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Genetic relationships among *Verticillium dahliae* isolates from cotton in Greece based on vegetative compatibility

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Abstract

Vegetative compatibility groups of a collection of 71 Greek *Verticillium dahliae* isolates obtained from cotton plants were tested. *Nit* mutants were generated from single spore wild strains by selecting chlorate-resistant sectors on minimal medium amended with potassium chlorate, 25 g/l. These mutants were tested against tester strains from the USA and Greece of the previously described VCGs 1, 2, 3 and 4. Forty-six of 71 isolates belonged to VCG2, because they were able to anastomose with the testers of this group, two isolates belonged to VCG4 and one to VCG1, while the 22 remaining strains could not be assigned to any of the identified VCGs. Our data demonstrated that wilt of cotton is caused only by *V. dahliae* in Greece, and VCG2 is the most commonly detected VCG. Some strains were found to be more virulent to cotton than other strains from the same VCG. This is the first report of VCG1 of *Verticillium* in Greece.

Introduction

Cotton (*Gossypium hirsutum* L.) is grown on a large acreage in Greece and is an economically important crop. Moreover, cotton is a widespread industrial crop among the countries of Western Asia and North Africa, surrounding the Mediterranean sea. Limiting factors of cotton cultivation in Greece are mainly soil-borne plant pathogens causing either seed and seedling diseases including *Pythium* spp., *Rhizoctonia solani* Kühn and *Chalara elegans* Nag Raj and Kendr. [*Thielaviopsis basicola* (Berk and Broome) Ferraris], or wilt by *Verticillium dahliae* Kleb. (Elena and Paplomatas, 1998; Tjamos and Kornaros, 1978).

Species of *Verticillium* are mainly characterized on the basis of morphology, while the genetic diversity is much less studied than in other fungi. *V. dahliae* is a widespread plant pathogen. In general, isolates of *V. dahliae* are not host specific but have a wide host range. Races 1 and 2 of *V. dahliae* have been defined in the case of tomato wilt, but race 2 is rare in Greece (Tjamos, 1980). In cotton, strains of *V. dahliae* also have been classified into two groups depending on whether they cause defoliation (Daayf et al., 1995). Puhalla (1985) proposed the use of vegetative compatibility assays to group isolates of the fungi. Nitrate-nonutilizing (*nit*) mutants are the most commonly used in these complementation tests. *nit* mutants can be recovered using a chlorate medium (Puhalla, 1985; Corell et al., 1987). Fungal strains that anastomose and form heterokaryons with one another are considered to be vegetatively compatible and are assigned to the same group. Strains that are incapable of anastomosing with one another and fail to establish heterokaryons are vegetatively incompatible. Recent studies have suggested that vegetative compatibility is also a useful tool to study genetic diversity among *V. dahliae* populations. Four vegetative compatibility groups (VCGs) of *V. dahliae* from various hosts, were found by Joaquim and Rowe (1990). All defoliating strains from cotton belonged to VCG1 and the non-defoliating strains to VCG2. These isolates had previously been classified into 15 groups using microsclerotial color mutants (Puhalla and Hummel, 1983). Joaquim and Rowe

(1991) also studied 187 strains isolated from potato plants and 47 isolates from potato field soil. These strains were assigned to VCGs 1, 2 and 4. In another study, a collection of 42 strains of *V. dahliae* obtained from ornamental woody plants belonged to VCGs 1, 2 and 4 (Chen, 1994). In a previous study in Greece the isolates of *V. dahliae* from different hosts were assigned to VCGs 2, 3 and 4 (Elena and Paplomatas, 1998). In preliminary tests, 23 of 28 isolates from cotton belonged to VCG2 and 5 isolates were self-incompatible (Elena, 1997). According to Daayf et al. (1995), all strains derived from cotton plants belonged to VCG1 and VCG2.

V. dahliae is widely distributed in Greece and causes extensive losses on cotton. Since cotton is a crop with high economic importance, it is useful to study and know the composition of the fungus population. The objectives of this study were to group more *V. dahliae* isolates from Greek cotton cultivations into VCGs, and to compare the results with those previously described in Greece or elsewhere.

