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Vegetative Compatibility Groups and Pathogenicity of *Verticillium dahliae* Isolates from Potato Plants in Erzurum Province

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ABSTRACT

One hundred eleven isolates of *Verticillium dahliae* were obtained from potato plants in Erzurum province, Turkey. The pathogen was isolated from 7.6% of the stems collected. All isolates were assigned to vegetative compatibility groups (VCGs) using nitrate-nonutilizing (*nit*) mutants. In total, 240 *nit* mutants were obtained from *V. dahliae* isolates, and classified as *nit*1 (71%) and *nit*M (29%). Two VCGs were found and identified as VCG 2B (34 isolates) and VCG 4B (77 isolates) by using tester isolates of known VCGs. Pathogenicity of *V. dahliae* isolates was tested on potato (cv. Marfona) by the root-dip method. Both VCG 2B and VCG 4B isolates showed similar aggressiveness on potato. This is the first study of VCGs of *V. dahliae* isolates from potato plants in Turkey.

Key words: Potato; *Verticillium dahliae; Nit* mutants; Vegetative compatibility groups; Pathogenicity

Erzurum İlinde Patates Bitkilerinden Elde Edilen *Verticillium dahliae* İzolatlarının Vejetatif Uyum Grupları ve Patojeniteleri

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ÖZET

Erzurum ilinde patates bitkilerinden 111 *Verticillium dahliae* izolatı elde edilmiştir. Toplanan gövdelerin % 7.6'sından patojen izole edilmiştir. Nitrat kullanamayan (*nit*) mutantlar kullanılarak tüm izolatların vejetatif uyum grupları (VCG) belirlenmiştir. *V. dahliae* izolatlarından toplamda 240 *nit* mutant elde edilmiş olup, bunların % 71'i *nit*1 ve % 29'u *nit*M olarak sınıflandırılmıştır. Bilinen test izolatları kullanılarak VCG 2B (34 izolat) ve VCG 4B (77 izolat) olmak üzere iki VCG'u belirlenmiştir. Kök daldırma metodu kullanılarak *V. dahliae* izolatlarının patojenitesi Marfona patates çeşidinde test edilmiştir. VCG 2B ve VCG 4B'ye ait izolatlar patateste benzer saldırganlık göstermiştir. Türkiye'de patates bitkilerinden elde edilen *V. dahliae* izolatlarının VCG'larının belirlendiği ilk çalışmadır.

Anahtar Kelimeler: Patates; *Verticillium dahliae; Nit* mutantlar; Vejetatif uyum grupları; Patojenite

1. Introduction

Verticillium dahliae Kleb. is a soilborne plant pathogen responsible for severe damage on many crop species including potato (Pegg & Brady 2002). Potato is an important crop in Erzurum province, where more than 3,000 ha of potatoes are planted. Verticillium wilt on potato caused by *V. dahliae* has been a serious problem in this area recently. On potato, this fungus causes early senescence of plants and a light brown discoloration in

the vascular ring of tubers (Rich 1986), and it can cause a reduction of both yield and quality. Many weed species also have been reported as hosts of *V. dahliae* (Pegg & Brady 2002; Ligoxigakis et al 2002). The pathogen has been isolated from five weed species in potato fields in Erzurum (Demirci & Genc 2009).

The identification of vegetative compatibility groups (VCGs) has proved to be a powerful tool in determining the fungal genetic structure of *V. dahliae*, an anamorphic fungus with no known sexual stage. By using nitrate-nonutilizing (*nit*) mutants, four major VCGs (VCG 1, VCG 2, VCG 3 and VCG 4) of *V. dahliae* have been reported. VCG 2 and 4 have been further divided into subgroups (2A and 2B, 4A and 4B, respectively) based on differential interactions between isolates (Joaquim & Rowe 1990&1991). VCGs 2, 2A, 2B, 4A, 4B and/or 4A/B have been detected among *V. dahliae* isolates from potato plants (Joaquim & Rowe 1991; Strausbaugh 1993; Korolev et al 2000; Tsror et al 2001; Zeise & Tiedemann Von 2002).

The aim of this research was to determine the VCGs of *V. dahliae* isolates from potato plants in Erzurum, Turkey and to investigate the pathogenicity of these isolates on potato.

