Phytopathol. Mediterr. (2002) 41, 170–178

Characterisation of *Verticillium dahliae* Kleb. isolates from *Olea europea* using RAPD markers

KHADIJA LACHQUER[1] and MY. HASSAN SEDRA[2]

[1]Laboratory of Plant Physiology, Department of Biology, Faculty of Sciences, Semlalia, P.O. Box 2390, Marrakesh, Morocco
[2]Laboratory of Genetic Phytopathology and microbial control, INRA, P.O. Box 533 Marrakesh, Morocco

Summary. The random amplification of polymorphic DNA fragments (RAPD) was used as a tool both for estimating the genetic variability of *Verticillium dahliae* Kleb. and for characterising this fungus. Forty *V. dahliae* isolates were examined, 33 from olive in the main olive-growing areas, 2 from tomato, one from aubergine, one from the soil of an infected olive grove in Morocco and 3 from olive in Algeria. A total of 95 polymorphic RAPD bands were obtained with ten primers selected among the 50 tested. The results revealed a clear polymorphism between the isolates, which were assigned to 4 RAPD groups. No correlation between RAPD markers, the geographic origin or the host plants of the isolates was observed.

Key words: *Verticillium* wilt, DNA polymorphism, olive, genetic diversity.

Introduction

Verticillium dahliae Kleb. causes *Verticillium* wilt in olive (*Olea europea* L.). The disease was first described by Ruggieri (1946) in Italy and has since been reported in many countries of the Mediterranean basin (Saydam and Copcu, 1972; Vigouroux, 1975; Blanco-Lopez *et al*., 1984; Ahmad, 1988; Matallah *el al*., 1996; Tosi and Zazzerini, 1998). In Morocco, where the olive is of great economic importance, *Verticillium* wilt is becoming an increasingly serious problem (Serghini and Zeroual, 1995; Lachqer and Sedra, 1996).

An effective strategy for the control of this disease consists in the use of resistant varieties and clones. Breeding for resistance requires knowledge about the genetic diversity within natural populations of the pathogen and about the relationships between its isolates. Part of the national programme to search for resistance in olive against *Verticillium* wilt is concerned with the characterisation of *V. dahliae,* which exhibits morphological and pathological variations (Lahlou and Boisson, 1984; Serghini and Zeroual, 1995; Lachqer and Sedra, 1996) and has a broad host range (Schnathorst, 1981). The characterisation of *V. dahliae* pathogenicity and/or morphology is difficult, and so is the identification of its sub-species, races, special forms or pathotypes. Physiological races of *V. dahliae* have only been defined in tomato (*Lycopersicon esculentum* Mill.) on the basis of their interaction with the *Ve* resistance gene (Alexander, 1962). Pathotypes have been first defined in cot-

Corresponding author: K. Lachqer
Fax: +212 044 436769
E-mail: Lachqer@ucam.ac.ma

ton, with a defoliating pathotype which causes severe defoliation on several cultivars of cotton and a nondefoliating pathotype which causes little or no defoliation (Schnathorst and Mathre, 1966). Several other characters, such as the optimal temperature of mycelial growth, the rate of conidia germination and the production of microsclerotia and conidia were added to the pathogenicity tests to distinguish these pathotypes (Wyllie and DeVay, 1970). About twenty years later, pathotypes were also defined in olive (Rodriguez-Jurado *et al*., 1993). Defoliating pathotypes can be lethal to the plants while non-defoliating ones are not (Rodriguez-Jurado *et al*., 1993). In addition, it was pointed out that in the case of olive, *Verticillium* wilt symptoms caused by artificial inoculations take two years to become evident (Vigouroux, 1975).

The analysis of DNA polymorphism using techniques of molecular biology can provide a better definition of the genetic characteristics of *Verticillium* populations. Restriction fragment length polymorphism (RFLP) and polymerase chain reaction (PCR) techniques have been used successfully to identify and distinguish *V. dahliae* from other *Verticillium* species (Carder and Barbara, 1991; Nazar *et al*., 1991; Carder *et al*., 1993; Li *et al*., 1994). Likewise, *Verticillium tricorpus* was genetically separated from *V. dahliae* and *V. albo-atrum* by PCR of the intergenic region 18–28 sequence ribosomal DNA (Moukhamedov *et al*., 1994). By using sub-repeat sequences in the RNA intergenic regions and sequences of the internal transcribed spacers, Morton *et al*. (1995a, b) were able to resolve the confusion between haploid and diploid strings of *V. dahliae*. The PCR technique has also been used for direct detection of *Verticillium* wilt pathogens in herbaceous host plants (Mokhamedov *et al*., 1994), in cotton plants (You Yong *et al*., 1998b) and in olive plants (Mercado-Blanco *et al*., 2002).

