Characterization of a Single Clonal Lineage of *Fusarium oxysporum* f. sp. *albedinis* Causing Bayoud Disease of Date Palm in Morocco

Abdelaziz Tantaoui, Mohamed Ouinten, Jean-Paul Geiger, and Diana Fernandez

First author: Laboratoire de Phytopathologie, Institut National de la Recherche Agronomique (INRA), B.P. 533 Marrakech, Morocco; second, third, and fourth authors: Laboratoire de Phytopathologie, Institut Français de Recherche Scientifique pour le Développement en Coopération (ORSTOM), B.P. 5045, 34032 Montpellier Cedex 1, France.

A. Tantaoui was supported by the Institut Français de Recherche Scientifique pour le Développement en Coopération (ORSTOM).

We thank our colleagues who provided us with molecular probes and H. C. Kistler (University of Florida), T. Langin (Institut de génétique et microbiologie, University of Paris XI), and M. Raymond (Institut des sciences de l'évolution, University of Montpellier II, France) for reviewing the manuscript.

Accepted for publication 23 April 1996.

ABSTRACT

Tantaoui, A., Ouinten, M., Geiger, J.-P., and Fernandez, D. 1996. Characterization of a single clonal lineage of *Fusarium oxysporum* f. sp. *albedinis* causing Bayoud disease of date palm in Morocco. Phytopathology 86: 787-792.

Bayoud, the Fusarium wilt of date palm, was first detected in southern Morocco (Draa Valley), after which it spread to most of the Moroccan palm groves. To assess whether the epidemic results from the spread of a single virulent clone, 42 isolates of *Fusarium oxysporum* f. sp. *albedinis* were collected from several cultivars of wilted palms at different locations in Morocco; two isolates were included from Algeria, where the disease also occurs. The isolates were tested for vegetative compatibility group (VCG), restriction fragment length polymorphism (RFLP), and random amplified polymorphic DNA (RAPD). No polymorphism was observed either in RFLP studies on mitochondrial DNA or in RAPD analysis, and all strains belonged to a single VCG (0170). Sequences homologous to the DNA transposable element Fot1 were found in the genome of the *F. oxysporum* f. sp. *albedinis* strains. Repetitive DNA patterns were produced when *Eco*RI-digested DNA of the isolates was probed with Fot1; 23 distinct hybridization patterns were established among the 44 isolates. Of these patterns, 4 accounted for more than 50% of the isolates, 1 was found twice, and 18 were represented by a single isolate each. Common hybridization patterns were found in the Moroccan palm groves surveyed; the two Algerian isolates had a pattern that also was found in the Draa Valley. Cluster analysis grouped most of the *F. oxysporum* f. sp. *albedinis* strains at a genetic distance of 0.11. Such close genetic relationships between the isolates provides evidence that Moroccan *F. oxysporum* f. sp. *albedinis* populations may belong to a single clonal lineage that originated in Moroccan palm groves and eventually reached the Algerian oases.

Additional keywords: genetic diversity, *Phoenix dactylifera*, population structure.

Bayoud, the fungal vascular wilt of date palm (*Phoenix dactylifera* L.) caused by *Fusarium oxysporum* Schlechtend.:Fr. f. sp. *albedinis* (Killian & Maire) Gordon, is widely distributed in all date palm-growing areas of Morocco and in the western and central parts of Algeria. The disease first appeared before 1870 in the Draa Valley, a large palm grove that extends for 300 km in southern Morocco. Epidemiological data (33) show new occurrences of the disease both in the western and eastern parts of Morocco a few years later; the oases of western Algeria were reached as early as 1898. It has been estimated that more than 10 million trees (representing two-thirds of the Moroccan palm grove) have been killed, threatening both the economic resources of the local human populations and the ecological maintenance of the oasis (reviewed in 25). Currently, disease control methods in Algeria and Morocco primarily involve preventitive measures, including strict phytosanitary rules at borders of date palm-growing countries that remain free of Bayoud.

Attempts to characterize and identify *F. oxysporum* f. sp. *albedinis* isolates showed that the pathogen vary in colony morphology and pathogenicity (9,10). No races within *F. oxysporum* f. sp. *albedinis* have been identified to date, mainly due to problems in performing pathogenicity tests because no well-defined differential series of host genotypes is available. Differentiation between *F. oxysporum* f. sp. *albedinis* and some formae speciales of *F. oxysporum* was made by conducting vegetative compatibility analyses (8). In a preliminary study, we showed that *F. oxysporum* f. sp. *albedinis* could be differentiated from nonpathogenic *F. oxysporum* isolated from date palm roots or soil by vegetative compatibility, mitochondrial DNA (mtDNA) restriction fragment length polymorphism (RFLP), or random amplified polymorphic DNA (RAPD) tests (16,31,32). These analyses also suggested that little genetic variability might exist in the forma specialis *albedinis*.

The objective of the current study was to test the hypothesis of the unique origin of *F. oxysporum* f. sp. *albedinis* isolates as a result of the invasion of the Moroccan oasis by a single virulent clone. Fourty-two *F. oxysporum* f. sp. *albedinis* isolates were collected from several date palm cultivars in Moroccan palm groves. Two isolates collected in Algeria also were included for comparison. We used vegetative compatibility group (VCG) genetic markers as well as molecular markers (RAPD and RFLP) to investigate the genetic relatedness of the *F. oxysporum* f. sp. *albedinis* isolates. Both the nuclear and the mitochondrial genome were examined, and repetitive hybridization patterns were established with the DNA transposable element Fot1 cloned from *F. oxysporum* f. sp. *melonis* (7). A preliminary report on this research has been published (15).

