Phytopathol. Mediterr. (2005) 44, 266–274

Genetic diversity of *Verticillium dahliae* isolates from olive trees in Algeria

Miloud Bellahcene[1], Komi Assigbetsé[2], Zohra Fortas[3], Jean-Paul Geiger[2], Michel Nicole[2] and Diana Fernandez[2]

[1]Département de Biologie, Faculté des Sciences, Université de Mostaganem, BP 227, Mostaganem, 27 000, Algeria
[2]Institut de Recherche pour le Développement (IRD), UMR 1097 Diversité et Génome des Plantes Cultivées, Equipe Résistance des Plantes, 911 Avenue Agropolis, BP64501, 34394 Montpellier Cedex 5, France
[3]Laboratoire de Biologie des Microorganismes et de Biotechnologie, Université d'Oran, Es-Sénia, 31 000, Oran, Algeria

Summary. *Verticillium* wilt of olive trees (*Olea europaea* L.), a wilt caused by the soil-borne fungus *Verticillium dahliae* (Kleb), is one of the most serious diseases in Algerian olive groves. To assess the pathogenic and genetic diversity of olive-infecting *V. dahliae* populations in Algeria, orchards from the two main olive-producing regions (north-western Algeria and Kabylia) were sampled and 27 *V. dahliae* isolates were recovered. For purposes of comparison, *V. dahliae* strains from France and Syria were added to the analysis. By means of PCR primers that specifically discriminate between defoliating (D) and non-defoliating (ND) *V. dahliae* pathotypes it was shown that all *V. dahliae* isolates belonged to the ND pathotype. The amount of genetic variation between the 43 isolates was assessed by random amplification of polymorphic DNA (RAPD). A total of 16 RAPD haplotypes were found on the basis of the presence or absence of 25 polymorphic DNA fragments. Genotypic diversity between the 27 Algerian isolates was low, with two RAPD haplotypes accounting for 70% of all isolates. Genotypic diversity was however greater between isolates from Kabylia than between isolates from north-western Algeria. Cluster analysis showed that most of the Algerian *V. dahliae* isolates grouped together with the French and Syrian isolates. On the basis of their ability to form heterokaryons with each other, a subset of 25 olive-pathogenic isolates was grouped into a single vegetative compatibility group (VCG). These results suggest that the olive-infecting *V. dahliae* populations in Algeria show limited diversity and that caution should be taken to prevent introduction of the D pathotype.

Key words: *Verticillium* wilt, *Olea europaea*, DNA polymorphism, vegetative compatibility group, non-defoliating pathotype.

Introduction

The fungus *Verticillium dahliae* (Kleb) is a broad host-range pathogen that infects many crop plants, including olive tree (*Olea europaea* L.) (Hiemstra and Harris, 1998). *V. dahliae* causes olive wilt in many Mediterranean countries (Vigouroux, 1975; Serghini and Zeroual, 1995; Hiemstra and Harris, 1998; Tosi and Zazzerini, 1998; Mercado Blanco *et al.*, 2002) and the fungus has recently also been detected in Algeria where it has reached epidemic proportions (Benchabane, 1990). An epidemiological survey found that *Verticillium* wilt of olive occurred in almost all olive-growing regions of Algeria, infecting 20 out of the 22 orchards surveyed (Bellahcene *et al.*, 2000), with characteristic wilt

Corresponding author: D. Fernandez
Fax: + 33 4 67 41 62 83
E-mail: diana.fernandez@mpl.ird.fr

symptoms, whether generalized or local. Old wilted branches were defoliated at the tips, while retaining some withered leaves at the base. Young shoots, as a rule, lost all their leaves at the base but might retain some brownish leaves at the shoot tip. In some cases however, wilted, very brittle and curled leaves also remained on young wilted shoots. Disease incidence varied from about 5% in north-western Algeria, to 30% in severely infected orchards in Kabylia. However, no significant differences in wilt resistance were found between the two main olive varieties grown in these regions, the variety 'Sigoise', used for food consumption, and the variety 'Chemlal', used for olive oil production (Bellahcene *et al*., 2000).

