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Full Length Research Paper

Vegetative compatibility of *Verticillium dahlia* isolated from olive trees (*Olea europea* L.) in Algeria

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25 isolates of *Verticillium dahliae* obtained from olive trees: 18 of them originating from two regions of Algeria (Nord-ouest and Kabylie), 4 isolates from France and 3 from Syria. They were investigated using complementation tests with nitrate-nonutilizing (Nit) mutants to know their vegetative compatibility. Among 250 chlorate-resistant sectors obtained, only 187 were Nit mutants. Three types of Nit mutants were obtained (Nit1, Nit3 and NitM) on the basis of the fungal phenotype. Nit1 mutants were the most frequent (71.6%), followed by NitM (16.6%) and Nit3 (11.8%). Based on their ability to form heterokaryons, all olive pathogenic isolates were grouped into a single vegetative compatibility groups (VCG). This is a good indication of the homogeneity of the Algerian *V. dahliae* population. The results also suggest the absence of a relationship between geographical origin of strains and VCG.

Key words: Nit mutants, VCG, *Verticillium dahliae*, Olive tree.

INTRODUCTION

Verticillium wilt, caused by the soil-borne fungus *Verticillium dahliae* (Kleb) threatens olive-growing in several Mediterranean Basin countries (Zazzerini and Tosi, 1994; Cirulli et al., 1998; Tosi and Zazzerini, 1998; Vigouroux, 1975; Serrhini and Zeroual, 1995; Hiemstra and Harris, 1998). In Algeria, the disease was first noted in the region of Kabylie (Benchabane, 1990). It is now widespread in main olive-growing belt, where it causes serious damage (Bellahcene et al., 2000). The control of *V. dahliae* is difficult because of the absence of specificity of host and the extreme variability of pathogenicity. The establishment of an effective effort to control this disease rests on the good knowledge of the structure of the population and genetic diversity of *V. dahliae.*

The identification of vegetative compatibility groups (VCG) allow for the identification of races, genetic diversity and variability of pathogenicity of the population (Chen, 1994). Two pathogenic fungi isolates are vegetatively compatible if their filaments are capable of fusion. The coupling of two different nuclei within a single cell (heterokaryosis) enables mutual complementation of the nutritional deficiencies brought by each nucleus, and possibly an exchange of genetic material via paraesexuality. Therefore, in an imperfect fungus such as *V. dahliae*, the vegetative compatibility of two isolates of different origins reflects a degree of genetic relatedness. Incompatibility, on the other hand, is a sign of a certain genetic isolation. Hence we determined the number of vegetative compatibility groups and their constitution as part of the *V. dahliae* population structure study. The purpose of this research was to study the population structure of *V. dahliae* which causes wilt to olive trees, based on VCG analysis.

MATERIALS AND METHODS

Fungal strains

The study was concentrated on 25 strains from 3 countries (Algeria, France and Syria) (Table 1). *V. dahliae* were isolated from olive trees on potato-dextrose-agar (PDA) at 20°C. Each isolate was transferred to PDA medium. Species identifications were based on

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Table 1. Geographical origin of the *V. dahliae* isolates obtained from olive trees and year of isolation.

Isolates	Department	Country	Var. *Olea europea*	Year of isolation
V1	Sig	Algeria	Sigoise	1998
V2	Sig	Algeria	Sigoise	1998
V3	Sig	Algeria	Sigoise	1998
V6	Mohammadia	Algeria	Sigoise	2000
V8	Mascara	Algeria	Sigoise	2000
V11	Relizane	Algeria	Sigoise	2000
V13	S. bel. Abbes	Algeria	Sigoise	2000
V15	Mostaganem	Algeria	Sigoise	2000
V18	Sig	Algeria	Sigoise	2000
V19	Tlemcen	Algeria	Sigoise	2000
V20	Tlemecen	Algeria	Sigoise	2000
V23	Sig	Algeria	Cornicabra	2000
V24	Sig	Algeria	Cornicabra	2000
V26	Cap-Djinet	Algeria	Chemlal	2000
V27	Cap-Djinet	Algeria	Chemlal	2000
V28	Cap-Djinet	Algeria	Chemlal	2000
V29	Tizi-Ouzou	Algeria	Chemlal	2000
V30	Tizi-Ouzou	Algeria	Chemlal	2000
VS1	Idleb	Syria	Sorani	2000
VS3	Damas	Syria	Sorani	2000
VS4	Damas	Syria	Sorani	2000
VF1	Béziers	France	Picholine	1999
VF2	Nîmes	France	Picholine	1999
VF8	Salon	France	Picholine	1999
VF9	Salon	France	Picholine	1999

morphological characteristics (Hawksworth and Talboys, 1970). One monoconidial culture was prepared from each isolate and used in this study.

