (Ashworth 1983; Grogan *et al.* 1979). However, data presented here provide evidence for recognition of at least two distinct pathotypes among populations of *V. dahliae* recovered from potatoes. One pathotype, to which most VCG4A isolates belong, leads to early and severe symptoms resulting in a rapid collapse and death of the plant. The other pathotype, composed of VCG4B and VCG2 isolates, leads to slower development of symptoms, which appear mainly as chlorotic areas on the leaves.

Our findings have broad implications for the Turkish potato industry. Extensive infection of potato seed lots with *V. dahliae* certainly explains the widespread

distribution of VCG4A isolates across Turkish potato production regions. It also brings into question the importance of tuberborne as compared with soilborne inoculum of *V. dahliae*, the latter being the target of most current management practices. Considerable resources are expended annually in some production regions to fumigate fields for control of Verticillium wilt prior to planting potato. The importance of tuberborne inoculum in the development of Verticillium wilt following fumigation warrants investigation. The use of partially resistant cultivars is the most practical disease management strategy (Dung & Johnson 2012; Omer *et al.* 2000; Powelson & Rowe 1993) and efforts are ongoing to develop cultivars with improved resistance to Verticillium wilt and high market acceptance. Better understanding of the genetic diversity that exists among populations of *V. dahliae* that affect potato and the use by breeders of the most aggressive isolates, *i.e.,* VCG4A, in screening germplasm will be essential to accomplish this goal.

Acknowledgments Our thanks are expressed to Mr. Adnan Özkan for technical assistance. This research was supported by funding from the Abant Izzet Baysal University Research Program (BAP/2012.24.01.562).

References

Agarwal, V. K., & Sinclair, J. B. (1997). *Principles of seed pathology.* Boca Raton, FL, USA: CRC Press.

Ashworth, L. J., Jr. (1983). Aggressiveness of random and selected isolates of *Verticillium dahliae* from cotton and the quantitative relationship of internal inoculum to defoliation. *Phytopathology, 73*, 1292–1295.

Azad, H. R., Davis, J. R., Schnathorst, W. C., & Kado, C. I. (1987). Influence of Verticillium wilt resistant and susceptible potato genotypes on populations of antagonistic rhizosphere and rhizoplane bacteria and free nitrogen fixers. *Applied Microbiology and Biotechnology, 26*, 99–104.

Bejarano-Alcázar, J., Blanco-López, M. A., Melero-Vara, J. M., & Jiménez-Díaz, R. M. (1995). Influence of inoculum density of defoliating and nondefoliating pathotypes of *Verticillium dahliae* in epidemics of Verticillium wilt of cotton in southern Spain. *Phytopathology, 85*, 1474–1481.

Bejarano-Alcázar, J., Blanco-López, M. A., Melero-Vara, J. M., & Jiménez-Díaz, R. M. (1997). The influence of Verticillium wilt epidemics on cotton in southern Spain. *Plant Pathology, 46*, 168–178.

Bhat, R. G., & Subbarao, K. V. (1999). Host range specificity in *Verticillium dahliae. Phytopathology, 89*, 1218–1225.

Botseas, D. D., & Rowe, R. C. (1994). Development of potato early dying in response to infection by two pathotypes of *Verticillium dahliae* and co-infection by *Pratylenchus penetrans. Phytopathology, 84*, 275–282.

Brooker, N. L., Leslie, J. F., & Dickman, M. B. (1991). Nitrate non-utilizing mutants of *Colletotrichum* and their use in studies of vegetative compatibility and genetic relatedness. *Phytopathology, 81*, 672–677.

Brunner, E., Domhof, S., & Langer, F. (2002). *Nonparametric analysis of longitudinal data in factorial experiments*. New York, NY: John Wiley & Sons.

Campbell, C. L., & Madden, L. V. (1990). *Introduction to plant disease epidemiology.* New York, NY: John Wiley & Sons.

Cappaert, M. R., Powelson, M. L., Christensen, N. W., & Crowe, F. J. (1992). Influence of irrigation on severity of potato early dying and associated yield loss. *Phytopathology, 82*, 1448–1453.