Corresponding author: D. Fernandez; E-mail address: fernande@orstom.rio.net

Publication no. P-1996-0528-01R

MATERIALS AND METHODS

Isolates. The geographic location, year of isolation, and host origin of the 44 isolates of *F. oxysporum* f. sp. *albedinis* examined are listed in Table 1. Nonpathogenic *F. oxysporum* strain A15 isolated from roots of a wilted date palm (16,31,32) was added to serve as an outgroup in Fot1 hybridization-pattern analysis (Table 2). These isolates are part of the Institut National de la Recherche Agronomique (INRA) fungal collection in Marrakech and have all been checked for pathogenicity on date palms. Fourteen locations were sampled in the Moroccan palm groves. Isolates collected during 1991 in Zagora (Draa Valley) were sampled at the INRA experimental station where date palms resulting from genetic crosses are tested for resistance to Bayoud. Hyphal mass isolates were obtained from the rachis of diseased palms by standard techniques (25). All cultures were single spored and were maintained as mycelia on potato dextrose agar slants for short-term storage or as chlamydospores in sand for long-term storage.

TABLE 1. *Fusarium oxysporum* f. sp. *albedinis* isolates tested and their corresponding Fot1 fingerprint as determined in this study

Isolate	Date palm host[a]	Geographic origin	Year of isolation	Fot1 hybridization pattern[b]
69	Boufg	Achoria	1983	P8
F'6	Khalt	Achoria	1992	P10
15.25	Khalt	Arromiate	1992	P11
15.19	Khalt	Arromiate	1992	P2
16.6	Jihel	Asrir	1992	P1
E4	Khalt	Eljorf	1992	P21
F'7	Bouslikhane	Eljorf	1992	P2
13.9	Bourar	Id-Ouchah	1992	P3
V.8	Khalt	Irchag	1992	P22
V.21	Male	Irchag	1992	P23
A3	Majhoul	Meski/Ziz	1992	P20
III.23	Khalt	Oaozagor	1992	P2
131	Bourar	Od MKadem	1983	P4
VII.3	Khalt	Tagounite	1992	P2
IV.6	Jihel	Tansikht	1992	P2
145	Sair-Layalate	Tata	1986	P13
BFGtj	Boufg	Tinjdad	1992	P2
133	Khalt	Tinzouline	1986	P18
132	Bouskri	Tinzouline	1986	P6
82	Aguellid	Zagora	1979	P9
9	Azegzao	Zagora	1979	P5
OTK	Otokdim	Zagora	1979	P10
50	Boufg	Zagora	1983	P15
11	Azegzao	Zagora	1984	P5
79	Ahardane	Zagora	1984	P7
BR	Barhi	Zagora	1986	P5
3Z	Boufg-omossa	Zagora	1992	P5
4Z	Boufg-omossa	Zagora	1992	P15
5Z	Boufg-omossa	Zagora	1992	P15
6Z	Iklane	Zagora	1992	P14
C11	P4.26/52	INRA station (Zagora)	1991	P15
C12	P4.34/77	INRA station (Zagora)	1991	P8
C1	P4.33/54	INRA station (Zagora)	1991	P10
C2	P4.1/89	INRA station (Zagora)	1991	P12
C3	P3.8/1	INRA station (Zagora)	1991	P5
C4	P3.7/17	INRA station (Zagora)	1991	P16
C5	P4.22/20	INRA station (Zagora)	1991	P5
C6	P3.2/27	INRA station (Zagora)	1991	P10
C7	P4.26/54	INRA station (Zagora)	1991	P19
C8	P3.22/4	INRA station (Zagora)	1991	P17
C9	P4.22/7	INRA station (Zagora)	1991	P15
C10	P3.4/17	INRA station (Zagora)	1991	P2
ALL	Allig	Algeria	1985	P5
DN	Deglat Nour	Algeria	1986	P5

[a] Names represent date palm cultivar designations, except for Khalt (the common name for spontaneously growing date palm trees, for which cultivar cannot be assessed); Male (a male tree for which cultivar cannot be assessed); and numbers (progeny from genetic crosses between date palm cultivars).

[b] DNA patterns are listed in Table 2.

VCG analysis. VCGs were determined according to Puhalla (29) and Correll et al. (5) with spontaneous nitrate nonutilizing (*nit*) mutants.

Genomic DNA extraction. Fungal mycelium was cultivated in 500-ml flasks containing 200 ml of GYP medium (glucose 2%, yeast extract 0.5%, and peptone 0.5%) for 5 days at 25°C. The mycelium was harvested by filtration, washed with sterile distilled water, and lyophilized for 48 h. Total DNA extraction was performed by a miniprep procedure (24).

Restriction endonuclease digestion, electrophoresis, and blotting. For each isolate, approximately 3 to 5 µg of total genomic DNA was digested with 20 to 30 units of the restriction enzymes (Boehringer Mannheim, Meylan, France) with the addition of 5 mM spermidine per reaction for 6 to 16 h at 37°C. Restriction fragments were separated by electrophoresis in 0.8 or 1.2% agarose gels in TAE buffer (40 mM Tris-acetate, 1 mM EDTA, pH 8.0) at 1 V cm^{-1} overnight. Gels stained with ethidium bromide (0.5 µg ml^{-1}) were photographed on a UV transilluminator. DNA fragments were blotted onto NylonN$^+$ membranes (Amersham, Les Ullis, France) by alkaline vacuum transfer (TE 80 TransVac, Hoefer Scientific Instruments, San Francisco).