Verticillium dahliae isolates from olive and cotton (*Gossypium hirsutum* L.) can be separated into two groups depending on their virulence towards cotton and olive respectively (Schnathorst and Mathre, 1966; Daayf *et al*., 1995; Jiménez-Díaz *et al*., 1998). *V. dahliae* isolates are also either of the defoliating (D) or the non-defoliating (ND) pathotype, depending on whether or not they defoliate the plant. The D pathotype is lethal to the plant, while olive plants infected with the ND pathotype may recover (Jiménez-Díaz *et al*., 1998; López-Escudero *et al*., 2004). Within the ND pathotype, however, variations in aggressiveness towards cotton or olive are reported (Korolev *et al*., 2001; Lachqer *et al*., 2002). Pathotype characterization of *V. dahliae* isolates is an important requirement for achieving control of olive wilt. However, virulence tests on olive plants are time-consuming and not always reliable. On the basis of the ability of *V. dahliae* isolates to form heterokaryons with each other, cotton isolates of both the D and ND pathotypes can be separated into vegetative compatibility groups (VCGs) (Daayf *et al*., 1995; Korolev *et al*., 2001). In addition, random amplified polymorphic DNA (RAPD) analysis differentiated the two pathotypes of *V. dahliae* isolates by their DNA banding patterns (Pérez-Artés *et al*., 2000). Specific PCR primers were designed that discriminated between D and ND pathotypes and that allowed direct detection of the parasite in infested olive plants (Pérez-Artés *et al*. 2000; Mercado-Blanco *et al*., 2001, 2002). This molecular technique permits the early and certain detection of D and ND pathotypes of *V. dahliae* on olive trees nurseries, irrespective of whether trees show wilt symptoms.

In the present study, we used vegetative compatibility tests, RAPD markers and cotton D- and ND-specific PCR primers to gain insight into the pathogenic and genetic structure of the Algerian *V. dahliae* population. In a survey conducted in 2000 we sampled 10 olive groves in the two main olive-growing regions in Algeria (north-western Algeria and Kabylia) and isolated a total of 27 *V. dahliae* strains from infected olive trees. To compare these Algerian isolates with *V. dahliae* isolates from other Mediterranean countries, we included 12 isolates from France and Syria in the study .

Materials and methods

Sampling of fungal isolates and DNA extraction

The 43 isolates studied (27 Algerian, 12 French and 4 Syrian) are listed in Table 1. Olive groves in Algeria were sampled in 2000, and 2–3 isolates were recovered from each grove. *V. dahliae* strains were isolated from pieces of infected shoots or branches, in slightly browning tissues. A pure culture of each isolate was grown in non-shade culture in the dark for 8 days at 25°C, in 200 ml of GYP medium (2% glucose, 0.5% yeast extract and 0.5% peptone). Genomic DNA was extracted as described in an earlier study (Assigbetsé *et al*., 1994). Purified DNA was quantified using UV absorbance at 260 nm. After quantification, the DNA was diluted in TE buffer to a final concentration of 20 ng μl^{-1} and kept at -20°C until use.

Selection, characterization and pairing of nit mutants

Nitrate nonutilizing (*nit*) mutants were used to assess the vegetative compatibility of the 25 *V. dahliae* isolates listed in Table 1. Mutant generation and characterization as well as pairings were done as described in Daayf *et al*. (1995).

RAPD analysis

RAPD reactions were carried out with 14 primers, (10-mer oligonucleotides) from the OPF primer set (Operon Technology, Alameda, CA, USA) listed in Table 2. The RAPD amplification experiments were conducted as described in Assigbetsé *et al*. (1994). The mixtures were amplified in a PTC-100 Programmable Thermal Controller thermocycler (MJ Research Inc., Watertown, MA, USA). Nega-

Table 1. Geographic origin, host genotype, vegetative compatibility group (VCG) and random amplification of polymorphic DNA (RAPD) haplotype number of the 43 *Verticillium dahliae* isolates tested.