Selection, characterization and pairing of Nit mutants

Nit mutants were used to assess the vegetative compatibility among isolates of *V. dahliae*. All isolates were grown on Puhalla's minimal medium (MM) at 22°C for 7 days. Following the technique described by Puhalla (1985) and Joaquim and Rowe (1990), small mycelial blocks from each isolate were transferred to MMC medium. Three concentrations of MM medium were prepared using 25, 30 and 35 g/l of KClO3 and 1.6 g/l L-asparagine was added to each medium.

Chlorate-resistant sectors emerged from the restricted colonies after incubation at 22°C for 8 to 15 days. The chlorate-resistant sectors producing a characteristically thin and expansive growth with no aerial mycelium on MM medium were considered Nit mutants. There were 3 sorts of mutant (Corell et al., 1987): Nit1 (nitrate reductase structure gene), Nit3 (nitrate reductase regulation gene) and Nit M (nitrate reductase cofactor structure gene).

The Nit mutant phenotypes Nit1, Nit3 or NitM were determined by their growth response on MM medium where sodium nitrate was replaced by sodium nitrite or hypoxanthine. Crossings were made between Nit1 and NitM mutants from the same strain (to check self-compatibility) or between 2 different strains. The Nit1 x NitM crosses were adopted because the success rate is higher than Nit1 x Nit3 or Nit3 x NitM.

Anastomosis and complementation were seen by formation of a dense and abundant aerial mycelium in the contact zone after 8 to 15 days of plating. In this case, the 2 strains crossed are considered to belong to the same compatibility group. If the opposite is true (no apparent complementation), and if the strain is not self-incompatible, checks were made to see whether crossing in the opposite direction (NitM from A crossed with Nit1 from B if the first cross was between Nit1 from A and NitM from B) leads to complementation, before concluding that the two strains tested belong to different compatibility groups.

RESULTS

Selection of Nit mutants

The majority of colonies obtained with 25 and 30 g/l of KClO3 developed a dense mycelium all around the periphery of colony. This result means that no mutants were obtained on MMC media amended with these two concentrations of $KClO_3$. On the MMC with 35 $g.l^{-1}$ all isolates of *V. dahliae* produced numerous chlorate-resistant sectors, but only 1 to 12 Nit mutants were

Table 2. Number of each *Nit* mutant type selected on characterization media.

Isolates	*Nit*1	*Nit*M	*Nit*3	Total
V1	5[a] (62.5)	1[a] (12.5)	2[a] (25)	08
V2	8 (72.7)	2 (18.2)	1 (9.1)	11
V3	1 (100)	0 (0)	0 (0)	01
V6	5 (62.5)	1 (12.5)	2 (25)	08
V8	8 (88.8)	1 (11.2)	0 (0)	09
V11	2 (40)	1 (20)	2 (40)	05
V13	7 (58.3)	3 (25)	2 (16.7)	12
V15	7 (100)	0 (0)	0 (0)	07
V18	4 (80)	1 (20)	0 (0)	05
V19	8 (72.7)	1 (9.1)	2 (18.2)	11
V20	9 (81.8)	2 (18.2)	0 (0)	11
V23	8 (66.7)	3 (25)	1 (8.3)	12
V24	7 (70)	1 (10)	2 (20)	10
V26	8 (88.8)	1 (11.2)	0 (0)	09
V27	3 (75)	1 (25)	0 (0)	04
V28	5 (71.4)	2 (28.6)	0 (0)	07
V29	3 (75)	1 (25)	0 (0)	04
V30	4 (80)	1 (20)	0 (0)	05
VS1	6 (100)	0 (0)	0 (0)	06
VS3	6 (60)	1 (10)	3 (30)	10
VS4	5 (55.6)	2 (22.2)	2 (22.2)	09
VF1	7 (77.8)	2 (22.2)	0 (0)	09
VF2	4 (66.6)	1 (16.7)	1 (16.7)	06
VF8	1 (20)	2 (40)	2 (40)	05
VF9	3 (100)	(0)	(0)	03
Total	134 (71.6)	22 (11.8)	31 (16.6)	187

