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Short communication

Molecular records of micro-evolution within the Algerian population of *Fusarium oxysporum* f. sp. *albedinis* during its spread to new oases

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Abstract

The genetic diversity of the date palm wilt pathogen *Fusarium oxysporum* f. sp. *albedinis* in Algeria was assessed using vegetative compatibility, restriction fragment length polymorphism (RFLP) of mitochondrial DNA (mtDNA), and random amplified polymorphic DNA (RAPD). Ninety-eight isolates were collected from the main infested regions, Touat, Gourara and Mzab, and 6 isolates from Morocco were added for comparison. All isolates were vegetatively compatible and belonged to VCG 0170. No variation was detected in the mtDNA of a subset of 73 isolates and the RAPD analysis indicated that they were genetically very closely related. However, some geographic substructuring was apparent, suggesting that local diversification of the pathogen might have occurred. These results provide evidence that the Algerian isolates of *F. oxysporum* f. sp. *albedinis* belong to a same clonal lineage and support the hypothesis that they were probably founded by a single virulent clone that originated from the Moroccan oases where the date palm wilt (Bayoud disease) was first detected. Based on similarity of RAPD patterns occurring in different oases, and on historical records of the Bayoud disease in Algeria, spread of the pathogen in the different regions is discussed.

Fusarium oxysporum Schlechtend.:Fr. f. sp. *albedinis* (Killian and Maire) Gordon, is the causal organism of Bayoud disease, the vascular wilt of date palm (*Phoenix dactylifera* L.), which has caused the death of millions of trees. The disease occurred since 1870 in Morocco and since the beginning of the XXth century in the western and central parts of the Algerian Sahara. It has never been reported in any other area of date palm cultivation throughout the world.

Vegetative compatibility and genomic analyses have allowed characterization of a single clonal lineage within the Moroccan *F. oxysporum* f. sp. *albedinis* (Tantaoui et al., 1996). All isolates tested belonged to a single vegetative compatibility group (VCG 0170) and displayed identical mitochondrial DNA (mtDNA) restriction fragment length polymorphism (RFLP) patterns and random amplified polymorphic DNA (RAPD) profiles (Tantaoui et al., 1996). In addition, two *F. oxysporum* f. sp. *albedinis* isolates from Algeria belonged to the same VCG and showed the same molecular profiles, suggesting that a single virulent clone spread throughout the Moroccan oases and reached Algeria (Tantaoui et al., 1996).

For decades, the epidemiology of *F. oxysporum* f. sp. *albedinis* has been well documented (Louvet and Toutain, 1981; Brac de la Perriere and Benkhalifa, 1991). In Algeria, the fungus is suspected to have been introduced along with contaminated date palm material from Morocco and to have been successively transported from one oasis to another (Louvet and Toutain, 1981). Spread of the Bayoud disease in the Algerian oases has been reported beginning in eastern Algeria as early as 1898 in Beni-Ounif, then in the regions of Gourara (1912), Mzab (1935) and Touat (1946) (Brac de la Perriere and Benkhalifa, 1991) (Figure 1). Fusariosis is still progressing in others Algerian areas

and foci are extending in diseased oases. The aim of this study was to assess the genetic diversity within the *F. oxysporum* f. sp. *albedinis* isolates in Algeria, in comparison with the clonal lineage of *F. oxysporum* f. sp. *albedinis* in Morocco. We used vegetative compatibility, mtDNA RFLP and RAPD to investigate the genetic relatedness of the *F. oxysporum* f. sp. *albedinis* isolates.

The main diseased regions in Algeria (Gourara, Mzab and Touat) were sampled during 1992-1994 (Figure 1) and 92 *F. oxysporum* f. sp. *albedinis* isolates were recovered from wilted palms of different varieties. Six additional isolates were gifts from collections and six isolates from Morocco, of which 5 had already been analysed (Tantaoui et al., 1996), were added for comparison. The geographical location, year of isolation and host origin of the 104 isolates examined are listed in Table 1. VCG testing and RAPD and mtDNA RFLP were performed as described in Tantaoui et al. (1996) unless stated hereafter.