Materials and methods

Fungal strains

The experiments included single-spore isolates of *V. dahliae* derived during 1995–1996 from the main cotton cultivation areas of Central Greece. Plants showing symptoms of *Verticillium* wilt were collected from 7 regions and 19 different fields (Table 1) and pieces of vascular tissue were excised and placed aseptically on Potato–Dextrose–Agar (PDA). Plates were incubated at 22 °C until colonies developed. Conidia from colonies were streaked on PDA plates and incubated at 22 °C until small colonies grew on the medium. From each isolate one monoconidial strain was obtained. PDA was also used to maintain cultures. The isolates were identified as *V. dahliae* based on their morphological characteristics (Hawksworth and Talboys, 1970).

Recovery of nit mutants

Following the technique described by Puhalla (1985) and Joaquim and Rowe (1990), small mycelial blocks from Minimal Medium (MM), for each isolate, were transferred to 9 cm diameter Petri dishes with MMC (chlorate medium) and incubated at 22 °C. This medium was based on MM amended with 25 g/l $KClO_3$ and 1.6 g/l L-asparagine. From the margin of chlorate-resistant sectors, small fragments were cut and transferred to separate Petri dishes with MM. The chlorate-resistant mutants with thin and expansive mycelium on MM were characterized as *nit* and were unable to metabolize nitrate. The *nit* mutants were identified as *nit*1, *nit*3 and NitM depending on their growth on nitrate, nitrite and hypoxanthine medium (Corell et al., 1987). In addition *nit* mutant testers from previously described Greek VCGs were used (Table 2) (from V7 and V8 isolates of VCG2, from V1 and V30 isolates of VCG4). The *nit* mutant strains from T9 and V44 isolates (VCG1), from PH (VCG2A) and 115 (VCG2B), from 70-21 and PCW (VCG3), from BB (VCG4A) and S39 (VCG4B) were the generous gift of R. Rowe and were used to compare the groups of our isolates to previously characterized VCGs (Table 2) (Joaquim and Rowe, 1990, 1991; Elena, 1997; Elena and Paplomatas, 1998). Three mutants were usually tested in a triangular manner and 3 triangles were put on each plate (9 cm diameter) containing MM. The distance between the mutants on the plates was 1–1.5 cm. Every pairing was repeated at least twice but usually more times and incubated at 22 °C.

Pathogenicity tests

Inocula from 69 single-spore isolates were prepared on SSN liquid medium (Sinha and Wood, 1968). The medium consisted of (per liter): 15 g sucrose, 1 g KH_2PO_4, 0.5 g $MgSO_4 7H_2O$, 2 g $NaNO_3$, 0.5 g KCl and 1 ml of microelement solution. Isolates of *V. dahliae* were grown in 250 ml erlenmeyer flasks with 100 ml SSN, for seven days at 24 °C in an orbital shaker incubator at 120 rpm. Pathogenicity of the isolates was tested on 20-day-old seedlings of cultivar Acala Sj-2, the most common cotton cultivar in Greece, using a suspension of 10^7 conidia/ml. The roots of control plants were dipped in tap water while those of the treated plants were dipped in a conidial suspension for 30 min. Nine plants per isolate were inoculated and transferred to pots 10 cm in diameter (1 plant/pot) with compost. The plants were kept in a glasshouse at 25 °C and 15 h photoperiod. Light was provided by white fluorescent Philips 400 W lamps.

The number of diseased plants was counted and recorded every 5 days starting at the 10th and continuing until the 42nd day after inoculation. The disease development was rated on a scale of 1–5, where 1 indicated a plant without symptoms and 5 indicated plants

Table 1. Pathogenicity of *Verticillium dahliae* isolates originating from cotton and characterization of their chlorate-resistant mutants