Random amplified polymorphic DNA (RAPD) has been shown to be useful for analysing genomic variations (Welsh and Clelland, 1990; Williams *et al*., 1990). The main advantage of this technique is that it does not require cloned DNA fragments as hybridisation probes, or sequence information for the synthesis of primers. RAPD has been used for the identification and characterisation of species, races and special forms of phytopathogenic fungi (Goodwin and Annis, 1991; Xianming *et al*., 1993; Assigbetse *et al*., 1994; Fernandez and Tantaoui, 1994). In the case of *V. dahliae*, the RAPD technique has made it possible to answer many questions concerning host specificity and the geographic origin and pathogenicity of isolates (Mokhamedov *et al*., 1994; Messner *et al*., 1996; Bellahcene *et al*., 2002).

The objective of our investigation was to study molecular variation of a collection of *V. dahliae* isolates mainly collected from olive using the RAPD technique and to find out more about genetic diversity within this population, which we investigated in an earlier work (Lachqer *et al*., 2002).

Materials and methods

Isolate origin

Forty isolates of *V. dahliae* were used, 33 from twigs of olive in the main olive-growing regions of Morocco, 1 from soil of an infected olive grove, 2 from tomato and 1 from aubergine, also from Morocco, and 3 from an Algerian olive grove. Field data about the isolates are given in Table 1. Prior to the experiments, isolates were single-spored, and one clone per isolate was retained for the test.

DNA extraction

For each isolate, mycelium was cultured in Erlenmeyer flasks each containing 100 ml glucose-yeast peptone (GYP) medium (2% glucose, 0.5% yeast extract, 0.5% peptone) on a rotary shaker (150 rpm). Mycelium was collected by filtration, and total DNA extraction was performed using the modified Lee *et al*. (1988) miniprep procedure. For each isolate, 0.1 g of mycelium was ground to a fine powder with a mortar and pestle in liquid nitrogen. The powder was transferred to Eppendorf tubes and mixed with 0.5 ml of lysis buffer (50 mM Tris-HCl pH 8; 50 mM EDTA; 3% SDS and 1% 2-mercapto-ethanol). The mixture was kept at 65°C for 30 min. DNA was extracted□three times with phenol:chloroform:isoamyl alcohol (25:24:1, v:v:v). After centrifugation for 10 min at 12,000 *g*, the supernatant was mixed with 0.1 volume of sodium acetate (3 M, pH 8) and 0.54 volume of isopropanol. The resulting pellet was rinsed with cold 70% ethanol and dissolved in 0.5 ml Tris-EDTA buffer (Tris-HCL 10 mM pH 7.5; EDTA 0.1 mM). The RNA was digested with ribo-

nuclease (final concentration 20 μg ml^{-1}) for one hour at 37°C. DNA was precipitated, rinsed with 70% ethanol, dried and dissolved in 100 ml TE buffer. In order to eliminate the polysaccharides from the DNA preps, 1/3 volume of ammonium acetate 7.5 M was added. The mixture was kept for 20 min in ice and centrifuged at 12,000 *g* for 15 min. The supernatant was then mixed with an equal volume of isopropanol. The resulting pellet was rinsed with cold absolute ethanol and dissolved in 50 μl TE buffer.

DNA was quantified using UV absorbance at

Table 1. Isolate designation, geographic origin, host and year of isolation of *Verticillium dahliae* isolates used in the study.

Isolate	Geographic origin	Host	Year of isolation
V57	Tamellalet (El Kelâa des Sraghna)	Olive	1994
V72	"	"	1995
V84	"	"	1995
V105	"	"	1996
V156	"	"	"
94	Ataouia (El Kelâa des Sraghna)	"	"
44	"	"	"
74	"	"	"
65	"	"	"
81	"	"	"
VB2	"	"	1996
147	"	"	"
M4	Mhamdia (Marrakesh)	"	"
S33	Souihla (Marrakesh)	"	1995
S41	"	"	"
V111	"	"	1997
V62	"	"	"
V92	"	"	"
R11	"	"	"
R21	"	"	"
R41	"	"	"
R32	"	"	"
Br22	Aghmat (Marrakesh)	"	1997
Br57	"	"	"
Ar4	Chouitre (Marrakesh)	"	"
A2	Marrakesh	"	"
TZ1	Tamzegleft (Marrakesh)	"	1995
C3	Marrakesh city	"	1996
MG3	Mghilia (Beni Mellal)	"	1995
B32	Oulad Ayag (Beni Mellal)	"	1997
B14	"	"	"
AT16	Aïn Taoujdat (Meknes)	"	1995
AT32	"	"	"
V11	Algeria	"	1996
V21	"	"	"
V34	"	"	"
VS	Tamellalet	Soil	1996
P32	Laboratory of Plant Pathology - University Mohamed V, Rabat	Tomato	Unknown
D2 (R44)	Laboratory of Botany University Iben Tofail, Kenitra	"	"
225	"	Aubergine	"

260 nm. After quantification, the DNA was diluted in TE buffer to a final concentration of 5 ng μl^{-1} and was kept at –20°C until use.