The 98 Algerian isolates and the 6 Moroccan isolates proved all vegetatively compatible, and belonged thus to the formerly described VCG 0170 (Tantaoui et al., 1996). A subset of 73 isolates (67 Algerian and 6 Moroccan) was used for mtDNA RFLP and RAPD experiments (Table 1). RFLP analyses were conducted with the two enzyme/probe combinations *BglII*/whole purified mtDNA and *HinfI*/pUF1-14 described in Tantaoui et al. (1996), and the additional combination *EcoRI*/whole purified mtDNA. No polymorphisms were detected among the 73 isolates with the three enzyme/probe combinations. The isolates displayed the same *BglII*- and *HinfI*-mtDNA restriction patterns as the Moroccan isolates previously tested (Tantaoui et al., 1996). Eight *EcoRI*-fragments (0.4, 1.8, 3.7, 3.9, 5.0, 6.0, 13.0 and 21-kb) hybridized with the whole purified mtDNA probe. The *F. oxysporum* f. sp. *albedinis* isolates from Algeria thus belonged to the same mtDNA RFLP group as detected in Morocco.

For RAPD analysis, we selected the three RAPD primers, OPF4, OPF12, and OPF13 previously used (Tantaoui et al., 1996) and the additional primers OPF1 (ACGGATCCTG), OPF5 (CCGAATTCCC), OPF6 (GGGAATTCGG), and OPF8 (GGGATATCGG) because they also produced bright reproducible bands. After analysis of PCR products on 1.4% agarose gels, no polymorphism was detected among the 73 isolates tested with the primers OPF5, OPF8 and OPF12 (Table 2). However, some polymorphisms were displayed in the RAPD patterns with the other primers tested. To enhance the separation and detection of the RAPD fragments, the amplification products were also resolved by polyacrylamide gel electrophoresis and stained with nitrate silver. For OPF1, OPF4 and OPF6, variation in amplification patterns was dependent on one band only, and generated two distinct amplification profiles among the 73 isolates. With primer OPF13, four bands were found to be polymorphic giving five RAPD patterns (Figure 2).

Table 1. Geographical repartition of the *Fusarium oxysporum* f. sp. *albedinis* isolates tested and their RAPD haplotypes determined in this study

Geographic region	Oasis	RAPD[a] haplotype	Number of isolates
Touat	Od Aissa	P6	1
		ND	8
	Mansor Bouda	P3	4
		P6	3
		ND	3
	Adgha	P4	2
		P5	1
		ND	3
	Tillilène	P4	2
		P6	2
		ND	4
	Mahdia	P1	1
		P6	4
	Tmantit	P3	1
		P4	1
		ND	6
	Od Hadj Mamou	P4	2
	Od Aarossa	ND	6
Taghit	Beni Ouarou	P7	1
Gourara	Ajdir	P2	2
		P6	3
	Alamellal	P6	4
	Ghiat	P6	3
Mzab	Ghardaia	P8	21
	Metlili	P2	2
		P6	6
	El Ateuf	P8	1
	Bounoura	P8	1
Morocco[b]	–	P6	6

[a] RAPD haplotypes, which are described in Table 2, are indicated for the 73 isolates which have also been tested in mtDNA RFLP.
[b] isolates of VCG0170 previously characterized (Tantaoui et al., 1996). ND: not determined; –: unknown.

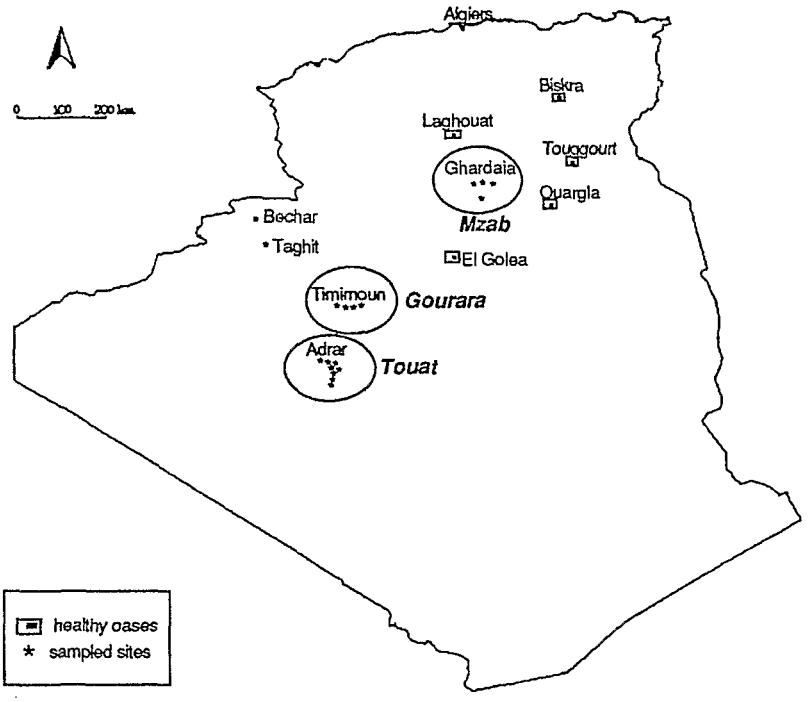

Figure 1. Map of Algeria showing geographical location of oases sampled.