Isolate	Site[1]	Chlorate resistant sectors	Number of mutants				Disease index[3]
			nit mutants[2]	*nit*1 mutants	*nit*3 mutants	NitM mutants	
V53	Karditsa 1	7	4	3	1	—	3.6
V54	Karditsa 1	5	5	2	—	3	2.2
V55	Karditsa 1	7	5	4	—	1	4.8
V56	Karditsa 1	5	5	2	—	3	2.8
V57	Karditsa 1	6	6	2	—	3	4.2
V59	Karditsa 1	7	7	2	—	5	4.2
V60	Karditsa 1	5	3	3	—	–	4.6
V61	Trikala 1	7	3	—	1	2	4.6
V62	Trikala 1	7	1	1	—	—	5
V63	Trikala 1	7	5	5	—	—	4.6
V64	Trikala 1	7	2	—	—	2	4
V65	Trikala 1	30	—	—	—	—	3
V66	Trikala 2	7	3	—	—	1	2.4
V67	Trikala 2	7	6	4	—	2	3.4
V77	Karditsa 1	7	4	—	1	3	3.4
V78	Karditsa 1	7	3	2	—	1	2.8
V80	Trikala 3	7	1	1	—	—	3
V81	Trikala 3	7	4	—	—	1	1.4
V83	Trikala 4	7	7	4	—	3	3
V84	Trikala 4	7	5	1	—	—	1.4
V85	Karditsa 2	7	5	—	—	2	3.2
V86	Karditsa 3	7	4	3	—	1	3.8
V87	Trikala 5	7	7	4	—	1	2.8
V88	Trikala 5	7	4	3	—	1	3.6
V89	Larissa	7	3	1	—	2	5
V90	Larissa	7	1	1	—	—	2.2
V91	Larissa	7	3	2	—	—	4.6
V92	Larissa	7	4	1	—	3	4.6
V93	Larissa	6	4	4	—	—	2.4
V94	Larissa	7	5	5	—	—	1.8
V95	Larissa	35	—	—	—	—	2.2
V96	Larissa	5	5	2	—	3	3.6
V97	Larissa	7	6	5	—	1	3
V98	Trikala 6	7	4	3	—	1	4.2
V99	Trikala 6	7	4	3	—	—	3.2
V100	Trikala 6	6	5	4	—	1	NT[4]
V101	Trikala 6	7	3	1	—	1	3.2
V102	Trikala 6	7	2	1	—	1	2.4
V103	Thiva 1	7	2	1	—	—	4
V104	Thiva 1	7	7	4	—	3	2.4
V105	Thiva 1	7	3	1	—	2	3.2
V106	Thiva 1	6	5	3	—	2	3.2
V107	Thiva 1	7	5	—	—	4	2.8
V108	Orhomenos 1	7	3	1	—	2	NT
V109	Orgomenos 1	7	2	2	—	—	2.8
V110	Thiva 2	7	6	5	1	—	2.8
V111	Thiva 2	6	2	2	—	—	3
V112	Thiva 2	7	4	4	—	—	3.2
V113	Thiva 2	—	—	—	—	—	1.4
V114	Thiva 2	7	7	2	—	2	4.4

Table 1. Continued

Isolate	Site[1]	Chlorate resistant sectors	Number of mutants				Disease index[3]
			nit mutant[2]	*nit*1 mutants	*nit*3 mutants	NitM mutants	
V115	Thiva 3	7	7	2	1	—	1.4
V116	Orhomenos 2	7	3	1	—	2	2.4
V117	Orhomenos 2	4	3	2	—	1	3
V118	Thiva 3	7	6	4	—	1	1.8
V119	Thiva 3	7	3	3	—	—	4.6
V120	Thiva 3	7	4	2	—	—	4.2
V121	Orhomenos 1	7	4	1	1	1	4.8
V141	Lamia	4	2	1	—	1	3.8
V142	Lamia	7	7	2	—	5	3.4
V143	Lamia	7	1	1	—	—	4
V144	Thiva 4	7	7	1	—	6	3.4
V145	Thiva 4	6	5	4	—	1	3.4
V146	Trikala 7	6	4	1	—	3	2.4
V147	Trikala 7	7	4	3	—	1	2.8
V148	Trikala 7	7	3	3	—	—	2.6
V149	Trikala 7	7	5	3	—	2	2.6
V150	Trikala 7	6	4	3	—	1	2.4
V151	Pella	7	3	3	—	—	1
V153	Trikala 7	7	3	3	—	—	4
V154	Trikala 7	6	5	1	—	4	1.6
V182	Trikala 7	6	4	1	—	3	2.2
Control		—	—	—	—	—	1

[1]Region and field number.
[2]Total number of mutants (some mutants have not been phenotyped).
[3]Mean of 9 replicates on a scale of 1–5: 1 = plant healthy, 2 = one cotyledon dead, 3 = cotyledons dead, 4 = cotyledons dead and necrosis more than one leaf, 5 = plant dead.
[4]NT: not tested.

killed by the fungus according to the final remarks. The experiment was repeated once.

Results

Seventy-one single-spore isolates of *V. dahliae* were isolated from diseased cotton plants. In preliminary tests 28 of these isolates grouped in VCG2 (Elena, 1997). From all the tested strains 517 chlorate-resistant mutants were recovered (Table 1).