Code	Geographic origin Algeria	City	*Olea europaea* variety	VCG	RAPD haplotype
V6	North West	Mohammadia	Sigoise	1	1
V7	North West	Mohammadia	Sigoise	nd[1]	1
V8	North West	Mascara	Sigoise	1	1
V9	North West	Mascara	Sigoise	nd	1
V10	North West	Mascara	Sigoise	nd	1
V11	North West	Relizane	Sigoise	1	4
V12	North West	Relizane	Sigoise	nd	1
V13	North West	Sidi-bel-abbes	Sigoise	1	1
V14	North West	Sidi-bel-abbes	Sigoise	nd	1
V15	North West	Mostaganem	Sigoise	1	1
V16	North West	Mostaganem	Sigoise	nd	1
V17	North West	Sig	Sigoise	nd	1
V18	North West	Sig	Sigoise	1	1
V19	North West	Tlemcen	Sigoise	1	1
V20	North West	Tlemcen	Sigoise	1	2
V21	North West	Tlemcen	Sigoise	nd	7
V22	North West	Tlemcen	Sigoise	nd	2
V23	North West	Sig	Cornicabra	1	2
V24	North West	Sig	Cornicabra	1	2
V25	North West	Sig	Cornicabra	nd	2
V26	Kabylia	Cap-Djinet	Chemlal	1	2
V27	Kabylia	Cap-Djinet	Chemlal	1	5
V28	Kabylia	Cap-Djinet	Chemlal	1	10
V29	Kabylia	Tizi-ouzou	Chemlal	1	3
V30	Kabylia	Tizi-ouzou	Chemlal	1	6
V31	Kabylia	Sidi-aich	Chemlal	nd	8
V32	Kabylia	Sidi-aich	Chemlal	nd	9
VS1	Syria	Idleb	Sorani	1	11
VS2	Syria	Idleb	Sorani	nd	12
VS3	Syria	Damascus	Sorani	1	12
VS4	Syria	Damascus	Sorani	1	12
VF1	France	Béziers	Picholine	1	14
VF2	France	Nîmes	Picholine	1	13
VF3	France	Nîmes	Picholine	nd	15
VF4	France	Var	Bouteillon	nd	15
VF5	France	Var	Bouteillon	nd	15
VF6	France	Var	Rovera	nd	15
VF7	France	Var	Rovera	nd	15
VF8	France	Salon	Unknown	1	15
VF9	France	Salon	Unknown	1	15
VF10	France	Salon	Unknown	nd	15
VF1D	France	Nîmes	Picholine	nd	16
VF8D	France	Nîmes	Picholine	nd	15

nd, not determined.

tive controls were included to check for contamination. Amplification products were electrophoresed in 1.2% agarose gels using 1×TAE buffer. A 1 kb DNA ladder was used to estimate the size of the amplified DNA bands.

Amplified fragments were scored as either present, 1, or absent, 0. Bands of the same electrophoretic mobility were scored as identical. Genetic distance analysis was based on the Simple matching index (Sokal and Michener, 1958) which measures the proportion of common discrete data (0 or 1) between isolates. A dendrogram was derived from the distance matrix using the UPGMA algorithm (Sneath and Sokal, 1973) contained in the software package TREECON, Version 1.3b (Van de Peer and De Wachter, 1994). The measure of genotypic diversity (Stoddart and Taylor, 1988) was based on the number of isolates with different RAPD haplotypes. The genotypic diversity of the Algerian population was estimated using the formula $G=1/\Sigma(f(x)\times(x/N)^2)$, where f(x) is the number of haplotypes observed x times in the sample, and N the number of isolates in the sample. To compare G in populations with different sample sizes, we divided G from each collection by N to calculate the percentage of maximum possible diversity that was obtained (Chen *et al.*, 1996). The maximum possible value for G, which occurs when each individual in the sample has a different genotype, is equal to the number of individuals in the sample (N).

Molecular characterization of V. dahliae *pathotypes*

The specific PCR oligonucleotide primers D-1/D-2 and ND-1/ND-2, which discriminated between D and ND strains of *V. dahliae* (Pérez-Artés *et al.*, 2000), were used to characterize the pathotype groups of *V. dahliae* from olive trees. PCR amplification reactions were conducted as described in Pérez-Artés *et al.* (2000) using 4 µl of fungal DNA. PCR reactions were performed in a PTC-100 Programmable Thermal Controller thermal cycler (MJ Research Inc., Watertown, MA, USA). Amplification products were separated on 1.2% agarose gels in 1×TAE buffer and visualized under UV light after staining with ethidium bromide.

Results

VCGs *nit* Mutants were obtained for a subset of 25 isolates and phenotypically characterized according to Correll *et al.* (1987). All strains produced *nit*1 mutants, 21 strains generated NitM mutants, but *nit*3 mutants were obtained for only 12 strains. Complementation tests were made between NitM and *nit*1 or *nit*3 of all strains (Table 2). These tests showed that the 25 *V. dahliae* isolates from Algeria, Syria or France were all vegetatively compatible and therefore belonged to the same VCG.