2. Material and Methods

2.1. Isolation of V. dahliae from potato plants

Potato plants generally showing wilt symptoms were collected from 9 locations (Table 1) in Erzurum province between August and September in 2003-2005 growing seasons. Plants were washed with tap water, and then stem sections 1 cm long were excised from potato plants. The tissue sections were surface disinfected with 0.5% sodium hypochlorite solution for 1 min, rinsed with sterile distilled water, dried on sterile filter paper and placed on water agar (WA, 2%) amended with 100 mg L^{-1} streptomycin sulfate in Petri plates. Plates were incubated at 24 °C in the dark for 7 days until verticillately branched conidiophores formed around the stem sections. Emerging fungi were subcultured on potato dextrose agar (PDA). Single-spore isolates of *V. dahliae* were obtained, identified as described previously (Hawksworth & Talboys 1970; Goud et al 2003), and maintained on PDA medium in tubes at 5 °C.

2.2. Generation and characterization of nit mutants

Nit mutants of *V. dahliae* were generated on cornmeal agar with 0.02% glucose amended with 3% potassium chlorate (CMC) as described previously (Korolev & Katan 1997). Mycelial discs (5 mm diam.) of *V. dahliae* isolates were removed from the margin of each actively growing colony on PDA and placed on CMC at six separate points in 9 cm diameter Petri plates. Plates were incubated in the dark at 24 ºC for 2-4 weeks. Chlorate-resistant sectors were transferred to Czapex-Dox Agar (CDA) plates. Sectors that grew on CDA as thin expansive colonies with no aerial 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). Mutants that grew profusely on sodium nitrite and hypoxanthine were classified as *nit*1, whereas mutants that grew profusely on sodium nitrite but sparsely on hypoxanthine were classified as *nit*M.

Table 1- Geographical distribution and vegetative compatibility groups (VCGs) of *Verticillium dahliae* isolates from potato plants during 2003-2005 in Erzurum province

Çizelge 1-Erzurum ilinde 2003–2005 yıllarında patates bitkilerinden elde edilen Verticillium dahliae izolatlarının coğrafik dağılımı ve vejetatif uyum grupları (VCGs)

Location	*Plants sampled*	*No. of isolates*	*Number of nit mutants*			*VCGs*	
			nitI	*nitM*	*Total*	*VCG 2B*	*VCG 4B*
Aşkale	180	19	33	10	43	7	12
Center	320	13	17	11	28	3	10
Horasan	120	7	10	6	16	-	7
Ilıca	60	1	2	0	2	-	1
İspir	40	1	0	1	1	1	-
Köprüköy	160	16	27	14	41	9	7
Narman	120	2	1	2	3	2	-
Pasinler	360	50	79	25	104	12	38
Tortum	100	2	2	0	2	-	2
Total	1460	111	171	69	240	34	77

2.3. Vegetative compatibility grouping

All *nit* mutants obtained in this study were paired with *nit* mutants (*nit*1 and *nit*M) of VCG tester isolates of *V. dahliae*. A set of VCG tester isolates [VCG 1 (T9), VCG 2A (PH), VCG 2B (115), VCG 3 (70-21), VCG 4A (BB, P103) and VCG 4B (S-39, MT)] was provided by Dr. R. C. Rowe (Department of Plant

Pathology, Ohio State University, Ohio Agricultural Research and Development Center, Wooster, Ohio, 44691, USA) and Dr. M. M. Jimenez-Gasco (Department of Plant Pathology, The Pennsylvania State University, University Park, PA 16802, USA). Phenotypically distinct mutants were placed 1.5 cm apart on CDA in 9 cm diameter Petri plates and incubated at 24 ºC for 2-4 weeks. Complementation was evident by the development of prototrophic growth where two mutant colonies met and formed a stable heterokaryon (Bao et al 1998). The degree of complementation was ranked as follows: (+) = dense prototrophic growth, (+/-) = small microsclerotial dots with or without a little aerial mycelium, (-) = prototrophic growth absent or inconspicuous (Korolev et al 2000). Each pairing was repeated at least twice. When mutants of two isolates formed a heterokaryon, their parents were assigned to the same VCG.