RAPD assay

In order to detect variations, three strains were randomly chosen for primer screening. Ten random primers were selected from those tested. All were random 10 mers (University of British Columbia, Vancouver, Canada).

Reaction of amplification was performed using the procedure of Willams *et al*. (1990) with modifications. A 25 µl reaction consisted of 50 µM each of ATP, CTP, GTP and TTP; 1 unit of Taq DNA polymerase; 0.2 of µM primer; 25 ng of genomic DNA; 50 mM of Taq polymerase buffer (Appligene-Oncor, France), with sterile distilled water added to reach a final volume of 25 µl. Negative controls were done to check for contamination. Conditions of amplification in the thermocycler (ThermojeT, Eurogentec, Seraing, Belgium) were one cycle of 4 min at 94°C for initial denaturation, followed by 40 cycles of 1 min at 94°C, 2 min at 34°C and 1 min at 72°C. After amplification, 10 µl of the amplification products was separated by electrophoresis on 1% agarose gel in 1× TBE buffer (10× Tris-borate 0.89 M pH 8.3, EDTA 0.025 M). The 1 Kb DNA ladder was used to estimate the size of the amplified DNA bands. The gel was stained with ethidium bromide (2 µg ml^{-1}), scanned and results were saved with the computer program Appligene.

Data from agarose gel electrophoresis were compared with a two-discrete-character matrix (0 for lack and 1 for presence of the RAPD-marker). This matrix was used for cluster analysis and global arrangement to calculate and plot the dendrogram using the Jukes-Cantor option (Jukes and Cantor, 1969) in the DNADIST program and application of the FITCH program (Fitch and Margoliash, 1967), in the Phylip-package by Felsenstein (1989).

Results

Selection of primers

Among the 50 tested primers, no amplification was obtained with UBC 35, 278, 247, 223, 276, 259, 2 and 3. Primers UBC 80, 23, 173, 213, 88 did not amplify the S33 DNA isolate; UBC 251, 4, 81 did not amplify the B14 DNA isolate, and UBC 235 produced no amplification of V156 DNA. Only primers amplifying all three isolates and giving at least three intense polymorphic bands were kept. This left 10 UBC primers (Table 2). The number and intensity of the bands varied according to the primer. Any bands present or those lacking in at least one of the isolates were considered polymorphic markers. The 10 selected primers gave a total of 95 polymorphic bands.

RAPD analysis

An example of the patterns obtained by primer UBC 83 is shown in Fig. 1. This showed significant DNA polymorphism among the isolates of *V. dahliae*. Combination of the amplification results of the ten primers made it possible to identify and classify the genotypes existing within the population studied. The hierarchical classification of the genetic profiles with the Phylip package program (DNADIST distance and FITCH arrangement method) enabled a dendrogram to be drawn comprising 40 isolates, subdivided into 4 RAPD groups (Fig. 2). Among these, RAPD3 included the majority of isolates, 23 out of 40 (Fig. 2). Clusters of isolates did not correlate with geographic origin. For example, in RAPD1 and RAPD2, some isolates from Morocco (Br57 and V111 of RAPD 1, 94 and C3 of RAPD2) and some from Algeria (V11 and V34 of RAPD1, V21 of RAPD2) clustered together. RAPD3 included isolates from Beni Mellal (MG3, B32) and the Haouz (V5, R11, R21, 147, Br22, Ar4, V62, V92, 65, VB, 74, 81, A2, S33, 44, V105, M4 and VS). RAPD4 included isolates from Meknes (AT32 and AT16), Figuig (F), Beni Mellal (B14) and the Haouz (V156, Tz1, S41, R41 and V72). On the other hand, no host specificity clusters were observed either.

Table 2. Selected UBC primers and their nucleotide sequences.