Labeling of probes and hybridization conditions. Two mtDNA probes obtained from *F. oxysporum* f. sp. *conglutinans* (20) were used for mtDNA RFLP analysis: the whole purified 52-kb molecule and pUF1-14 (6.1-kb *Eco*RI-fragment in pUC119). Probe Fot1 was a *F. oxysporum* f. sp. *melonis* 4.6-kb *Eco*RI-fragment containing the 1.9-kb Fot1 sequence (7). DNA probes were labeled with ^{32}P-dCTP by random-priming according to the manufacturer's specifications (Megaprime kit, Amersham) and hybridized to membrane-bound DNA fragments in standard hybridization buffer (Amersham) for at least 16 h at 65°C. Blots were washed twice with 2× SSC (1× SSC is 0.15 M sodium chloride plus 0.015 sodium citrate, pH 7.0) and 0.1% sodium dodecyl sulfate (SDS) for 20 min at room temperature, once with 1× SSC and 0.1% SDS for 20 min at 65°C, and twice with 0.5× SSC for 20 min at 65°C. Hybridized membranes were exposed to X-ray film (Amersham) at –80°C with intensifying screens.

mtDNA RFLP analysis. Previously (32), we found that the combination *Hin*fI/pUF1-14 allowed detection of mtDNA length variations between *F. oxysporum* f. sp. *albedinis* and other *F. oxysporum* isolates, and the combination *Bgl*II/whole purified mtDNA gave the most readable mtDNA hybridization patterns in *F. oxysporum* f. sp. *albedinis*. In addition, the combination *Bgl*II/whole purified mtDNA revealed the highest number of restriction site polymorphisms in *F. oxysporum* f. sp. *vasinfectum* (14). Based on these results, we chose the enzyme/probe combinations *Bgl*II/whole purified mtDNA and *Hin*fI/pUF1-14 to test the *F. oxysporum* f. sp. *albedinis* isolates in this study.

Fot1 hybridization-pattern analysis. DNA from the 44 *F. oxysporum* f. sp. *albedinis* isolates and from nonpathogenic *F. oxysporum* strain A15 was digested with *Eco*RI and hybridized to the Fot1 probe. Fot1-hybridizing restriction fragments were scored either as present (1) or absent (0); unique band patterns were identified with specific pattern numbers. Bands of the same electrophoretic mobility were scored as identical. Analyses were based on Jaccard's distance (18), which measures the proportion of common discrete data (C) between the isolates: $d = 1 - C/(2N - C)$, in which N is the total number of different bands in the two compared hybridization patterns. A dendrogram was derived from the distance matrix by the the unweighted paired-group method, arithmetic mean (UPGMA) algorithm (30) contained in the computer program package Phylip 3.4 (developed by J. Felsenstein, Department of Genetics, University of Washington, Seattle).

RAPD assay. The primers used were random 10-base oligomers (kit F, Operon Technologies, Alameda, CA). We selected three RAPD primers, OPF04 (5′GGTGATCAGG3′), OPF12 (5′ACGGTACCAG3′), and OPF13 (5′GGCTGCAGAA3′) from a set of 20 previously analyzed (16) because these primers differentiated

F. oxysporum f. sp. *albedinis* from *F. oxysporum*, and they produced bright, reproducible bands. Amplification reactions were performed as described previously (2). After the reaction, either 20 µl of the amplification products was separated by electrophoresis on 1.4% agarose gels stained with ethidium bromide and then photographed under UV light, or 10 µl of the amplification products was separated by electrophoresis on 7% polyacrylamide gels and stained with silver nitrate. RAPD assays were performed at least twice for each isolate with each primer to ensure that amplification patterns were reproducible.

RESULTS

Vegetative compatibility tests. For each isolate, a *nit*1 mutant was generated and paired with a NitM mutant tester from the four VCGs identified previously (31). Complementation tests showed that the 44 isolates were all vegetatively compatible and belonged to the group formerly designated VCG1 (31), which, henceforth, we will call *F. oxysporum* f. sp. *albedinis* group VCG0170, to follow the designation first used by Puhalla (29) and others (12,19,34).

mtDNA RFLP analysis. No polymorphisms were detected among the 44 isolates with the two enzyme/probe combinations tested (Fig. 1). Fourteen *Bgl*II-restriction fragments (with estimated sizes of 13, 12.5, 7.8, 4.2, 3.8, 3.6, 2.4, 2.2, 1.5, 1.3, 1.2, 1.1, 0.9, and 0.5 kb, respectively) hybridized with the whole purified mtDNA probe, and four *Hin*fI-restriction fragments (with estimated sizes of 1.25, 0.8, 0.6, and 0.4 kb, respectively) hybridized with the pUF1-14 probe. The length of *F. oxysporum* f. sp. *albedinis* mtDNA, estimated to be 55 kb (32), was confirmed.

RAPD analysis. Initially, polymerase chain reaction (PCR) amplification products were separated by electrophoresis in 1.4% agarose gels. Depending on the primer tested, three to four DNA bands were obtained for each isolate, and no polymorphism was detected among isolates within our collection. To enhance separation and detection of the RAPD fragments, amplification products also were resolved by polyacrylamide gel electrophoresis and stained with silver nitrate. Five to eight amplified DNA fragments were identified for each isolate, and again, no polymorphism was found (Fig. 2).