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

Correll, J. C., Klittich, C. J. R., & Leslie, J. F. (1987). Nitrate nonutilizing mutants of *Fusarium oxysporum* and their use in vegetative compatibility tests. *Phytopathology, 77*, 1640–1646.

Daayf, F., Nicole, M., & Geiger, J. (1995). Differentiation of *Verticillium dahliae* populations on the basis of vegetative compatibility and pathogenicity on cotton. *European Journal of Plant Pathology, 101*, 69–79.

Dane, E., & Demirci, E. (2012). Vegetative compatibility groups and pathogenicity of *Verticillium dahliae* isolates from potato plants in Erzurum Province. *Journal of Agricultural Sciences, 18*, 110–114.

Demirci, E., & Genç, T. (2009). Vegetative compatibility groups of *Verticillium dahliae* isolates from weeds in potato fields. *Journal of Plant Pathology, 91*, 671–676.

Derviş, S., Mercado-Blanco, J., Erten, L., Valverde-Corredor, A., & Pérez-Artés, E. (2010). Verticillium wilt of olive in Turkey: a survey on disease importance, pathogen diversity and susceptibility of relevant olive cultivars. *European Journal of Plant Pathology, 127*, 287–301.

Derviş, S., Yetisir, H., Yıldırım, H., Tok, F. M., Kurt, S., & Karaca, F. (2009). Genetic and pathogenic characterization of *Verticillium dahliae* isolates from eggplant in Turkey. *Phytoparasitica, 37*, 467–476.

Dobinson, K. F., Harrington, M. A., Omer, M., & Rowe, R. C. (2000). Molecular characterization of vegetative compatibility group 4A or 4B isolates of *Verticillium dahliae* associated with potato early dying. *Plant Disease, 84*, 1241–1245.

du Toit, L. J., Derie, M. L., & Hernandez-Perez, P. (2005). Verticillium wilt in spinach seed production. *Plant Disease, 89*, 4–11.

Dung, J. K. S., Ingram, J. T., Cummings, T. F., & Johnson, D. A. (2012). Impact of seed lot infection on the development of black dot and Verticillium wilt of potato in Washington. *Plant Disease, 96*, 1179–1184.

Dung, J. K. S., & Johnson, D. A. (2012). Roles of infected seed tubers and soilborne inoculum on Verticillium wilt of 'Russet Burbank' potato. *Plant Disease, 96*, 379–383.

El-Bebany, A. F., Alkher, H., Adam, L. R., & Daayf, F. (2013). Vegetative compatibility of *Verticillium dahliae* isolates from potato and sunflower using nitrate non-utilizing (*nit*) mutants and PCR-based approaches. *Canadian Journal of Plant Pathology, 35*, 1–9.

Elmer, W. H., & Stephens, C. T. (1989). Classification *of Fusarium oxysporum* f. sp. *asparagi* into vegetatively compatible groups. *Phytopathology, 79*, 88–93.

Gómez, K. A., & Gómez, A. A. (1984). *Statistical procedures for agricultural research* (2nd ed.). New York, NY: John Wiley and Sons.

Göre, M. E. (2007). Vegetative compatibility and pathogenicity of *Verticillium dahliae* isolates from the Aegean Region of Turkey. *Phytoparasitica, 35*, 222–231.

Göre, M. E. (2009). Vegetative compatibility and pathogenicity of *Verticillium dahliae* isolates from chrysanthemum in Turkey. *Phytoparasitica, 37*, 87–94.

Göre, M. E., Erdoğan, O., Altın, N., Aydın, M. H., Caner, Ö. K., Filizer, F., *et al*. (2011). Seed transmission of Verticillium wilt of cotton. *Phytoparasitica, 39*, 285–292.

Grogan, R. G., Ioannouu, D., Schneider, R. W., Sall, M. A., & Kimble, K. A. (1979). Verticillium wilt on resistant tomato cultivars in California: Virulence of isolates from plants and soil and relationship of inoculum density to disease incidence. *Phytopathology, 69*, 1176–1180.