Molecular characterization of V. dahliae *pathotypes*

DNA of the 43 *V. dahliae* isolates was tested in PCR experiments using the D- and ND-primers (Pérez-Artés *et al.*, 2000). Results are shown in Figure 1. A 1.5-kb DNA fragment was amplified with the ND-primers for all isolates tested. No amplification was obtained with the D-primers. The results indicated that all the isolates tested were genetically related to the ND pathotype of *V. dahliae* and might belong to that pathotype.

Table 2. Number and percentage (in brackets) of each *Verticillium dahliae nit*- mutants selected on characterization media.

Isolates	*nit*-1	Nit-M	*nit*-3	Total
V1	5 (62.5)	1 (12.5)	2 (25)	8
V2	8 (72.7)	2 (18.2)	1 (9.1)	11
V3	1 (100)	0 (0)	0 (0)	1
V6	5 (62.5)	1 (12.5)	2 (25)	8
V8	8 (88.8)	1 (11.2)	0 (0)	9
V11	2 (40)	1 (20)	2 (40)	5
V13	7 (58.3)	3 (25)	2 (16.7)	12
V15	7 (100)	0 (0)	0 (0)	7
V18	4 (80)	1 (20)	0 (0)	5
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)	9
V27	3 (75)	1 (25)	0 (0)	4
V28	5 (71.4)	2 (28.6)	0 (0)	7
V29	3 (75)	1 (25)	0 (0)	4
V30	4 (80)	1 (20)	0 (0)	5
VS1	6 (100)	0 (0)	0 (0)	6
VS3	6 (60)	1 (10)	3 (30)	10
VS4	5 (55.6)	2 (22.2)	2 (22.2)	9
VF1	7 (77.8)	2 (22.2)	0 (0)	9
VF2	4 (66.6)	1 (16.7)	1 (16.7)	6
VF8	1 (20)	2 (40)	2 (40)	5
VF9	3 (100)	1 (0)	1 (0)	3
Total	134 (71.6)	22 (11.8)	31 (16.6)	187

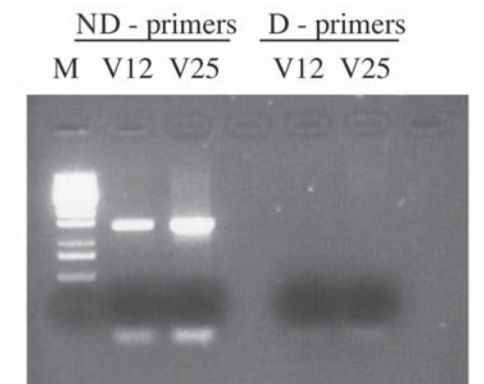

Fig. 1. Ethidium bromide-stained agarose gel showing result of PCR experiments with the specific ND- and D- primer pairs. M, molecular-weight marker 1 kb ladder (Eurogentec, Angers, France). Note the selective amplification of a 1.5-kb fragment with ND-primers, only.

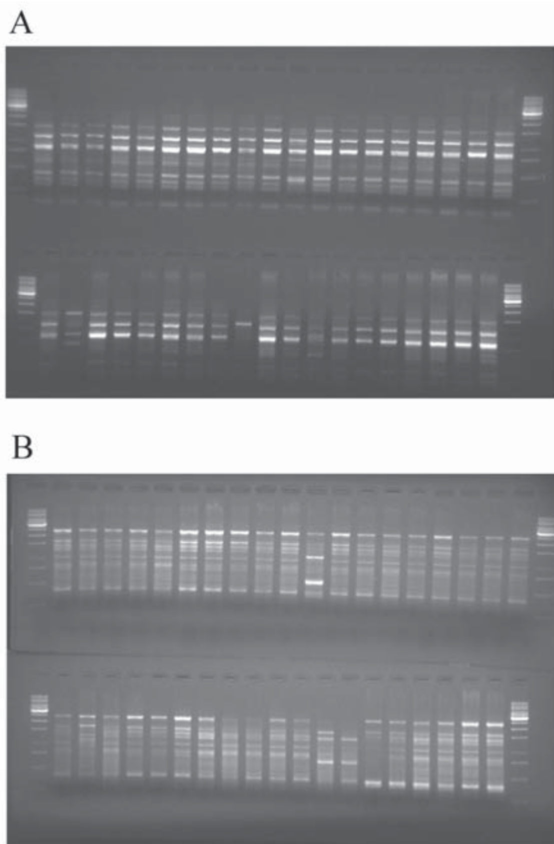

Fig. 2. Gel stained with ethidium bromide showing amplification products generated from the *Verticillium dahliae* isolates with primer OPF-13 (A) and OPF-14 (B)