Fot1 hybridization-pattern analysis. For *F. oxysporum* f. sp. *albedinis* isolates, an average of 20 *Eco*RI fragments per isolate, ranging from 0.8 to 15 kb, hybridized with the Fot1 probe. A total of 27 distinct restriction fragments were obtained among the 44 isolates, allowing detection of 23 DNA hybridization patterns (Fig. 3; Table 2). Only 7 of 27 fragments were common to all isolates tested, and the repetitive DNA patterns differed from each other by one to six bands. For *F. oxysporum* strain A15, only four *Eco*RI fragments hybridized with the Fot1 probe (Table 2). Cluster analysis showed that all *F. oxysporum* f. sp. *albedinis* isolates were very similar and formed a very tight cluster (Fig. 4). The isolates were grouped at a genetic distance of 0.11, except one isolate (15.25, displaying pattern P11) that clustered at 0.17. *F. oxysporum* strain A15 clustered at 0.46 (Fig. 4). The high similarity detected among the 23 hybridization patterns indicates that the 44 *F. oxysporum* f. sp. *albedinis* isolates belong to a single genetic group. Among the 23 hybridization patterns, 4 patterns (P2, P5, P10, and P15) accounted for more than 50% of the isolates, and 18 were represented by single isolates (Table 2). Common hybridization patterns (P2, P8, and P10) were found in the Moroccan locations. The two Algerian isolates exhibited a pattern (P5) also found in Zagora (Draa Valley) (Table 1).

DISCUSSION

The aim of this study was to test whether the *F. oxysporum* f. sp. *albedinis* isolates causing Bayoud disease on date palm in Morocco originate from a unique virulent clone that spread throughout the oasis.

We used a large set of genetic and molecular markers to reveal polymorphisms in *F. oxysporum*. All the *F. oxysporum* f. sp. *albedinis* isolates grouped in a single VCG (0170) and displayed the same mtDNA restriction patterns and RAPD profiles. Sequences homologous to the DNA transposable element Fot1 (7) were found in the genome of the *F. oxysporum* f. sp. *albedinis* strains, revealing 23 closely related repetitive DNA patterns among the 44 isolates (Figs. 3 and 4). VCG, RAPD, and RFLP analyses have proved useful in detecting polymorphisms among pathogenic and nonpathogenic *F. oxysporum* isolates and have increased our knowledge of the population biology of *F. oxysporum* (2–4,12–14,17,19,21,23,26–28,34). Our analyses clearly showed that the *F. oxysporum* f. sp. *albedinis* isolates tested belong to a single genetic group. The high level of genetic similarity found among

TABLE 2. Fot1 hybridization patterns (presence [1]/absence [0] of each *Eco*RI fragment) obtained among 44 *Fusarium oxysporum* f. sp. *albedinis* isolates and *F. oxysporum* strain A15 (outgroup)

Pattern no.	Haplotype																												No. of fragments	No. of isolates
P1	1	1	0	1	1	1	0	1	1	1	1	0	0	0	1	1	1	1	1	0	0	1	1	1	0	1	1	1	20	1
P2	1	1	1	1	1	1	0	1	1	1	1	0	0	0	1	1	1	1	1	1	0	1	1	1	0	1	1	1	22	7
P3	1	1	1	1	1	1	0	1	1	1	1	0	0	1	1	1	1	1	1	1	0	1	1	1	0	1	1	1	23	1
P4	1	1	1	1	1	1	0	0	1	1	1	0	0	0	0	0	1	1	1	1	0	1	1	1	0	1	1	1	19	1
P5	1	1	1	1	1	1	0	1	1	1	1	0	0	0	1	1	1	1	1	1	0	1	0	1	1	1	1	1	22	8
P6	1	1	1	1	0	1	0	1	1	1	1	0	0	0	1	1	1	1	1	1	0	1	1	1	0	1	1	1	21	1
P7	1	1	1	1	0	1	0	1	1	1	1	0	0	0	1	1	1	1	1	1	1	1	1	1	0	1	1	1	22	1
P8	1	1	0	1	1	1	0	1	1	1	1	0	0	0	1	1	1	1	1	1	0	1	0	1	1	0	1	1	20	2
P9	1	1	1	1	1	1	0	1	1	1	1	0	0	0	1	0	1	1	1	1	0	1	1	1	0	1	1	1	21	1
P10	1	1	1	1	1	1	0	1	1	1	1	0	0	0	1	1	1	1	1	1	0	1	0	1	0	1	1	1	21	4
P11	1	1	0	0	1	1	0	0	1	1	1	0	0	0	1	1	1	1	1	0	0	1	1	1	0	0	0	0	15	1
P12	1	1	1	1	1	1	0	1	1	1	1	0	0	0	1	1	1	1	0	1	0	1	0	1	0	1	1	1	20	1
P13	1	1	1	1	0	1	0	0	1	1	1	0	0	0	1	1	1	1	1	1	0	1	1	1	0	1	1	1	20	1
P14	1	1	0	1	1	1	0	1	1	1	1	0	0	0	1	1	1	0	1	1	0	1	0	1	1	1	1	1	20	1
P15	1	1	0	1	1	1	0	1	1	1	1	0	0	0	1	1	1	1	1	1	0	1	0	1	1	1	1	1	21	5
P16	1	1	0	0	0	1	0	1	1	1	1	0	0	0	1	1	1	1	1	0	0	1	1	1	0	1	1	1	18	1
P17	1	1	0	1	1	1	0	0	1	1	1	0	0	0	1	1	1	1	1	1	0	1	0	1	1	0	0	0	17	1
P18	1	1	1	1	1	1	0	1	1	1	1	0	0	0	1	1	1	0	1	1	0	1	1	1	0	1	1	1	21	1
P19	1	1	1	1	0	1	0	1	1	1	1	0	0	0	1	0	1	1	1	1	0	1	1	1	0	1	1	1	20	1
P20	1	1	0	1	1	1	0	1	1	1	1	0	1	0	1	1	1	1	1	0	0	1	1	1	0	1	1	1	21	1
P21	1	1	1	1	0	1	0	1	1	1	1	0	0	0	1	1	1	1	1	1	0	1	0	1	0	1	1	1	20	1
P22	1	1	1	1	1	1	0	1	1	1	1	0	0	0	1	1	0	1	1	1	0	1	1	1	0	1	1	1	21	1
P23	1	1	1	1	1	1	0	1	1	1	1	1	0	0	1	1	1	1	1	1	0	1	1	1	0	1	1	1	23	1
Outgroup	0	0	1	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	4	1