Primer	Sequence of nucleotides	No. of polymorphic bands
UBC-77	GAGCACCAGG	10
UBC-145	TGTCGGTTGC	7
UBC-2	CCTGG GCTTG	6
UBC-222	AAGCCTCCCC	11
UBC-95	GGGG GGTTGG	6
UBC-83	GGGCTCGTGG	16
UBC-111	AGTAGACGGG	16
UBC-250	CGACAGTCCC	6
UBC-215	TCACACGTGC	3
UBC-361	GCGAGGTGCT	14

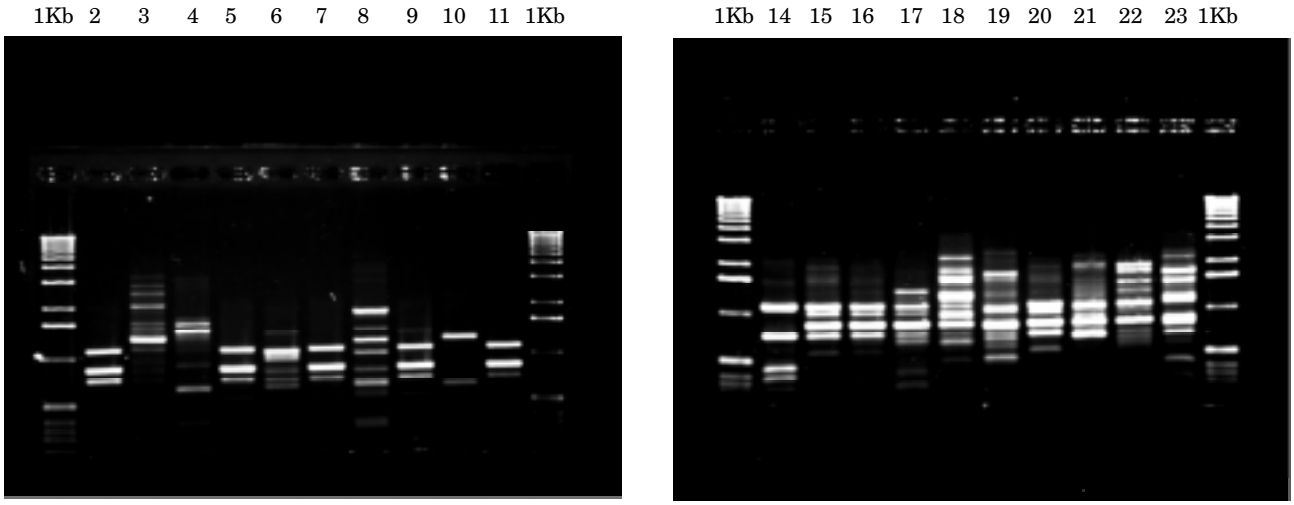

Fig. 1. Example of Random amplified polymorphic patterns of genomic DNA amplified with primer UBC 83 from 20 *Verticillium dahliae* isolates. Lane 2, R21; lane 3, D2; lane 4, 225; lane 5, 147; lane 6, VS; lane 7, 81; lane 8, V72; lane 9, 44; lane 10, B32; lane 11, 94; lane 14, V62; lane 15, AT16; lane 16, Ar4; lane 17, AT32; lane 18, S33; lane 19, F; lane 20, Br22; lane 21, B14; lane 22, Tz1; lane 23, V111.

In RAPD3 and RAPD4, isolates from olive clustered with isolates from aubergine (225) and tomato (D2 and P32).

Discussion

The results showed genetic variation within the population of *V. dahliae* from olive in Morocco. DNA polymorphism was detected among isolates but the diversity of the RAPD groups was limited. Only four RAPD groups were identified. About 57% of the isolates belonged to one group, RAPD3.

When these results were compared with our previous findings on vegetative compatibility groupings (VCGS), it was found that most isolates also belonged to a single VCG, VCG 001, but isolates from RAPD groups did not coincide with those from VCGs. Similar results were also found with *Fusarium oxysporum* (Kohn, 1992; Fernadez and Tantaoui, 1994). Why vegetatively compatible isolates should be spread over different RAPD groups is more difficult to explain. Gordon and Okamoto (1992) concluded that possible mutations at the VC locus of vegetatively incompatible isolates can result in this discordance between RAPD and VC groups.

Genetic variation in *V. dahliae* has been reported previously (Okoli *et al*., 1993; Subbarao *et al*., 1995; Pramateftaki *et al*., 2000). A limited diversity of RAPD groups was also reported in a study involving *V. dahliae* isolates from different host genera (Messner *et al*., 1996), while isolates from cotton plants in Australia were divided into 15 RAPD groups (You-Yung *et al*., 1998a).