Polymorphism between* V. dahliae *isolates revealed by RAPDs

Of the 14 primers tested, only primers OPF-04, 06, 07, 11, 13, 14, 19 and 20 amplified DNA bands from the DNA of all 43 isolates. Polymorphic patterns of amplification between isolates were shown with 4 primers: OPF-04, 06, 13 and 14 (Fig. 2), while only monomorphic bands were obtained with OPF-07, 11, 19 and 20. RAPD analysis of DNA from all 43 *V. dahliae* isolates using the 4 primers revealed 25 polymorphic RAPD fragments. A total of 16 RAPD haplotypes were obtained based on the presence or absence of the 25 DNA fragments (Table 3). Among the 27 Algerian isolates, 10 haplotypes were revealed, but two of these (RAPD1 and 2) accounted for 70% of the isolates (Table 3). The estimated genotypic diversity (G) calculated for the whole of Algeria was low, being only 12.6% of the maximum possible value (Table 4). On a regional scale, the value of G was much higher in Kabylia than in north-western Algeria (Table 4). In Kabylia, G attained its maximum possible value, whereas in north-western Algeria it reached only 18.6% of this value. A study of the genetic similarity of the genotypes showed that most of the Algerian *V. dahliae* isolates (20 out of 27 - RAPD1 and 2, displaying 95% similarity) clustered together with the French and Syrian isolates (Fig. 3). The

Table 3. Random amplified polymorphic DNA (RAPD) haplotypes obtained among the 43 *Verticillium dahliae* isolates tested.

Pattern No.	Haplotype	No. of isolates
RAPD1	0110110011001101011110010	13
RAPD2	0110110011001101001110010	6
RAPD3	0110110011001101101110010	1
RAPD4	0110111011011101111101011	1
RAPD5	0110000010001101001010010	1
RAPD6	0110000010001101001110010	1
RAPD7	1111110000001101001110000	1
RAPD8	0100100011000101000100110	1
RAPD9	0110100011001101000000110	1
RAPD10	0000110001000010001010010	1
RAPD11	0110100011001101001100011	1
RAPD12	0110100011001101001110011	3
RAPD13	0110110011001101001110011	1
RAPD14	0110100011001101001110010	1
RAPD15	0110110111001101111110011	3
RAPD16	0110110111101101111110010	1

Table 4. Frequency distribution of RAPD haplotypes in Algerian *V. dahliae* populations.

RAPD haplotype	Frequency distribution[1]		
	Algeria	North-West	Kabylia
1	8	2	7
5	0	1	0
6	1	0	0
13	1	1	0
No. of genotypes	10	4	7
N[2]	27	20	7
G[3]	3.42	3.73	7
(G/N) %[4]	12.6%	18.6%	100

[1] Number of times each genotype is present in the population.
[2] Total number of isolates in sample.
[3] Index of genotyping diversity G (Stoddart and Taylor, 1988).
[4] Percentage of maximum possible value for G.

36 isolates were subdivided into 9 RAPD haplotypes grouped at a genetic distance of 0.14. Other branches of the dendrogram separated 7 Algerian isolates (v11 and v21 from the north-west and v27, v30, v31, v32 and v28 from Kabylia) as having a higher level of genetic diversity. No direct relationship was found between the geographic origin of the Algerian *V. dahliae* isolates and the RAPD clustering. The two most common haplotypes, RAPD1 and RAPD2, occurred in both parts of Algeria (Table 1).

Discussion

Overall, the olive-infecting *V. dahliae* populations in Algeria displayed a moderate level of diversification. All the isolates tested were vegetatively compatible and thus belonged to the same VCG. The use of pathotype-specific PCR primers (Pérez-Artés *et al.*, 2000) suggested that the isolates might belong to the ND pathotype described for cotton. At the molecular level, RAPD analysis revealed the presence of several genotypes, which shared 70% genetic similarity. Some two thirds of the isolates belonged to 2 genetically related predominant RAPD haplotypes dispersed throughout the country, and the overall level of genotypic diversity was low. However, there was a regional diversification of isolates in Kabylia, where the maximum level of genotypic diversity was reached. Ecological conditions and agricultural practices differ between Kabylia and north-western Algeria. Kabylia is a mountainous area with small olive groves, whereas in the north-west olive groves extend over large plains. In addition, different olive varieties are grown in these regions. The *V. dahliae* strains from Kabylia were isolated from the Chemlal variety, used for producing olive oil, whereas the strains from the north western area were isolated from the Sigoise variety, whose olives are grown for direct consumption. Whether these varietal differences account for the differences observed in the genetic diversity of *V. dahliae* isolates from these two regions is unclear.