[a]: Number of *V. dahliae Nit* mutants.
In parentheses: percentage of mutants.

obtained from each isolate. Among the 250 chlorate-resistant only 187 (74%) were characterized as Nit. 134 (71.6%) were characterized as Nit1, 31 (16.6%) as NitM and 22 (11.8) as Nit3. We have noticed that these averages showed a strong disparity from one strain to the other. The investigation of Nit mutants using the isolates (V3, V15, VS1 and VF9) gave only Nit1 mutants. The NitM was obtained by 21 strains from the 25 used (Table 2).

Complementation of *Nit* mutants and VCG determination

All posibilities of complementations between the *Nit*M of the 21 strains and *Nit*1 or *Nit*3 of the all strains were done. The results showed that all the *V. dahliae* strains coming from the three different countries were assembled in one vegetative compatibility group (Table 3).

DISCUSSION

Verticillium species, which have no sexual form as for all fungi named 'imperfect', can only exchange their genetic material by a vegetative manner. The parasexuality includes the formation of a heterocayon followed by a mitotic recombination. The first stage is anastomosis by fusion between mycelial filaments belonging to two isolates. This requires compatibility between these isolates. Mutations affecting one of the genes which control the compatibility of a strain leads to the incompatibility with all other strains (not having undergone the same mutation) and then to its genetic isolation. Two incompatible strains are classified into two groups of different vegetative compatibility (VCG).

Different concentrations of KClO3 were tested but only the 35 g l^{-1} level gave Nit mutants. Daayf (1993) selected *V. dahliae* mutants using 30 g l^{-1} of $KClO_3$. The number of Nit mutants which obtained (187), was less than the

Table 3. Results of crossings on a nitrate minimum medium (MM) between complementary mutants generated from clones of *V. dahliae* isolated from olive trees.

Clones		V1	V2	V3	V6	V8	V11	V13	V15	V18	V19	V20	V23	V24	V26	V27	V28	V29	V30	S1	S3	S4	F1	F2	F8	F9
	V1	+	+	+	+	-	+	+	+	+/-	+/-	+/-	+	+	+	+	+	+	+	+	+	+	-	-	-	+
	V2		+	+	+	+	-	+	+	+	-	+	+	+	+	+/-	+/-	+/-	+	+	+	+	+/-	+	+	+
	V3			•	+	-	-	+	+	+	+/-	+/-	+/-	+/-	+	+	+	+/-	+	-	-	+	+/-	+	+	-
	V6				+	+	+	+	+	+	+	-	+	+/-	+	+	-	+	+	+/-	+	-	-	-	-	+
	V8					+	+	+	+	-	-	+	-	+/-	+	-	+	+/-	+	+	+	-	-	+	+	+
	V11						+	-	+	+	+/-	+	+	-	-	-	+	+	+/-	+	+	+	+	-	+	+
	V13							+	+	+	+	+	-	+	+	+	+	-	-	-	+	+	-	-	+	-
	V15								•	-	+	-	+	+	+/-	+/-	+	+	+	-	-	+	-	+	-	+
Algérie	V18									+	+	+	+	-	+	+	+	+	+	+	+/-	+	-	+	-	-
	V19										+	+	+	-	+/-	+	+/-	-	+	-	-	+	-	-	-	+
	V20											+	+	-	+	+	+	+	-	+/-	+	+	+	-	-	-
	V23												+	+	+	+	-	-	+	+	+	+	+	+/-	+/-	-
	V24													+	+	+	+	+	+	-	-	+/-	-	+/-	-	-
	V26														+	+	+	+/-	+/-	+	+	-	-	+	-	-
	V27															+	+	+	+	+	-	+	+	+/-	-	-
	V28																+	+/-	+/-	+/-	+	-	+	-	-	-
	V29																	+	+	+	+	+/-	+/-	-	+	-
	V30																		+	+/-	+/-	+/-	-	-	+	+
	S1																			•	+	+	+/-	+/-	-	-
Syrie	S3																				+	+	+	+/-	-	+
	S4																					+	+	+	-	-
	F1																						+	+	-	+
France	F2																							+	+	+
	F8																								+	+
	F9																									•

+: Formation of heterokarotic mycelium in the contact zone.
+/-: Weak reaction.
- : No complementation between the mutants tested.
*: Confrontation not caried out.