Among these 517 mutants, only 281 (54%) grew as expansive colonies without aerial mycelium (*nit* growth). Inability to recover *nit* mutants from strain V113 was because of the failure to obtain chlorate-resistant sectors on MMC and on CMA (corn meal agar amended with 1.5–2% $KClO_3$). The latter medium generated more mutants than did MM (Chen, 1994). The chlorate-resistant sectors of strains V65 and V95 failed to yield colonies with *nit* growth on MM. Among the 281 *nit* mutants, 149 were characterized as *nit*1, 95 as NitM, 6 as *nit*3 and 31 were not characterized (Table 1). Some of the non-characterized mutants had wild-type growth on hypoxanthine medium but did not grow on the nitrite medium and were characterized as *nit*1 or *nit*3. Others showed *nit*-type mycelium on nitrite and hypoxanthine medium or had a very slow growth on MM. Among the 281 *nit* mutants and the tester strains, more than 1600 pairings were performed. Multiple mutants from wild strains were employed in complementation tests. This approach was used because the mutants of the same parent strain may not always give the same prototrophic growth when paired with the same tester (Chen, 1994). The first heterokaryon was seen 6 days after pairing and observations were continued up to 30 days. Complementation was evident by development of a dense aerial mycelium or formation of microsclerotia with or without aerial mycelium.

Table 2. Host and geographic origin of the tester mutant strains of *Verticillium dahliae* previously assigned to vegetative compatibility groups (Joaquim and Rowe 1990, 1991; Elena and Paplomatas 1998)

Isolate	Host of origin	Geographical origin	Mutant phenotype	VCG[1]
T9	Cotton	USA	*nit*1 and NitM	1
V44	Cotton	USA	*nit*1 and NitM	1
V7	Tomato	Greece	$NitM_6$	2
V8	Tomato	Greece	$NitM_1$	2
PH	Pistachio	USA	*nit*1 and NitM	2A
115	Cotton	Syria	*nit*1 and NitM	2B
70-21	Pepper	USA	*nit*1 and NitM	3
PCW	Pepper	USA	*nit*1 and NitM	3
V1	Cotton	Greece	$NitM_3$[2]	4
V30	Tomato	Greece	$NitM_3$	4
BB	Potato	USA	*nit*1 and NitM	4A
S39	Potato or potato soil	USA	*nit*1 and NitM	4B

[1]Vegetative compatibility group.
[2]The number of the NitM mutant. The mutants from testers obtained from the USA have not been enumerated.

Mutants from 46 *V. dahliae* isolates were able to anastomose with one or more of the tester *nit* mutants from PH, 115, V7 and V8 isolates of VCG2 as characterized by Joaquim and Rowe (1990) and by Elena and Paplomatas (1998). The mutants from V104 and V151 isolates formed heterokaryons with the VCG4 testers. Only the 5 *nit* mutants of isolate V63 were compatible with both VCG1 testers from T9 and V44 isolates and did not form heterokaryons with any of the other VCG tester strains, or other isolates from these groups (Table 3). All the strains failed to anastomose with the VCG3 testers. Anastomoses also formed between the mutants of Greek isolates belonging to the same group. The pairings with the VCG1 testers were repeated 5 times. The VCGs of 19 strains were not determined because the mutants from these isolates did not form heterokaryons with the American or Greek testers or other Greek isolates which belonged to the characterized groups.

Pathogenicity tests showed that the isolates were pathogenic at various levels to cotton (Table 1). Only isolate V151 was not pathogenic (disease index: 1). The isolates 63 (VCG1) and 90 (not assigned to a VCG) were given disease index 5 because all the plants were dead. Variation in pathogenicity was observed among isolates from diseased plants obtained from the same field.

Discussion

Our study demonstrated that wilt of cotton plants is caused by *V. dahliae* in Greece as indicated in a previous study by Tjamos and Kornaros (1978).

Recovery of *nit* mutants from *Verticillium* isolates has been more difficult than from other species because chlorate does not greatly restrict the growth of many strains and neither sectoring nor the proportion of *nit* mutants were increased by raising the chlorate concentration in the medium (Korolev and Katan, 1997). Some strains produced *nit* mutants readily, others produced a few mutants and three produced none (Table 1). *nit*3 mutants were rarely isolated in this study while *nit*1 and NitM mutants predominated. Some *nit*3 mutants were unable to be distinguished from *nit*1 because they did not grow on nitrite medium. In other studies on *V. dahliae*, *nit*3 mutants were never recovered (Joaquim and Rowe, 1991; Korolev and Katan, 1997; Strausbough et al., 1992; Chen, 1994).