On a broader geographical scale, a comparison of the Algerian isolates with the 12 French and 4 Syrian isolates in the study indicated that the olive–infecting *V. dahliae* isolates were genetically closely related. All isolates were vegetatively compatible and the RAPD profiles of the French and Syrian isolates shared more than 80% similarity with the Algerian isolates. In addition, a positive amplification was obtained with the ND primers for the non Algerian isolates, suggesting that these isolates also belonged to the ND pathotype. Moreover, a study conducted in Morocco on 33 indigenous olive-infecting *V. dahliae* populations and 3 isolates from Algeria (including V11 and V21 also used in this study) showed that all these isolates could be separated into 4 RAPD groups displaying limited genetic diversity (Lachqer and Sedra, 2002). The same isolates were found to belong to 1 major VCG and 2 minor

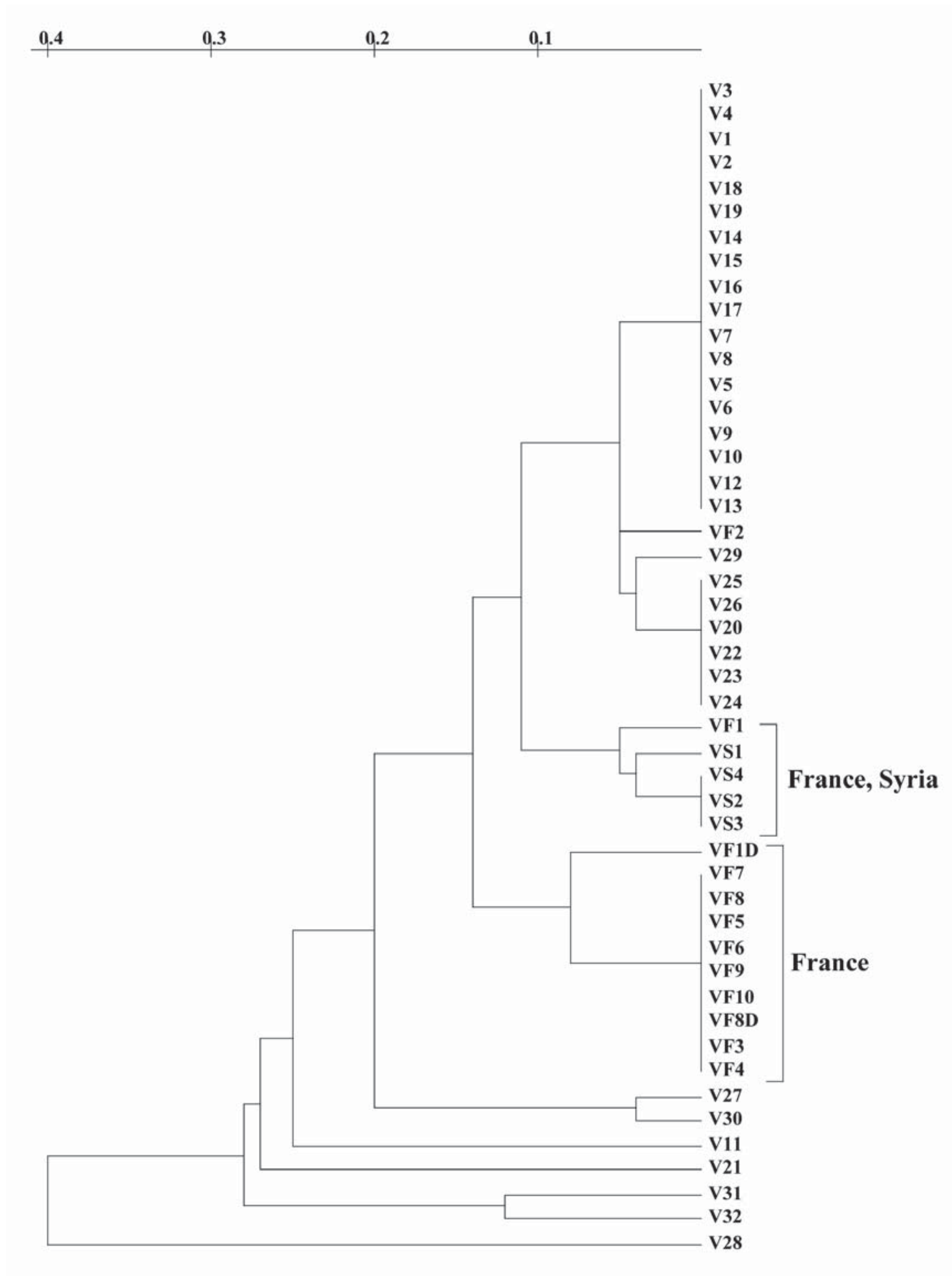

Fig. 3. Dendrogram showing the genetic relationships among the 43 *Verticillium dahliae* isolates. Cluster analysis was performed using the unweighted paired-group method with arithmetic averaging (UPGMA) and genetic distances were obtained based on Simple Matching coefficient of 25 individual DNA bands produced by random amplified polymorphic DNA (RAPD).

VCGs (Lachqer *et al.*, 2002). Interestingly, the *V. dahliae* isolates from Algeria could not be differentiated from the *V. dahliae* Moroccan isolates, except V21 which did not form heterokaryons with any other strains (Lachqer and Sedra, 2002; Lachqer *et al.*, 2002). These observations suggest that olive-infecting *V. dahliae* populations are genetically poorly diversified in the Mediterranean region. A few studies report higher levels of pathogenic and genetic diversity in *V. dahliae* populations isolated from other hosts. In Spain, for instance, the two pathotypes D and ND coexist in cotton, and isolates are separated into 3 VCGs (Korolev *et al.*, 2001). Generally, a low level of VCG diversity has been reported in *V. dahliae* isolates (Joaquim and Rowe, 1990; 1991; Korolev *et al.