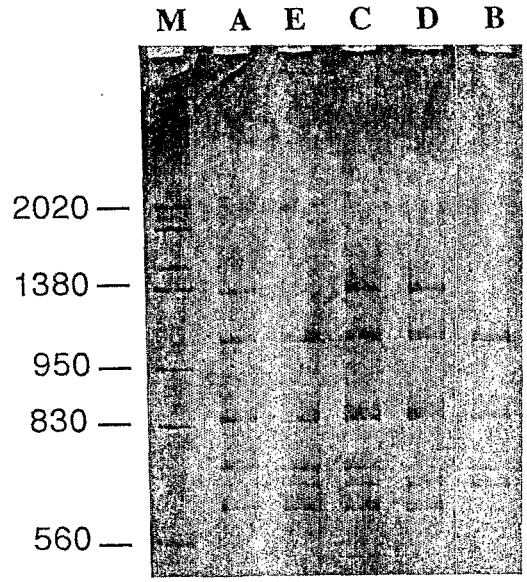

Figure 2. Polyacrylamide gel stained with silver-nitrate showing the RAPD patterns A, B, C, D and E generated from the *Fusarium oxysporum* f. sp. *albedinis* isolates with primer OPF-13. M, molecular weight marker (*Eco*RI-*Hind*III-digested phage lambda DNA); the fragment size in basepairs is indicated on the right.

By combining the results obtained with the 6 primers, 43 band positions were scored for presence versus absence (1/0) for all the isolates studied, and only 7 were polymorphic (OPF13-1380; OPF13-750; OPF13-700; OPF13-600; OPF6-700; OPF1-600 and OPF4-400). Eight distinct haplotypes were obtained, noted P1 to P8 (Table 2). The combined data from all isolates were analyzed by a simple matching coefficient (Sokal and Michener, 1958), which measures proportion of common discrete data (either 0 or 1) between the isolates, to produce a dendrogram (Figure 3). We used the simple matching coefficient as in Assigbetse et al. (1994) because we estimated it would be more suitable for RAPD analysis where absence of amplification may also be an indicator of genetic similarity. All the isolates were grouped at a genetic distance of 0.032, indicating they are genetically closely related. The isolates studied fell into two main groups, the first comprising isolates from Touat (haplotypes P1, P3, P4 and P5) and the second, isolates from all areas studied including Touat (haplotypes P2, P6, P7 and 8).

The results showed that the Algerian *F. oxysporum* f. sp. *albedinis* isolates belong to a single genetic group. In addition, the genetic relatedness evidenced between the Algerian and the Moroccan isolates tested suggests that the isolates of the *forma specialis albedinis* derive from a common ancestor. The high level of genetic similarity detected confirms that all *F.*

Table 2. RAPD haplotypes of *Fusarium oxysporum* f. sp. *albedinis* isolates obtained by combining the amplification patterns displayed with primers OPF1, OPF4, OPF5, OPF6, OPF8, OPF12 and OPF13, respectively

Pattern no.	Haplotype						
P1	1111111	111111	111	11111011111	1111	111111	111101
P2	1111111	111111	111	11111011111	1111	111111	011110
P3	1111111	111111	111	11111011111	1111	111111	111111
P4	1111111	111111	111	11111011111	1111	111111	111011
P5	1110111	111111	111	11111011111	1111	111111	111011
P6	1111111	111111	111	11111011111	1111	111111	011111
P7	1111111	111110	111	11111011111	1111	111111	011111
P8	1111111	111111	111	11111111111	1111	111111	011111