368 and 457 obtained by Daayf (1993) and Lachquer et al. (2002), respectively. According to Strausbagh et al. (1992), some loci can be mutated more frequently. The frequency of Nit1 and NitM mutants is higher than the Nit 3 mutants. The dominance of Nit1 has also been reported by several authors (Daayf, 1993; Chen, 1994; Korolev and Katan, 1997; Elena and Paplomatas, 1998; Lachquer et al., 2002; Tsror and Levin, 2003).

The comparison of selected Nit mutants produced only one vegetative compatibility group (VCG). This shows that *V. dahliae* population is homogeneous and that the isolates are genetically closely related. The presence of low vegetative compatibility groups (VCG) in *V. dahliae* has been reported by different authors. Joaquim and Rowe (1990) identified 4 VCG out of a population of 22 isolates previously classified in 15 groups by Puhalla and Hummel (1983). Joaquim and Rowe (1991), Strausbaugh et al. (1992), Quingjii and Chiyi (1990) have also showed the presence of a small number of VCG inside a population of *V. dahliae*. Chen (1994), Daayf et al. (1995), Elena and Paplomatas (1998) have also classified 30 to 40 isolates of the pathogenic agent in 3 VCGs.

Even though the *V. dahliae* tested strains come from different geographical sites, they all belong to the same VCG suggesting the absence of relation between VCG and their geographic origin. This is in accordance with the result of Joaquim and Rowe (1990). The absence of VCG-geographical relation was also observed by Daayf et al. (1995) inside a population of 27 *V. dahliae* strains from Africa, Asia, Europe and the United States. Corell et al. (1988) also reported the absence of such relation in a population of V. albo-atrum.

The obtained results show the existence of relationship between the VCG and their host plant. Identical results were reported by Puhalla (1979), who classified 7 cotton isolates among the 10 into the same VCG. Also among 8 isolates from tomato coming from 7 different countries, 7

were classified in the same VCG. Joaquim and Rowe (1991), Strausbagh et al. (1992) found that of 47 V. dahliae isolated from potato, 45 were classified into the same group. This observation showed that vegetative compatibility is related to the existence of favorite hosts for the majority of *V. dahliae* (Daayf et al., 1995). The study of VCG by Korolev et al. (2000) in a collection of 565 *V. dahliae* isolates (from 47 Israeli sites and from 13 different species) classified these isolates in two groups; VCG4B and VCG2. This classification is strictly related to the pathogenicity and phenotypical peculiarities. Working on 44 V. dahliae olive trees isolates (41 from Morocco and 3 from Algeria), Lachquer et al. (2002) classified them into only one VCG (VCG001), finding no correlation between VCG and geographical origin or pathogenicity.

In Isrëal, Bao et al. (1998) classified 34 isolates of *V. dahliae*, taken from 6 different hosts into 2 VCG groups: one group was from isolates taken from cotton, aubergine, olive and chrysanthemum, the other from potato, olive and cotton. Also Elena (2000) regrouped 17 *V. dahliae* isolates obtained from a melon culture from 8 regions in Greece to one VCG.

In conclusion, we are of the opinion that the vegetative compatibility gene which govern the formation of VCG are dependent of the geographical origin of strains. The complementation of the selected Nit mutants gave only one vegetative compatibility group. This indicates that the studied population of *V. dahliae* from olive trees is homogenous and the strains are genetically related. This can be explained by the existence of a clonal population descended from the same “parent” which spread to other countries.

REFERENCES

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

Bellahcene M, Fortas Z, Geiger JP, Matallah A, Henni D (2000). *Verticillium* wilt in olive tin Algeria : Geographical distribution and extent of the disease. Olivae 82: 41 – 43.