Because asexual multiplication is the only known means of reproduction for *V. dahliae*, spontaneous mutations could be a source of genetic variation. Parasexual recombination, demonstrated for *Verticillium* (Hastie, 1964; McGeary and Hastie, 1982), could also lead to variation. In this connection, *V. dahliae* isolates from olive could have a common origin that may have undergone a divergent genetic evolution due to host genotype selection. Vanderplank (1982) reported that the parasite underwent mutations to enhance its virulence by making changes in its metabolism□that would not trigger the host defence reaction. This author also postulated that for each host gene that mutates in the host so as to confer resistance to a parasite, there is a gene in the parasite that mutates so as to overcome this resistance. And indeed, Moroccan olive plantations are prevalently planted with a heterogeneous variety, Moroccan Picholine. Its heterogeneity is shown by its variability in both phenotypes and productivity (Boulouha *et al*., 1992). Another possible explanation for the genetic

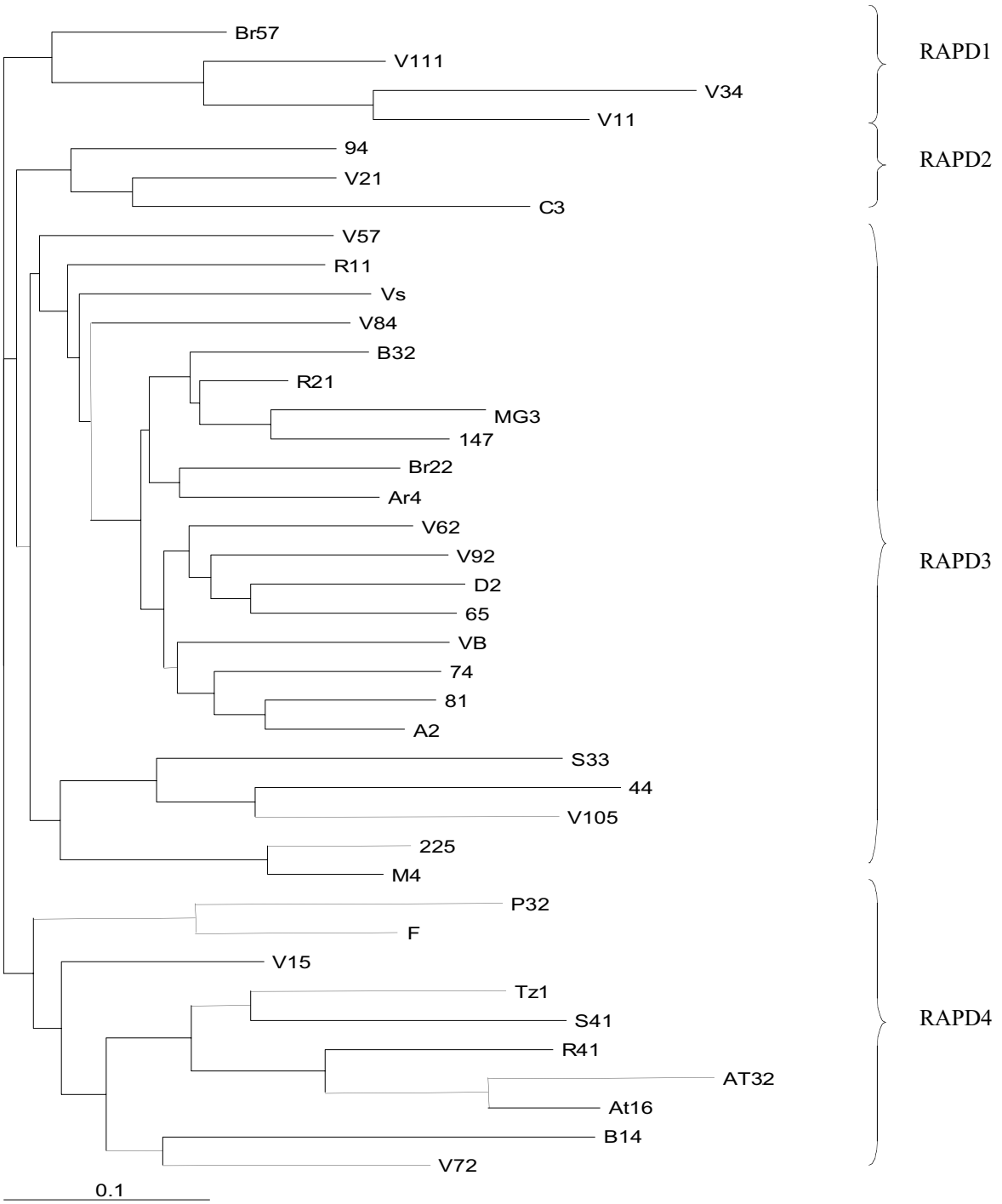

Fig. 2. Dendrogram of the 40 isolates of *Verticillium dahliae* listed in Table 1. The dendrogram was drawn using the DNADIST and FITCH programs of the Phylip package.

diversity of *V. dahliae* is undergrowth vegetation in olive groves. Such vegetation usually includes other hosts of *V. dahliae*. This favours the introduction of parasite genotypes that are adapted to olive. The passage of a parasite to an unusual host may increase the pathogenic capacity of that parasite (Rufty *et al.,* 1981). Successive confrontations of *V. dahliae* with unusual or even resistant hosts affects some parasite capacities, especially protein and pectocellulolytic enzyme synthesis, and may give the parasite increasing virulence against a new host (Elaissami, 1998).