the isolates is consistent with the hypothesis that all *F. oxysporum* f. sp. *albedinis* isolates belong to a unique clonal lineage and that the present Moroccan populations may derive from a single clone.

In addition, isolates from Morocco and Algeria had the same multilocus genotype, and identical Fot1 hybridization patterns were found in distinct palm groves (Table 1). Additional data obtained by the same techniques on a large sampling of Algerian isolates confirm the existence of a unique genetic group among *F. oxysporum* f. sp. *albedinis* isolates ([16], M. Ouinten, *unpublished data*). Bayoud was first described in the Draa Valley in southern Morocco (33); *F. oxysporum* f. sp. *albedinis* is suspected to have been introduced along with contaminated date palm material in other locations and to have been successively transported from one oasis to another (25,33). Our results are consistent with the scenario involving evolution in Morocco of a virulent clone and its subsequent geographic spread.

The special form *albedinis*, thus, presents a remarkable level of genetic homogeneity compared with those described in other *F. oxysporum*. In most of the special forms studied, several genetic groups, which can be referred to as different clonal lineages, have been identified on the basis of VCG, RFLP, and RAPD analyses (2,12–14,19,26–28,34). To our knowledge, the existence of a unique clonal lineage within a special form is restricted to the crucifer-infecting formae speciales *conglutinans*, *raphani*, and *matthioli* (3,20,21,23). These special forms were classified into the forma specialis *conglutinans* by Armstrong and Armstrong (1) because they share common host specificities.

The limited geographic area of extension of Bayoud disease and the dispersal of *F. oxysporum* f. sp. *albedinis* to only two countries could account for the lack of diversity in this special form but might not be the only explanation. There are several examples in pathogenic *F. oxysporum* populations in which genetic diversity is distributed on a small scale (13,14,19,26,27,34). Our results from *F. oxysporum* f. sp. *albedinis* can be compared with those obtained from another Arecaceae-attacking special form. *F. oxysporum* f. sp. *elaeidis*, the oil palm (*Elaeis guinensis*) wilt pathogen is distributed in Africa and America, and no races have been described so far (11). At least 10 genetic groups have been identified based on VCG (11,12) and fingerprinting analysis with the *F. oxysporum* f. sp. *elaeidis* cloned transposable element Palm (28). These clonal lineages coexist in at least two African countries (Ghana and Zaire), and two genetically distinct clones have been isolated from the same tree in Ghana. On the other hand, some clonal lineages may be dominant on a regional or continental scale; analyses strongly suggest that the recently discovered American populations of *F. oxysporum* f. sp. *elaeidis* were introduced from Africa (11,12,28).

Whether several clones were the origin of Bayoud disease in the Draa Valley in Morocco and then lost by random genetic drift during the past 120 years is questionable and cannot be assessed easily. Loss of genotypes through genetic drift can be particularly important in strictly asexually reproducing populations. In the same way, reduction in host population genetic diversity by the dramatic disappearance of some highly susceptible date palm cultivars could have led to the reduction of genetic diversity in the actual pathogen population. In this study, the *F. oxysporum* f. sp. *albedinis* isolates were collected from several date palm cultivars as well as from trees resulting from genetic crosses at the INRA experimental station in Zagora. We could not find particular associations between the Fot1 hybridization patterns and the date palm cultivars from which the isolates were collected, including the highly Bayoud-susceptible Bouffegous (Boufg) and Deglat Nour cultivars.

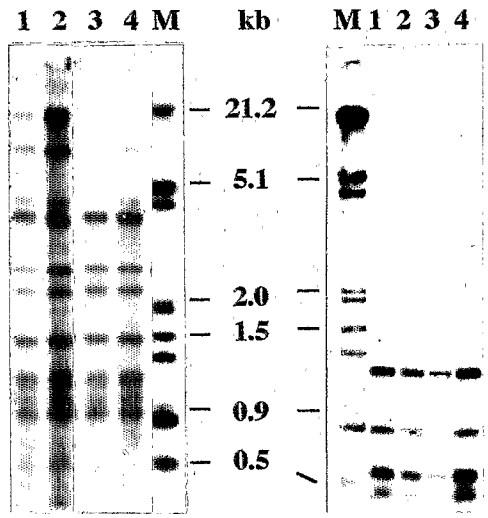

Fig. 1. Mitochondrial DNA (mtDNA) restriction patterns of *Fusarium oxysporum* f. sp. *albedinis* isolates. Left: hybridization patterns of the whole purified mtDNA on *Bgl*II-digested DNAs. Right: hybridization patterns of probe pUF1-14 on *Hin*fI-digested DNAs. Lanes 1 to 4, isolates OTK, C1, 145, and ALL, respectively. Lanes M, molecular weight marker (radiolabeled *Eco*RI-*Hin*dIII–digested phage lambda DNA); the fragment size in kilobases is indicated in the middle.