*, 2001; Hiemstra and Rataj-Guranowska, 2003). Four VCGs, numbered 1 to 4, were initially described (Joaquim and Rowe, 1990); these groups were further subdivided into subgroups (Joaquim and Rowe, 1991; Korolev *et al.*, 2000). So far, VCG1 is reported to contain the cotton D isolates (Daayf *et al.*, 1995; Korolev *et al.*, 2001) and is predominant among *V. dahliae* isolated from ornamental woody hosts in the USA (Chen, 1994). Cotton ND isolates display more variation and have been classified as belonging to VCG2 and VCG4 (Daayf *et al.*, 1995; Korolev *et al.*, 2001). It would be useful to test olive-infecting *V. dahliae* isolates with testers of these VCGs in order to further our understanding of the genetic relationships between *V. dahliae* isolates infecting olive, and *V. dahliae* isolates from other hosts.

As regards pathogenicity, significant variations in aggressiveness on olive trees were found between *V. dahliae* isolates displaying the mildly aggressive ND pathotype in Morocco (Lachqer and Sedra, 2002; Lachqer *et al.*, 2002). The highly aggressive D pathotype of *V. dahliae* has recently been detected in Spain (Mercado-Blanco *et al.*, 2002). Absence of the D pathotype among the Algerian strains tested must be verified by screening a larger number of isolates. If its absence should be confirmed, strict prophylactic measures should be taken to prevent introducing the D pathotype into Algeria. Experiments are currently under way to achieve a better understanding of the diversity of *V. dahliae* from olive trees, with emphasis on a larger collection of strains from Algeria and other North African countries.

Acknowledgements

This study was supported by the French-Algerian inter-university cooperation program (92 MDU 204).

Literature cited

Assigbetsé K., D. Fernandez, M.P. Dubois and J.P. Geiger, 1994. Differentiation of *Fusarium oxysporum* f. sp. *vasinfectum* races on cotton by random amplified polymorphic DNA (RAPD) analysis. *Phytopathology* 84, 622–626.

Bellahcene M., Z. Fortas, J.P. Geiger, A. Matallah and D. Henni, 2000. *Verticillium* wilt in olive in Algeria: geographical distribution and extent of the disease. *Olivae* 82, 41–43.