Though the number of Solanaceae isolates was low, there was no relation between any RAPD group and the *V. dahliae* host plant. The grouping of isolates in RAPD groups 3 and 4 indicated that the isolates from olive were genetically close to those from tomato and aubergine. This supports the idea that the origin of olive verticillium wilt was probably the same as that of the undergrowth vegetation, or of previous olive cultures. This is consistent with You Yung *et al*. (1998a) who stated that the correlation between the RAPD groups of *V. dahliae* and its host cotton cultivars was very weak. Messner *et al*. (1996) reported that of 34 isolates from 9 genera, only *Brassica napus* isolates were separately classified from the others, which were all gathered in one single RAPD group. Carder and Barbara (1991) and Okoli *et al*. (1993) also found no correlation between the original host of *V. dahliae* and any particular RFLP group.

There was also no relation between the geographic origin of *V. dahliae* isolates and specific RAPD groups. Isolates from the northern, southern and central olive belts in Morocco were all found together in the same RAPD group (RAPD4). Farmers in fact confirmed that those plants in the southern olive belt which most probably introduced *V. dahliae* there came from a northern olive grove. Moreover, the Algerian and Moroccan isolates belonged to the same RAPD groups (RAPD1 and RAPD2). This suggests that in the Moroccan and Algerian olive belts clonal subpopulations are widespread, probably as a result of exchanges of plant material. The importance of commercial exchanges in bringing about the spread of phytopathogenic agents was demonstrated by Hanson (1987). The absence of a relation between the geographic origin of *V. dahliae* isolates and molecular markers has been observed by various authors: Okoli *et al*. (1993), Messner *et al*. (1996), You-Yung *et al*. (1998b), Bellahcen *et al*. (2002).

In conclusion, *V. dahliae* of olive in Morocco is characterised by genetic diversity with the predominance of one clone, which is probably the one best adapted to olive. RAPD groups did not possess any biological or ecological meaning. If more genetic, physiological, and biochemical traits were known, or if additional genetic markers were examined, *V. dahliae* isolates from olive might be placed in more meaningful biological groups.

Acknowledgements

The authors express their sincere thanks to Dr. Wolfgang Schweigkofler (Italy) for statistical treatment of the results and valuable remarks; to Dr. Abdelaziz Tantaoui for his help, to Professor Allal Douira and Houria Lahlou for providing aubergine and tomato isolates, and to Professors Abdelatif Boussaid and Abdellah Yassir for critical reading of the manuscript.

Literature cited

Ahmad M., 1988. Evaluation quantitative de la verticilliose de l'olivier dans le sud de Syrie. *Arab Plant Protection* 6,□27–32.

Alexander L.J., 1962. Susceptibility of certain *Verticillium* resistant tomato varieties to an Ohio isolate of the pathogen. *Phytopathology* 52, 98–100.

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

Bellahcen M., Z. Fortas, A. Matallah, J.P. Geiger, M. Nicole and K. Assigbetse, 2002. Divérsité génétique de quelques populations de *Verticillium dahliae* issues de trois régions du bassin méditerranéen. In: *Proceedings of VIII e Journées Scientifiques du Réseau "Biotechnologies des Plantes et Sécurité Alimentaire"-AUF. Biotechnologie Végétales: Valorisations pour une Agriculture Durable,* 7–9 October, Marrakesh, Morocco, 27.

Blanco-Lopez M.A., R.M. Jiménez-Díaz and J.M. Caballero, 1984. Symptomatology, incidence and distribution of *Verticillium* wilt of olive trees in Andalucia. *Phytopathologia Mediterranea* 23, 1–8.

Boulouha B., R. Lousser and A. Saadi, 1992. Etude de la variabilité phénotypique de la variété Picholine marocaine dans la région du Haouz. *Olivae* 43, 30–33.

Carder J.H. and D.J. Barbara, 1991. Molecular variation and restriction fragment length polymorphisms (RFLPs) within and between six species of *Verticillium*. *Mycological Research* 95(8), 935–942.

Carder J.H., R. Serers, T.M. Butt, D.J. Barbara, N. von Mend and J. Coosemans, 1993. Taxonomy of the nematophagous fungi *Verticillium chlamydosporium* and *Verticillium suchlasporium* based on secreted enzyme activities and RFLP analysis. *Journal of Invertebrate Pathology* 62, 178–184.