Complementation between *nit* mutants of *V. dahliae* is complex because not all strains within a VCG complement one another. According to Joaquim and Rowe (1990) and our observations, the tester strains from PH and 115 isolates (VCG2) are weakly compatible with each other but several *nit* mutants from other *Verticillium* strains were capable of complementing

Table 3. Heterokaryon formation between *nit* mutants derived from *V. dahliae* isolates from cotton and testers of previously described VCGs from Greece and the USA

Isolates[1]	*nit* mutants from tester strains										Characterized VCG
	VCG1		VCG2				VCG4				
	T9	V44	V7	V8	PH	115	V1	V30	BB	S39	
V53	+/−	+/−	+	+	+/−	+	−	−	−	−	2
V54	−	−	−	−	−	+	−	−	−	−	2
V55	−	−	+/−	−	+/−	+	−	−	−	−	2
V56	−	−	+/−	−	+/−	+	−	−	−	−	2
V57	−	−	+	−	+	+	−	−	−	−	2
V59	−	−	−	−	−	−	−	−	−	−	NC
V60	−	+/−	+/−	+	+/−	+	−	−	−	−	2
V61	−	−	+	+	+/−	+	−	−	−	−	2
V62	−	−	−	−	−	−	−	−	−	−	NC
V63	+	+	−	−	−	−	−	−	−	−	1
V64	−	−	+	−	+/−	+	−	−	−	−	2
V66	−	−	−	+	−	+	−	−	−	−	2
V67	−	+/−	+	+	+	+	−	−	−	−	2
V77	−	−	−	−	−	−	−	−	−	−	NC
V78	−	−	+/−	+	+/−	+	−	−	+/−	−	2
V80	−	−	+	+	+/−	+	−	−	−	−	2
V81	+/−	+/−	+	+	+	+	−	−	−	−	2
V83	−	−	−	+/−	+/−	+	−	−	−	−	2
V84	−	−	−	−	−	−	−	−	−	−	NC
V85	−	−	+/−	+	−	+	−	−	−	−	2
V86	−	−	+/−	+	+/−	+	−	−	−	−	2
V87	−	−	−	−	−	−	−	−	−	−	NC
V88	−	−	−	−	−	−	−	−	−	−	NC
V89	−	−	+	+	+/−	+	−	−	−	−	2
V90	−	−	−	−	−	−	−	−	−	−	NC
V91	−	−	+/−	+	+/−	+	−	−	−	−	2
V92	−	−	+	+	+/−	+	−	−	−	−	2
V93	−	−	+	+	+/−	+	−	−	−	−	2
V94	−	−	+/−	+	+/−	+	−	−	+/−	−	2
V96	−	−	+	−	+	+	−	−	−	−	2
V97	−	−	−	−	−	−	−	−	−	−	NC
V98	−	−	+	+	+/−	+	−	−	−	−	2
V99	+/−	+/−	+	+	+	+	−	−	−	−	2
V100	−	−	−	−	−	−	−	−	−	−	NC
V101	−	−	+/−	−	+	+	−	−	−	−	2
V102	−	−	+/−	+	+/−	+	−	−	−	−	2
V103	−	−	−	+	+/−	+	−	−	−	−	2
V104	−	−	−	−	−	−	+	+	+	+/−	4
V105	−	−	+	+	+/−	+	−	−	−	−	2
V106	−	−	−	−	−	−	−	−	−	−	NC
V107	−	−	−	−	+/−	+	−	−	−	−	2
V108	+/−	+/−	+	+	+/−	+	−	−	−	−	2
V109	−	−	+/−	+	+/−	+	−	−	−	−	2
V110	−	+/−	+	+	+/−	+	−	−	−	−	2
V111	−	−	−	−	−	−	−	−	−	−	NC
V112	−	−	+/−	+/−	+/−	+	−	−	−	−	2
V114	−	−	−	+	−	+	−	−	−	−	2
V115	−	−	+	+	+	+	−	−	−	−	2
V116	−	−	+	+	+/−	+	−	−	−	−	2