Benchabane M., 1990. Observation des cas de verticilliose à Cap-Djinet et Sidi-Aich, rapport de mission, ITAF, Alger, Algeria, 5 p.

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

Chen R.S. and B. McDonald, 1996. Sexual reproduction plays a major role in the genetic structure of populations of the fungus *Mycosphaerella graminicola*. *Genetics* 142, 1119–1127.

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

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

Jiménez-Díaz R.M., E.C. Tjamos and M. Cirulli, 1998. Verticillium wilt of major tree hosts: olive. In: *A Compendium of Verticillium Wilts in Tree Species* (J.A. Hiemstra, D.C. Harris, ed.), Ponsen and Looijen, Wageningen, The Netherlands, 13–16.

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

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

Hiemstra J.A. and D.C. Harris (ed.), 1998. *A Compendium of Verticillium Wilts in Tree Species*. Ponsen and Looijen, Wageningen, the Netherlands, 250 pp.

Hiemstra J.A. and M. Rataj-Guranowska, 2003. Vegetative compatibility groups in *Verticillium dahliae* isolates from the Netherlands as compared to VCG diversity in Europe and in the USA. *European Journal of Plant Pathology* 109, 827–839.

Korolev N., E. Pérez-Artés, J. Bejarano-Alcázar, D. Rodríguez-Jurado, J. Katan, T. Katan and R.M. Jiménez-Díaz, 2001. Comparative study of genetic diversity and path-

ogenicity among populations of *Verticillium dahliae* from cotton in Spain and Israel. *European Journal of Plant Pathology* 107, 443–456

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

Lachquer K. and M.H. Sedra, 2002. Characterisation of *Verticillium dahliae* Kleb isolates from *Olea europaea* using RAPD markers. *Phytopathologia Mediterranea* 41, 170–178.

Lachquer K., M.H. Sedra and A. Tantaoui, 2002. Vegetative compatibility and pathogenicity of *Verticillium dahliae* isolates from olive (*Olea europaea*) in Morocco. *Phytopathologia Mediterranea* 41, 170–178.

López-Escudero F.J., C. del Río, J.M. Caballero and M.A. Blanco-López, 2004. Evaluation of olive cultivars for resistance to *Verticillium dahliae*. *European Journal of Plant Pathology* 110, 79–85.

Mercado-Blanco J., D. Rodriguez-Jurado, E. Pérez-Artéz and R.M. Jiménez-Díaz, 2001. Detection of the nondefoliating pathotype of *Verticillium dahliae* in infected olive plants by nested PCR. *Plant Pathology* 50, 609–619.

Mercado-Blanco J., D.Rodriguez-Jurado, E. Pérez-Artéz and R.M. Jiménez-Díaz, 2002. Detection of the defoliating pathotype of *Verticillium dahliae* in infected olive plants by nested PCR. *European Journal of Plant Pathology* 108, 1–13.

Pérez-Artéz E., M.D. Garcia-Pedrajas, J. Bejarano-Alcazar and R.M. Jiménez-Díaz, 2000. Differentiation of cotton-defoliating and non defoliating pathotypes of *Verticillium dahliae* by RAPD and specific analysis. *European Journal of Plant Pathology* 106, 507–517.

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

Serghini M.N. and A. Zeroual, 1995. La verticilliose de l'olivier au Maroc. *Olivae* 58, 58–61.

Sneath P.A. and R.R. Sokal (ed.), 1973. *Numerical Taxonomy: the Principles and Practice of Numerical Classification*. Freeman WH and Company, San Francisco, CA, USA, 573 pp.

Sokal R.R and C.D. Michener, 1958. A statistical method for evaluating systematic relationships. *University of Kansas Science Bulletin* 38, 1409–1438.

Stoddart J.A. and J.F. Taylor, 1988. Genotypic diversity: estimation and prediction in samples. *Genetics* 118, 705–711.

Tosi L. and A. Zazzerini, 1998. Etude de la verticilliose de l'olivier en Italie centrale. *Olivae* 71, 50–55.

Van de Peer Y. and R. De Wachter, 1994. TREECON for Windows: a software package for the construction and drawing of evolutionary trees for the Microsoft Windows environment. *Computational Applications in Biosciences* 10, 569–570.

Vigouroux A., 1975. *Verticillium dahliae*, agent d'un dépérissement de l'olivier en France. *Annales de Phytopathologie* 7, 27–44.

Accepted for publication: October 25, 2005