Elaissami A., F. Brhada and H. Lahlou, 1998. Effet de l'adaptation progressive à la luzerne d'un isolat de *Verticillium dahliae* d'origine tomate sur la synthèse in vitro de ses enzymes pectocellulotiques. *Cryptogamie Mycologie* 19(3), 227–234.

Felsenstein J., 1989. PHYLIP-Phylogeny inference pakage (Version 3.2). *Cladistics* 5, 164–166.

Fernandez D. and A. Tantaoui, 1994. Random Amplified Polymorphic DNA (RAPD) analysis: a tool for rapid characterization of *Fusarium oxysporum* f. sp. *albedinis* isolates. *Phytopathologia Mediterranea* 33, 223–229.

Fitch W.M. and E. Margoliash, 1967. Construction of phylogenetic trees. *Science* 155, 279–284.

Goodwin P.H. and S.L. Annis, 1991. Rapid identification of genetic variation and pathotype of *Leptosphaeria maculans* by Random Amplification Polymorphic DNA Assay. *Applied and Environmental Microbiology* 57(9), 2482–2486.

Gordon T.R. and D. Okamoto, 1992. Variation in mitochondrial DNA among vegetatively compatible isolates of *Fusarium oxysporum*. *Experimental Mycology* 16, 245–250.

Hastie A.C., 1964. The parasexual cycle in *Verticillium alboatrum*. *Genetical Research* 5, 305–315.

Hanson E.M., 1987. Speciation in plant pathogenic fungi. The influence of agricultural practice. *Canadian Journal of Plant Pathology* 9, 403–410.

Jukes T.H. and C.R. Cantor, 1969. Evolution of protein molecules. In: *Mammalian Protein Metabolism*. (H.N. Munro, ed.), Academic Press, NewYork, NY, USA, 21–131.

Kohn L.M., 1992. Developing new characters for fungal systematics: An experimental approach for determining the rank of resolution *Mycologia* 84, 139–153.

Lachqer K. and M.H. Sedra, 1996 Importance de la verticilliose de l'olivier dans la région du haouz au Maroc, répartition et caractérisation des isolats de *Verticillium dahliae* Kleb. In: *Proceedings of the IV^e Congrès de Phytopathologie*, 19–22 November, Nice, France, C12.

Lachqer K., M.H. Sedra, A. Tantaoui, 2002. Vegetative compatibility and pathogenicity of *Verticillium dahliae* Kleb. isolates from olive (*Olea europea*) in Morocco. *Phytopathologia Mediterranea* 41, 19–27.

Lahlou H. and C. Boisson, 1984. Variabilité intraclonale du pouvoir pathogène du *Verticillium albo-atrum* R. et B. forme à microsclérotes, vis-à-vis de la tomate. In: *Proceedings of the 26^e Colloque SFP. Variation et Variabilité des Agents Phytopathogènes,* 10–11 mai, Avignon, France, 70–78.

Lee S., M. Milgroom and J. Taylor, 1988. A rapid high yield miniprep method for isolation of total genomic DNA from fungi. *Fungal Genetics Newsletter*, 35, 352–358.

Li K.N., D.I. Rouse and T.L. German, 1994. PCR Primers that allow intergeneric differentiation of Ascomycetes and their application to *Verticillium* spp. *Applied and Environmental Microbiology* 60, 4324–4331.

Maatalah A., Z. Fortas, E. Ehnned, H. Sedra and J.P. Geiger, 1996. La verticilliose de l'olivier dans l'ouest Algérien. Histopathologie des interactions hôte-parasite. In: *Proceedings of the IV^e Congrès de Phytopathologie*, Nice, France, E13.

McGeary F.M. and A.C. Hastie, 1982. Hybridization of *Verticillium alboatrum* strains from tomato and lucerne. *Physiology and Plant pathology* 21, 437–444.

Mercado-Blanco J., D. Rodriguez-Jurado, E. Perez-Artes, R.M. Jimenez.Dìaz, 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. Perez-Artes, R.M. Jiménez-Díaz, 2002. Detection of the defoliating pathotypes of *Verticillium dahliae* in olive. *European Journal of Plant Pathology* 108(1), 1–13.

Messner R., W. Schweigkofler, M. Ibl and H. Prillinger, 1996. Molecular characterization of the plant pathogen *Verticillium dahliae* Kleb. using RAPD-PCR and sequencing of the 18Sr RNA-Gene. *Journal of Phytopathology* 144, 347–354.