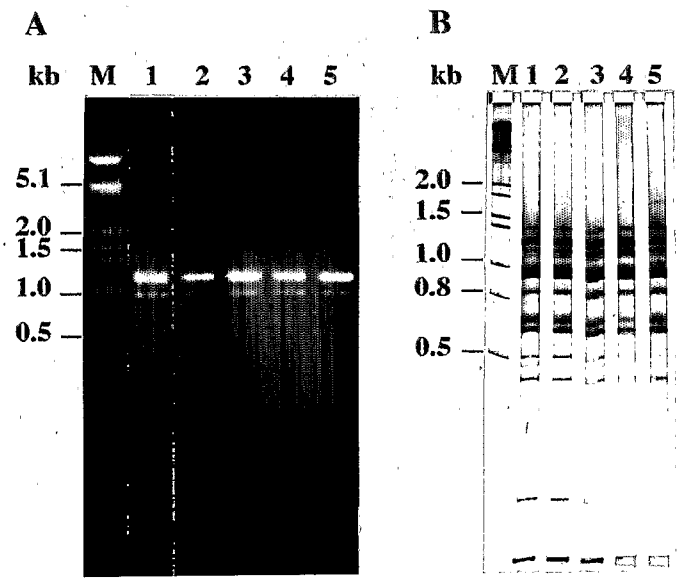

Fig. 2. Random amplified polymorphic DNA patterns generated from *Fusarium oxysporum* f. sp. *albedinis* isolates with primer OPF-12. **A,** Agarose gel stained with ethidium bromide; **B,** polyacrylamide gel stained with silver nitrate. Lanes 1 to 5, isolates 133, OTK, C1, 145, and ALL, respectively. Lanes M, molecular weight marker (*Eco*RI-*Hin*dIII–digested phage lambda DNA); the fragment size in kilobases is indicated to the left of **A and B**.

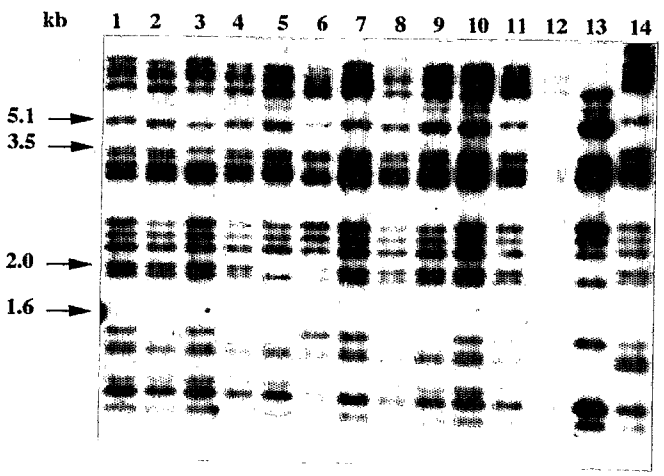

Fig. 3. Fot1 hybridization patterns obtained with 14 *Fusarium oxysporum* f. sp. *albedinis* isolates (lanes 1 through 14). The fragment size is indicated to the left.

Several questions need to be addressed. First, the possible occurrence of races in *F. oxysporum* f. sp. *albedinis* and the pathogenic status of the isolates have to be determined. The genetic similarity detected by molecular markers or VCG does not necessarily mean there is no variation with respect to pathogenicity. In several other special forms of *F. oxysporum*, isolates belonging to distinct races belong to the same VCG or RFLP or RAPD group (4,13,17,19,27). Thus, we cannot exclude the possible existence of races in *F. oxysporum* f. sp. *albedinis* based on the current results.

Second, will this genetic homogeneity be maintained over time? We have shown that the genome of the fungus contains several sequences homologous to the DNA transposable element Fot1, which is known to be active in *F. oxysporum* (7). The high number of Fot1 hybridization patterns in *F. oxysporum* f. sp. *albedinis* strains (23 patterns versus 44 isolates) compared to the strict genetic homogeneity observed with other analyses (VCG, mtDNA, RFLP, and RAPD) suggests that some copies of Fot1 may still be able to move or that some recombination events may occur during mitosis or via a parasexual process between vegetatively compatible strains (6,22). If new Fot1 patterns can be generated by several molecular mechanisms associated with the presence of active transposable elements in the genome, then it would be possible for local genetic diversification of the fungus to occur. We have shown that the Fot1 hybridization patterns make it possible to analyze *F. oxysporum* f. sp. *albedinis* populations on a local scale (oasis).