Joaquim, T. R., & Rowe, R. C. (1990). Reassessment of vegetative compatibility relationships among strains of *Verticillium dahliae* using nitrate-nonutilizing mutants. *Phytopathology, 80*, 1160–1166.

Joaquim, T. R., & Rowe, R. C. (1991). Vegetative compatibility and virulence of strains of *Verticillium dahliae* from soil and potato plants. *Phytopathology, 81*, 552–558.

Johnson, D. A., & Dung, J. K. S. (2010). Verticillium wilt of potato – the pathogen, disease and management. *Canadian Journal of Plant Pathology, 32*, 58–67.

Johnson, K. B., Radcliffe, E. B., & Teng, P. S. (1986). Effects of interacting populations of *Alternaria solani*, *Verticillium dahliae*, and the potato leafhopper (*Empoasca fabae*) on potato yield. *Phytopathology, 76*, 1046–1052.

Katan, T. (2000). Vegetative compatibility in populations of *Verticillium*—an overview. In E. C. Tjamos, R. C. Rowe, J. B. Heale, & D. R. Fravel (Eds.), *Advances in Verticillium research and disease management* (pp. 69–86). St. Paul, MN, USA: APS.

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.

Korolev, N., Pérez-Artés, E., Mercado-Blanco, J., Bejarano-Alcázar, J., Rodríguez-Jurado, D., Jiménez-Díaz, R. M., *et al*. (2008). Vegetative compatibility of cotton defoliating *Verticillium dahliae* in Israel and its pathogenicity to various hosts. *European Journal of Plant Pathology, 122*, 603–617.

Nadakavukaren, M. J., & Horner, C. E. (1959). An alcohol agar medium selective for determining Verticillium microsclerotia in soil. *Phytopathology, 49*, 527–528.

Nitzan, N., Hazanovsky, M., Tal, M., & Tsror (Lakhim), L. (2002). Vegetative compatibility groups in *Colletotrichum coccodes*, the causal agent of black dot on potato. *Phytopathology*, *92*, 827-832.

Nitzan, N., Tsror (Lahkim), L., & Johnson, D. A. (2006). Vegetative compatibility groups and aggressiveness of North American isolates of *Colletotrichum coccodes*, the causal agent of potato black dot. *Plant Disease*, *90*, 1287-1292.

Omer, M. A., Johnson, D. A., & Rowe, R. C. (2000). Recovery of *Verticillium dahliae* from North American certified seed potatoes and characterization of strains by vegetative compatibility and aggressiveness. *American Journal of Potato Research, 77*, 325–331.

Powelson, M. L., & Rowe, R. C. (1993). Biology and management of early dying of potatoes. *Annual Review of Phytopathology, 31*, 111–126.

Rowe, R. C. (1995). Recent progress in understanding relationships between Verticillium species and subspecific groups. *Phytoparasitica, 23*, 31–38.

Rowe, R. C., & Powelson, M. L. (2002). Potato early dying: management challenges in a changing production environment. *Plant Disease, 86*, 1184–1193.

Schnathorst, W. C., & Mathre, D. E. (1966). Host range and differentiation of a severe form of *Verticillium albo-atrum* in cotton. *Phytopathology, 56*, 1155–1161.

Shah, D. A., & Madden, L. V. (2004). Nonparametric analysis of ordinal data in designed factorial experiments. *Phytopathology, 94*, 33–43.

Strausbaugh, C. A. (1993). Assessment of vegetative compatibility and virulence of *Verticillium dahliae* isolates from Idaho potatoes and tester strains. *Phytopathology, 83*, 1253–1258.

Tomsett, A., & Garret, R. H. (1980). The isolation and characterization of mutants defective in nitrate assimilation in *Neurospora crassa. Genetics, 95*, 649–660.

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

Wicklow, D. T., & Horn, B. W. (2007). Association between vegetative compatibility and aflatoxin production by *Aspergillus* species during intraspecific competition. *Mycoscience, 48*, 267–273.

Zhengjun, P. N., Achar, P. N., & Benkang, G. (1998). Vegetative compatibility groupings of *Verticillium dahliae* from cotton in mainland China. *European Journal of Plant Pathology, 104*, 871–876.