Bellahcene M, Matallah A, Fortas Z, Geiger J.P, Nicole M, Assigbétsé K (2001). Molecular and histological characterization of *Verticillium dahliae* (Kleb) on olive tree. In: Proceeding of 8th Int. Verticillium Symp., November 5-8, Cordoba, Spain. p. 40.

Benchabane M (1990). Observation des cas de verticilliose de l’olivier à Cap-Djinet et Sidi-Aîch . Rapport de mission, ITAF, Algérie, p. 5.

Chen W (1994). Vegetative compatibility group of Verticillium dahliae from ornemental woody plants. Phytopathology 84: 214-219.

Cirulli M, Amenduni M, Colella C (1998). La verticilliosi del pesco e reazioni di Prunus spp. verso Verticillium dahliae Kleb. Atti Convegno su Innovazione e sviluppo per la peschicoltura meridionale, 2-3, Luglio, Peastum (Salerno), 32-35.

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

Correll JC, Gordon TR, McCain AH (1988). Vegetative compatibility and pathogenicity of Verticillium albo-atrum. Phytopathology 78: 1017 – 1021.

Daayf F (1993). La verticilliose du cotonnier. Pouvoir pathogène et diversité génétique de *Verticillium dahliae*, réactions de la plante à l’infection. Thèse de doctorat, Univ. Montpellier, France. p. 202.

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.

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

Elena k, Paplomatas EJ (1998). Vegetative compatibility groups within *Verticillium dahliae* isolates from different hosts in Greece. Plant Pathol. 47: 635 – 640.

Hiemstra JA, Harris DC (1998). A compendium of Verticillium wilts in tree species. (eds.) CPRO-DLO/HRI, Ponsen and Looijen, Wageningen/West-Malling, the Netherlands, 80p.

Hawksworth DL, Talboys PW (1970). *Verticillium dahliae*. Descriptions of pathogenic fungi and bacteria. C.M.I. N° 256.

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

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

Korolev N, Katan T (1997). Improved medium for selecting nitrate nonutilising (nit) mutants of *Verticillium dahliae*. Phytopathology 87: 1067 – 1070.

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

Lachquer K, Sedra MH, Tantaoui A (2002). Vegetative compatibility and pathogenicity of *Verticillium dahliae* isolates from olive *(Olea europea* L.) in Morocco. Phytopathol. Mediterr. 41: 19 – 27.

Puhalla, JE (1979). Classification of isolates of *Verticillium dahliae* based on heterokaryon incompatibility. Phytopathology 69: 1186 – 1189.

Puhalla, JE (1985). Classification of strains of Fusarium oxysporum on the basis of vegetative compatibility. Can. J. Bot. 63: 179 – 183.

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

Quingjii L, Chiyi C (1990). Species, physiological form and vegetative compatibility group of cotton Verticillium wilt pathogen in China. In: Proceeding of 5th Int. Verticillium Symp. Leningrad, URSS, p.17.

Serrhini MN, Zeroual A (1995). Verticillium wilt of olive trees in Marocco. Olivea 58: 58 -61.Strausbagh CA, Schroth MN, Weinhold AR, Hancock JG (1992). Assessment of vegetative compatibility of *Verticillium dahliae* tester strains and isolates fromCalifornia potatoes. Phytopathology 82: 61 – 68.

Tosi L, Zazzerini A (1998). An epidemiological study on Verticillium wilt of olive in central Italy. Olivae 71: 50-55.

Tsror L, Levin AG (2003). Vegetative compatibility and pathogenicity of *Verticillium dahliae* Kleb. Isolates from olive in Israël. J. Phytopathology 151 : 451 – 455.

Vigouroux A (1975). *Verticillium dahliae*, agent d’un dépérissement de l’olivier en France. Ann. Phytopathol. 7 : 37 – 44.

Zazzerini A, Tosi L (1994). Ricerche epidemiologiche su *Verticillium dahliae* e Phoma incompta su olivo nell’Italia centrale. Convegno ‘Innovazioni e prospective nella difesa fitosanitaria’, Ferrara, 24 – 25 october, 235 – 257.

Zhengjun X, Achar NP, Benkang G (1998). Vegetative compatibility grouping of Verticillium dahliae from cotton in mainland China. Eur. J. Plant Pathol. 104: 871–876.