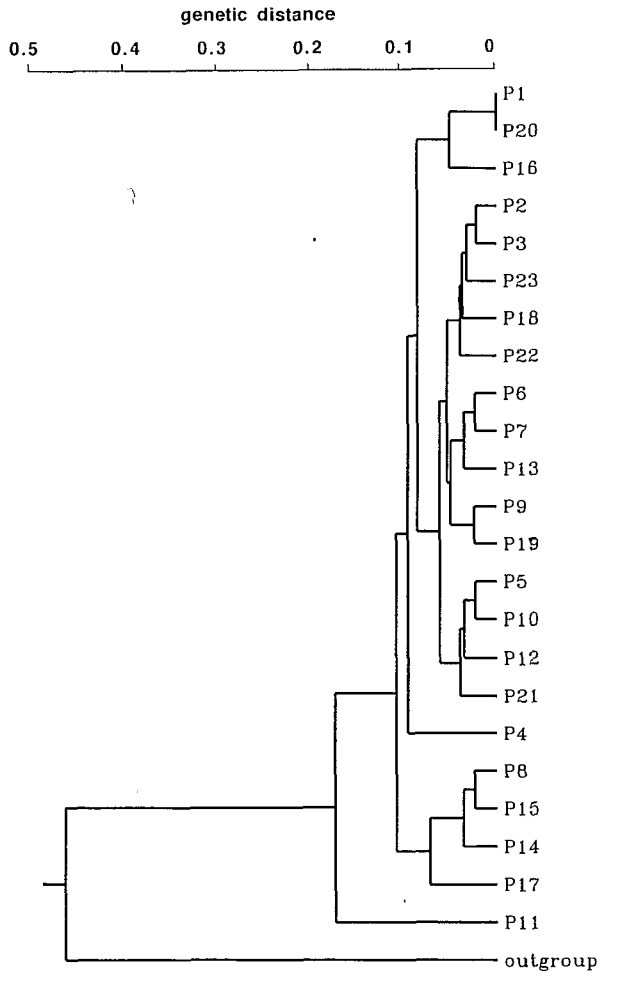

Fig. 4. Dendrogram showing the genetic relationships among *Fusarium oxysporum* f. sp. *albedinis* isolates and nonpathogenic *F. oxysporum* isolate A15 ("outgroup"). Cluster analysis was performed by the unweighted paired-group method with arithmetic mean, and genetic distances were obtained based on Jaccard's similarity coefficient of 27 individual DNA bands produced by hybridization with the Fot1 probe. For convenience, isolates are represented by their corresponding hybridization-pattern numbers (Table 2).

Tracking genotypes would be useful to detect genetic changes over time and would allow testing for *F. oxysporum* f. sp. *albedinis* population differentiation in the oases of Morocco and Algeria.

LITERATURE CITED

1. Armstrong, G. M., and Armstrong, J. K. 1981. *Formae speciales* and races of *Fusarium oxysporum* causing wilt diseases. Pages 391-399 in: *Fusarium*: Diseases, Biology and Taxonomy. P. E. Nelson, T. A. Toussoun, and R. J. Cook, eds. The Pennsylvania State University Press, University Park.
2. Assigbetse, K. B., Fernandez, D., Dubois, M. P., and Geiger, J. P. 1994. Differentiation of *Fusarium oxysporum* f. sp. *vasinfectum* races on cotton by random amplified polymorphic DNA (RAPD) analysis. Phytopathology 84:622-626.
3. Bosland, P. W., and Williams, P. H. 1987. An evaluation of *Fusarium oxysporum* from crucifers based on pathogenicity, isozyme polymorphism, vegetative compatibility, and geographic origin. Can. J. Bot. 65: 2067-2073.
4. Correll, J. C. 1991. The relationships between formae speciales, races, and vegetative compatibility groups in *Fusarium oxysporum*. Phytopathology 81:1061-1064.
5. Correll, J. C., Klittich, C. J. R., and Leslie, J. F. 1987. Nitrate non-utilizing mutants of *Fusarium oxysporum* and their use in vegetative compatibility tests. Phytopathology 77:1640-1646.
6. Daboussi, M. J., and Langin, T. 1994. Transposable elements in the fungal plant pathogen *Fusarium oxysporum*. Genetica 93:49-59.
7. Daboussi, M. J., Langin, T., and Brygoo, Y. 1992. Fot1, a new family of fungal transposable elements. Mol. Gen. Genet. 232:12-16.
8. Djerbi, M., and Den Braber, K. 1990. A new method of identification of *Fusarium oxysporum* f. sp. *albedinis* on the basis of vegetative compatibility. Page 513 in: Proceedings of the 8th Congress of the Mediterranean Phytopathological Union.
9. Djerbi, M., El Ghorfi, M. A., and El Idrissi Ammari, M. A. 1985. Etude du comportement du Henné (*Lawsonia inermis* L.) et de la Luzerne (*Medicago sativa* L.) et de quelques espèces de palmiers à l'égard du *Fusarium oxysporum* f. sp. *albedinis* agent du bayoud. Ann. Inst. Natl. Rech. Agron. Tunis. 58:1-11.
10. Djerbi, M., Sedra, M. H., and El Idrissi Ammari, M. A. 1985. Caractéristiques culturales et identification du *Fusarium oxysporum* f. sp. *albedinis*, agent causal du Bayoud. Ann. Inst. Natl. Rech. Agron. Tunis. 58:1-8.
11. Dossa, C. 1993. Variabilité de la morphologie et du pouvoir pathogène et diversité génétique chez *Fusarium oxysporum* Schlecht. f. sp. *elaeidis* Toovey, agent de la fusariose du Palmier à huile. Ph.D. thesis. Université Montpellier II, Montpellier, France.
12. Dossa, C., Pando-Bahuon, A., Renard, J. L., and Boisson, C. 1991. Détermination des groupes de compatibilité végétative chez des souches africaines de *Fusarium oxysporum* isolées de palmiers à huile fusariés. Oléagineux 46:145-147..
13. Elias, K. S., Zamir, D., Lichtman-Pleban, T., and Katan, T. 1993. Population structure of *Fusarium oxysporum* f. sp. *lycopersici*: Restriction fragment length polymorphisms provide genetic evidence that vegetative compatibility group is an indicator of evolutionary origin. Mol. Plant-Microbe Interact. 6:565-572.
14. Fernandez, D., Assigbetse, K. B., Dubois, M. P., and Geiger, J. P. 1994. Molecular characterization of races and vegetative compatibility groups in *Fusarium oxysporum* f. sp. *vasinfectum*. Appl. Environ. Microbiol. 60: 4039-4046.
15. Fernandez, D., Ouinten, M., Tantaoui, A., Lourd, M., and Geiger, J.-P. 1995. Population genetic structure of *Fusarium oxysporum* f. sp. *albedinis*. (Abstr.) Fungal Genet. Newsl 42A:34.
16. Fernandez, D., and Tantaoui, A. 1994. Random amplified polymorphic DNA (RAPD) analysis: A tool for rapid characterization of *Fusarium oxysporum* f. sp. *albedinis* isolates? Phytopathol. Mediterr. 33:223-229.
17. Grajal-Martin, M. J., Simon, C. J., and Muehlbauer, F. J. 1993. Use of random amplified polymorphic DNA (RAPD) to characterize race 2 of *Fusarium oxysporum* f. sp. *pisi*. Phytopathology 83:612-614.
18. Jaccard, P. 1908. Nouvelles recherches sur la distribution florale. Bull. Soc. Vaudoise Sci. Nat. 44:223-270.
19. Jacobson, D. J., and Gordon, T. R. 1991. *Fusarium oxysporum* f. sp. *melonis*: A case study of diversity within a forma specialis. Phytopathology 81:1064-1067.
20. Kistler, H. C., and Benny, U. 1989. The mitochondrial genome of *Fusarium oxysporum*. Plasmid 22:86-89.
21. Kistler, H. C., Bosland, P., Benny, U., Leong, S., and Williams, P. 1987. Relatedness of strains of *Fusarium oxysporum* from crucifers measured by examination of mitochondrial and ribosomal DNA. Phytopathology 77:1289-1293.