Morton A., J.H. Carder and D.J. Barbara, 1995a. Sequences of the internal transcribed spacers of the ribosomal RNA gene and relationships between isolates of *Verticillium alboatrum* and *V. dahliae*. *Plant Pathology* 44, 183–190.

Morton A., A.M. Tabrett, J.H. Carder and D.J. Barbara, 1995b. Sub-repeat sequences in the ribosomal RNA intergenic regions of *Verticillium alboatrum* and *V. dahliae*. *Mycological Research* 99, 257–266.

Moukhamedov R., X. Hu, R.N. Nazar, and J. Robb,1994. Use of polymerase chain reaction-amplified ribosomal intergenic sequences for the diagnosis of *Verticillium tricorpus*. *Phytopathology* 3, 256–259.

Nazar R.N., X. Hu, J. Schmidt, D. Culham and J. Robb, 1991. Potential use of PCR- amplified ribosomal intergenic sequences in the detection and differentiation of Verticillium wilt pathogens. *Physiological and Molecular Plant Pathology* 39, 1–12.

Okoli C.A.N., J.H. Carder and D.J. Barbara, 1993. Molecular variation and sub-specific grouping within *Verticillium dahliae*. *Mycological Research* 97, 233–239.

Pramateftaki P.V., P.P. Antoniou and M.A. Typas, 2000. The complete DNA sequence of the nuclear ribosomal RNA gene complex of *Verticillium dahliae:* intraspecific heterogeneity within the intergenic spacer region. *Fungal Genetic Biology* 29(2), 135–143.

Rodriguez-Jurado D., M.A. Blanco-Lopez, H.F. Rapoport, R.M. Jiménez-Díaz, 1993. present status of Verticillium wilt of olive in Andalucia. *Bullettin OEPP/EPPO Bullettin* 23, 513–516.

Rufty R.C., T.T. Herbert and C.F. Murphy, 1981. Evaluation of resistance to *Septoria nodorum* in wheat. *Plant Disease* 65, 406–409.

Ruggieri G., 1946. In: Blanco-Lopez M.A., R.M. Jiménez-Díaz and J.M. Caballero,1984. Symptomatology, inci-

dence and distribution of *Verticillium* wilt of olive trees in Andalucia. *Phytopathologia Mediterranea* 23, 1–8.

Saydam C. and M. Copcu, 1972. *Verticillium* wilt of olive in Turkey. *Journal of Turkish Phytopathology* 1, 45–49.

Schnathorst W.C., 1981. Life cycle and epidemiology of *Verticillium*. In: *Fungal Wilt Diseases of Plants*. (M.E. Mace, A.A. Bell, C. Beckman, ed.), Academic Press, New York, NY, USA, 81–111.

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

Subbarao K.V., A. Chassot, T.R. Gordon, J.C. Hubbard, P. Bonello, R. Mullin, D. Okamoto, R.M. Davis and S.T. Koike, 1995. Genetic relationships and cross pathogenicities of *Verticillium dahliae* isolates from cauliflower and other crops. *Phytopathology* 85(10), 1105–1112 .

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

Vanderplank J.E., 1982. Host-pathogen interactions. In: *Plant Disease*. Academic Press, New York, NY, USA,

Vigouroux A., 1975. *Verticillium dahliae*, agent d'un dépérissement de l'olivier en France. *Annales de Phytopathologie* 7, 37–44.

Welsh J. and M. McClelland, 1990. Fingerprinting genomes using PCR with arbitrary Primers. *Nucleic Acids Research* 18(24), 7213–7218.

Williams J., A. Kubilik, K. Livak, J. Rafalski and S.Tingey, 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Research* 18(22), 6531–6535.

Wyllie T.D. and J.E. DeVay, 1970. Growth characteristics of several isolates of *Verticillium albo-atrum* and *Verticillium nigrescens* from cotton. *Phytopathology* 60, 907–910.

Xianming C., F.L. Roland and L. Hei, 1993. Relationship between virulence variation and DNA polymorphism in *Puccinia striiformis*. *Phytopathology* 12, 1489–1495.

You-Yong Zhu, S.M. Dilbag and R.L. Bruce, 1998a. DNA polymorphism, geographical origin and host cultivar relationships of Australian isolates of *Verticillium dahliae* from cotton plants. In: *Proceedings of the Kunming Satellite Conference, 18 ICG*. 16–18 August, Kunming, China (abstract).

You-Yong Zhu, Yun Yue Wang and R.L. Bruce, 1998b. PCR detection of pathogen of *V. dahliae* from diseased plants. In: *Proceedings of the Kunming Satellite Conference, 18 ICG*. 16–18 August, Kunming, China (abstract).

Accepted for publication: July 30, 2002