2.4. Pathogenicity of V. dahliae isolates on potato

Pathogenicity of 10 isolates of each VCG, selected at random on the basis of geographical origin, was determined on potato plants (cv. Marfona) by the root-dip method. This cultivar has been grown in Erzurum for a long time. Surface-disinfected potato tubers (1 min in 2% formaldehyde and rinsed in sterile distilled water) were planted in 15 cm diameter pots containing a sterile soil mix of topsoil and sand (1:1, v/v) in a growth chamber. After 5 weeks, 10 to 20 cm tall plants were selected for inoculation (Joaquim & Rowe 1991). The isolates were grown on PDA (9-cm plates) at 24°C in the dark for 10 days. Conidia were washed off the agar surface with sterile distilled water, and the inoculum density adjusted to 10^6 conidia mL^{-1} with a hemacytometer and sterile distilled water (Strausbaugh 1993). Potato plants were uprooted from soil mix, rinsed in sterile distilled water, and dipped in a conidial suspension for 30 min. Inoculated plants were transplanted into 15 cm-diam pots containing a sterile soil mix. Control plants were dipped in sterile distilled water before transplanting. Plants were grown in a growth chamber at 24 °C under a 16 h photoperiod. A completely randomized design with four replicate pots per isolate was used. Sixty five days after inoculation, disease severity was rated on a scale 0 to 3 (0= no symptoms, 1= vascular discoloration without apparent leaf symptoms, 2= vascular discoloration with leaf-wilt symptoms, 3= dead plant) as described previously (Bao et al 1998). After disease evaluations, small sections from all above-ground parts (stem, petiole and leaf) of each plant were surface disinfected and placed on WA to determine the presence of *V. dahliae*. Statistical analysis was performed by SAS Software (SAS Institute Inc., Cary, NC, USA). The General Linear Models procedure was used to test effects at the 0.05 level of probability and means were compared by *t* test.

3. Results and Discussion

3.1. Isolates of V. dahliae from potato plants

Stem samples were collected from potato fields in Erzurum province. The number of potato stems excised from plants onto culture media in the laboratory totaled 1460 during the 3 years, and *V. dahliae* was isolated from 7.6% of the stems examined. Totally, 111 isolates of *V. dahliae* were obtained from potato stems from 9 locations (Table 1). Most of these isolates were collected from Pasinler.

3.2. Generation and characterization of nit mutants

In total, 240 *nit* mutants were obtained from 111 isolates of *V. dahliae*, ranging from 1 to 7 mutants per isolate. *Nit* mutants were identified based on their phenotype; 171 mutants were classified as *nit*1 and the remainder as *nit*M (Table 1). Similar frequencies of *nit*1 and *nit*M classes were found for *V. dahliae* from various hosts including potato (Bao et al 1998; Zeise & Tiedemann Von 2001; Demirci & Genc 2009). In this study, twelve isolates produced both types of mutants, 68 the *nit*1 type mutant and 31 the *nit*M type mutant only (data not shown). No *nit*3 mutants were recovered.

3.3. Vegetative compatibility grouping

The genetic diversity among one hundred eleven *V. dahliae* isolates was determined. After complementation with the tester isolates of known VCGs, 34 isolates were assigned to VCG 2B, and 77 to VCG 4B (Table 1). Isolates assigned to VCG 2 showed strong complementation only with tester isolates of VCG 2B. Cross-reactions occurred between isolates VCG 4 from potato plants and tester isolates of VCG 4 (subgroups A and B), VCG 4 isolates showed strong complementation (+) with the tester isolates of VCG 4B, but all were also weakly compatible (+/-) with the tester isolates of VCG 4A. Unfortunately a mistake was made on the determination of the VCG of a number of isolates of *V. dahliae* from potato plants (Dane 2007). In this thesis, isolates of *V. dahliae* were typed as VCG 4A due to problems with some tester isolates. After we were aware of this problem, new VCG tester isolates [VCG 4A (BB, P103) and VCG 4B (S-39, MT)] of *V. dahliae* were provided by Dr. M. M. Jimenez-Gasco. The vegetative compatibility of isolates previously typed as VCG 4A was re-evaluated. All *nit* mutants of VCG 4 obtained in this study were paired with *nit* mutants of new tester isolates of VCG 4A and 4B. Eventually, all VCG 4 isolates from potato plants in Erzurum were re-classified as VCG 4B in this article.

Both VCG 2B and VCG 4B isolates were identified from Center, Aşkale, Köprüköy and Pasinler. Only VCG 2B isolates were identified from İspir and

Narman, and only VCG 4B isolates were identified from Horasan, Ilıca and Tortum. Both VCG 2B (Zeise & Tiedemann Von 2002) and VCG 4B (Joaquim & Rowe 1991; Strausbaugh 1993; Korolev et al 2000; Tsror et al 2001; Zeise & Tiedemann Von 2002) isolates have been reported before on potato. In a study from Erzurum, *V. dahliae* isolates from some of the common weeds in potato fields also were assigned to VCG 2B and VCG 4B (Demirci & Genc 2009; 2011). The results suggest that the population of *V. dahliae* isolates from potato plants and weeds in potato fields is the same. This is the first study of vegetative compatibility of *V. dahliae* isolates from potato plants in Turkey.