22. Kistler, H. C., and Miao, V. P. W. 1992. New modes of genetic change in filamentous fungi. Annu. Rev. Phytopathol. 30:131-152.
23. Kistler, H. C., Momol, E. A., and Benny, U. 1991. Repetitive genomic sequences for determining relatedness among strains of *Fusarium oxysporum*. Phytopathology 81:331-336.
24. Lee, S., Milgroom, M., and Taylor, J. 1988. A rapid, high yield miniprep method for isolation of total genomic DNA from fungi. Fungal Genet. Newsl. 35:23-24.
25. Louvet, J., and Toutain, G. 1981. Bayoud, *Fusarium* wilt of date palm. Pages 13-20 in: *Fusarium*: Diseases, Biology and Taxonomy. P. E. Nelson, T. A. Toussoun, and R. J. Cook, eds. The Pennsylvania State University Press, University Park.
26. Manicom, B., and Baayen, R. P. 1993. Restriction fragment length polymorphisms in *Fusarium oxysporum* f. sp. *dianthi* and other *fusaria* from *Dianthus* species. Plant Pathol. 42:851-857.
27. Mes, J. J., Van Doorn, J., Roebroeck, E. J. A., Van Egmond, E., Van Aartrijk, J., and Boonekamp, P. M. 1994. Restriction fragment length polymorphisms, races and vegetative compatibility groups within a worldwide collection of *Fusarium oxysporum* f. sp. *gladioli*. Plant Pathol. 43:362-370.
28. Mouyna, I. 1994 . Caractérisation moléculaire et étude de la diversité génétique des *Fusarium oxysporum* forme spéciale *elaeidis* responsables de la fusariose du Palmier à huile. Ph.D. thesis. Université Paris VII, Paris.
29. Puhalla, J. E. 1985. Classification of strains of *Fusarium oxysporum* on the basis of vegetative compatibility. Can. J. Bot. 63:179-183.
30. Sneath, P. H. A., and Sokal, R. R. 1973. Numerical Taxonomy. W. H. Freeman & Co., San Francisco.
31. Tantaoui, A., and Boisson, C. 1991. Compatibilité végétative d'isolats du *Fusarium oxysporum* f. sp. *albedinis* et des *Fusarium oxysporum* de la rhizosphère du Palmier dattier et des sols de palmeraies. Phytopathol. Mediterr. 30:155-163.
32. Tantaoui, A., and Fernandez, D. 1993. Comparaison entre *Fusarium oxysporum* f. sp. *albedinis* et *Fusarium oxysporum* des sols de palmeraies par l'étude du polymorphisme de longueur des fragments de restriction (RFLP). Phytopathol. Mediterr. 32:235-244.
33. Toutain, G. 1965. Note sur l'épidémiologie du Bayoud en Afrique du Nord. Al Awamia 15:37-45.
34. Woudt, L. P., Neuvel, A., Sikkema, A., van Grinsven, M. Q. J. M., de Milliano, W. A. J., Campbell, C. L., and Leslie, J. F. 1995. Genetic variation in *Fusarium oxysporum* from cyclamen. Phytopathology 85:1348-1355.