Table 3. Continued

Isolates[1]	*nit* mutants from tester strains										Characterized VCG
	VCG1		VCG2				VCG4				
	T9	V44	V7	V8	PH	115	V1	V30	BB	S39	
V117	−	+/−	+	−	+	+	−	−	−	−	2
V118	−	−	+	+	−	+	−	−	−	−	2
V119	−	−	−	−	−	−	−	−	−	−	NC
V120	−	−	+	+	+/−	+	−	−	−	−	2
V121	−	−	−	−	−	−	−	−	−	−	NC
V141	−	−	+/−	+/−	+/−	+	−	−	−	−	2
V142	+/−	+/−	+	+/−	+/−	+	−	−	−	−	2
V143	−	−	−	+	+/−	+	−	−	−	−	2
V144	−	−	−	−	+	+	−	−	−	−	2
V145	−	−	+	+/−	+/−	+	−	−	−	−	2
V146	−	−	−	−	−	−	−	−	−	−	NC
V147	+/−	−	+/−	+/−	+/−	+	−	−	−	−	2
V148	−	−	+	+	+/−	+	−	−	−	−	2
V149	−	−	−	−	−	−	−	−	−	−	NC
V150	−	−	−	−	−	−	−	−	−	−	NC
V151	−	−	−	−	−	−	−	+	+/−	+	4
V153	−	−	−	−	−	−	−	−	−	−	NC
V154	−	−	−	−	+/−	+	−	−	−	−	2
V182	−	−	−	−	−	+/−	−	−	−	−	NC

[1]Culture collection abbreviations are given in Table 2. The mutants from T9 and V44 isolates are the USA testers of VCG1, from PH and 115 of VCG2, from BB and S39 of VCG4 (Joaquim and Rowe, 1990). Mutants from V7 and V8 are Greek testers of VCG2, V1 and V30 of VCG4 (Elena and Paplomatas, 1998).
+: strong reaction; +/−: weak reaction; −: no reaction; NC = Not characterized.
All the strains failed to make anastomosis with the testers of VCG3.

strongly with both (Table 3). Furthermore, some weak complementation reactions were observed in pairings among strains of different VCGs (Table 3). Weak heterokaryons when produced between strains assigned to the same or especially to different VCGs could be attributed to transitory heterokaryosis (Joaquim and Rowe, 1991).

If a mutant of a tested strain complemented strongly with at least one of the testers of one of the previously characterized groups, it was assumed to belong to that VCG, even if the same strain reacted weakly or failed to yield any reaction with the other testers of the same VCG. We avoided the subdivision of VCG2 and VCG4. All the isolates which belonged to VCG2 formed strong reactions with the tester strain from 115 isolate (VCG2B) but their reactions with other testers of VCG2 were variable. The strains that were not strongly compatible with one of the testers of all VCGs may represent new VCGs.

A limited diversity of VCGs was reported in the study involving *V. dahliae* isolates from different hosts and diverse geographical locations in Greece. In this study based on complementarity of *nit* mutants, only three VCGs from Greek isolates from different hosts were identified: VCGs 2, 3 and 4 (Elena and Paplomatas, 1998). The present study demonstrated that 46 isolates (about 94% of the characterized strains and 65% of the total sample) were assigned to VCG2, two were assigned to VCG4 and one to VCG1.

nit mutants from strain V63 were only compatible with testers from T9 and V44 isolates of VCG1. From tester 138 of this group it was impossible to derive *nit* mutants. This is the first report of VCG1 in Greece. The defoliating strains of this fungus belongs to this group. All the strains were incompatible with the VCG3 testers. Strausbaugh et al. (1992) re-assigned the tester strains of VCG3 to VCG4, but in our study (Elena and Paplomatas, 1998) two isolates of *V. dahliae* from cocklebur (*Xanthium* sp.) reacted strongly with both testers of VCG3. There was an uneven distribution of isolates among the three VCGs in strains from ornamental plants (Chen, 1994). Similar results were presented

in other studies if *nit* mutants were used for characterization, while a large number of VCGs were identified when using microsclerotial color mutants induced by ultraviolet light (Joaquim and Rowe, 1990, 1991; Puhalla and Hummel, 1983).

The pathogenicity tests indicated that not all strains of *V. dahliae* had the same potential for virulence to cotton plants. Some strains of the same VCG were more virulent than others. The tested isolates caused no symptoms or moderate symptoms on tomato seedlings while they were highly virulent on water melon plants (Vloutoglou et al., 1997). Variation in pathogenicity of isolates from a given host plant was a common phenomenon (Tjamos, 1981).

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