3.4. Pathogenicity of V. dahliae isolates on potato

The pathogenicity of twenty isolates representing VCG 2B and VCG 4B was determined on potato plants by the root-dip method. Potato plants all exhibited typical Verticillium wilt symptoms in response to inoculation with the tested *V. dahliae* isolates. Disease symptoms were visible 5-6 weeks after inoculation and developed over time from chlorosis to necrosis and wilting. When stem sections of these plants were dissected, they all showed vascular discoloration. Based on the results of pathogenicity tests, all isolates were pathogenic on potato at various levels of aggressiveness (Table 2). Disease severity ranged from 1.5 to 2.8 for VCG 2B isolates, and from 2.0 to 2.5 for VCG 4B isolates. No significant differences ($F_{4,80}$=0.66, P=0.42) were observed among the tested VCG 2B and 4B for disease severity. Control plants showed no disease symptoms. *V. dahliae* was recovered from all the inoculated plants but not from control plants.

The present study showed that most isolates of VCG 2B and VCG 4B from potato plants were highly aggressive on potato cv. "Marfona", and there was no difference between the isolates for disease severity.

Disease severity of potato plants infected with *V. dahliae* isolates from weed species in Erzurum ranged from 1.6 to 2.3 for both VCG 2B and VCG 4B isolates (Demirci & Genc, 2009 & 2011). However, symptom severity was significantly higher in potato plantlets inoculated with VCG 4B than VCG 2A and VCG 2B (Tsror et al 2001).

Table 2- Pathogenicity of *Verticillium dahliae* isolates on potato cv. Marfona
Çizelge 2-Marfona patates çeşidinde Verticillium dahliae izolatlarının patojenitesi

VCGs[a]	*Isolates*	*Location*	*Disease severity*[b]	*Plant colonization*[c]		
				Stem	*Petiole*	*Leaf*
VCG 2B	OA04-1-8	Aşkale	2.3	4/4	3/4	0/4
	OA04-2-2	Aşkale	2.5	4/4	3/4	1/4
	OA05-1-3	Aşkale	1.5	4/4	0/4	0/4
	4.Kuyu05-1	Center	2.0	4/4	3/4	0/4
	Bi1-2	İspir	2.3	4/4	4/4	0/4
	KöDç05-2-4	Köprüköy	2.3	4/4	1/4	0/4
	NM05-4-2	Narman	2.3	4/4	2/4	0/4
	PM04-1	Pasinler	2.0	4/4	4/4	0/4
	EP04-4	Pasinler	2.0	4/4	2/4	0/4
	PTi04-2	Pasinler	2.8	4/4	4/4	1/4
VCG 4B	OA04-2-3	Aşkale	2.3	4/4	3/4	0/4
	OA05-4-2	Aşkale	2.5	4/4	4/4	1/4
	Tu04-2	Center	2.5	4/4	4/4	1/4
	HÇ05-1	Horasan	2.5	4/4	4/4	2/4
	I05-1-1	Ilıca	2.5	4/4	4/4	0/4
	Kö04-2	Köprüköy	2.0	4/4	3/4	0/4
	AP05-2-2	Pasinler	2.0	4/4	3/4	1/4
	PM04-7	Pasinler	2.5	4/4	4/4	2/4
	PM04-8	Pasinler	2.0	4/4	3/4	0/4
	TA05-2	Tortum	2.0	4/4	3/4	0/4

[a] Vegetative compatibility groups of *V. dahliae* isolates.
[b] Disease severity was on a scale of 0 to 3; 0= no symptoms, 1= vascular discoloration without apparent leaf symptoms, 2= vascular discoloration with leaf-wilt symptoms, 3= dead plant (Bao et al 1998).
[c] Number of plants colonized by *V. dahliae* / total number of examined plants.

In other researches, VCG 4A isolates were more virulent on potato than VCGs 2 and 4B (Joaquim & Rowe 1991) or VCGs 4B and 4A/B isolates (Strausbaugh 1993).

Rotation is one of the major components of

Verticillium wilt management. However, many field crops, vegetables and weeds are susceptible to the pathogens causing this disease. In addition, microsclerotia produced by *V. dahliae* in the dying tissues of the infected plant can survive in the soil for many years (Pegg & Brady 2002). Moreover, one study has reported that formation of microsclerotia in senescent tissues of infected weeds could be an important factor in the failure of rotation programs to control *V. dahliae* effectively (Johnson et al 1980). Another study showed that some of the common weeds in potato fields can act as potential hosts of *V. dahliae* and potentially play important role in the survival of this pathogen (Demirci & Genc 2009). Therefore, weed control in potato fields is also very important.

4. Conclusion

The results of this study show that *V. dahliae* isolates from potato in Erzurum were classified as VCG 2B and VCG 4B. There are no statistically significant differences between VCG 2B and 4B isolates on disease severity.

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