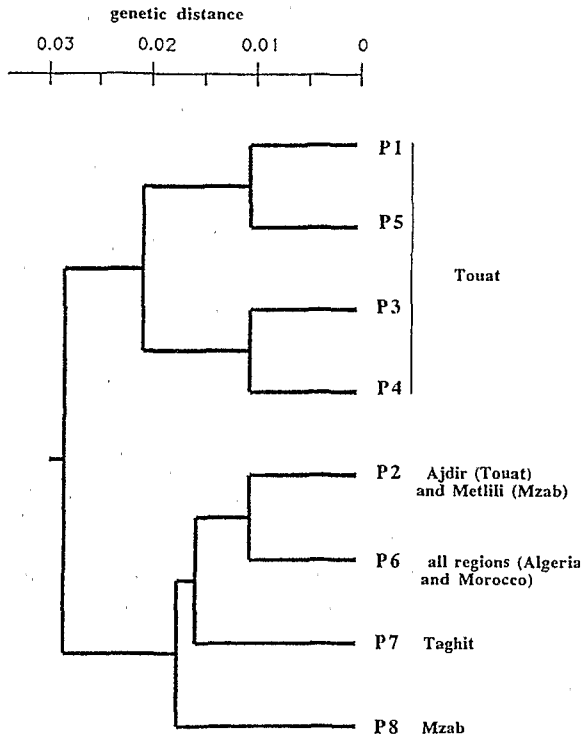

Figure 3. Dendrogram showing the genetic relationships among *Fusarium oxysporum* f. sp. *albedinis* isolates. Cluster analysis was performed using the unweighted paired-group method with arithmetic averaging (UPGMA) and genetic distances were obtained based on Simple Matching coefficient of 43 individual DNA bands produced by RAPD. For convenience, isolates are represented by their corresponding amplification patterns.

oxysporum f. sp. *albedinis* isolates belong to a single clonal lineage (Tantaou et al., 1996) and is consistent with the hypothesis that the present Algerian populations result from the dissemination of a single virulent clone originating from Morocco, where the disease was first detected.

The presence of identical RAPD patterns in several oases is an indicator of past (or present) migrations of the pathogen among localities in Algeria and between Morocco and Algeria. Pattern P6 was found in all Algerian regions sampled as well as in Morocco, in accordance with the supposed contamination of the Algerian oases by Moroccan isolates. Of particular interest is the situation in the region of the Mzab. First, isolates from Metlili (Mzab) and Ajdir (Gourara) shared pattern P2, suggesting that specific exchanges have occurred between the two localities. Second, the isolates originating from the other localities sampled in the Mzab (Ghardaia, Bounoura, El Ateuf) exhibited a distinct RAPD pattern (P8). Because the Bayoud disease has been recorded earlier in Ajdir (1912) than in Metlili (1935) (Brac de la Perriere and Benkhalifa, 1991), it is likely that the *F. oxysporum* f. sp. *albedinis* population of Metlili was founded by isolate(s) originating from Ajdir. Finally, given that the other localities in the Mzab were contaminated later (Ghardaia, 1965; El Ateuf, 1967; Bounoura, 1970; Brac de la Perriere and Benkhalifa, 1991), it had been suspected that these oases were contaminated from Metlili. Our results support the hypothesis that the population of Ghardaia has a distinct origin and that the oases of Bounoura and El Ateuf were probably infected from Ghardaia.

Previous results have shown that the *F. oxysporum* f. sp. *albedinis* isolates could be differentiated from other soilborne *F. oxysporum* non-pathogenic to date palms by using either vegetative compatibility grouping (Tantaoui and Boisson, 1991), mtDNA RFLP analysis (Tantaoui and Fernandez, 1993), or RAPD markers (Fernandez and Tantaoui, 1994). In addition, the mtDNA restriction patterns of the *F. oxysporum* f. sp. *albedinis* differed from those characterized in other *formae speciales* (Marriott et al., 1984; Kistler

and Benny, 1989; Jacobson and Gordon, 1990; Kim et al., 1992; Fernandez et al., 1994). The Algerian populations of the special form *albedinis* are thus composed of members of a single clonal lineage, which share unique genetic determinants. Predominance of a single genetic lineage at a large geographical scale has been found in other *F. oxysporum* special forms, such as *vasinfectum* (Assigbetse et al., 1994; Fernandez et al., 1994) and *elaeidis* (Dossa et al., 1991; Mouyna et al., 1996). However, these pathogenic forms, and most others, also contained several distinct lineages distributed worldwide (Assigbetse et al., 1994; Dossa et al., 1991; Jacobson and Gordon, 1991; Elias et al., 1993; Fernandez et al., 1994; Mes et al., 1994; Mouyna et al., 1996; Woudt et al., 1995; Woo et al., 1996). The genetic homogeneity observed in the *forma specialis albedinis* thus represents a special and original case among the plant pathogenic *F. oxysporum*.

The genetic diversity detected by RAPD analysis among the isolates also suggests that some local genetic differentiation has occurred since the Algerian populations were founded. Five distinct amplification patterns were displayed with primer OPF13 and three (A, C and D) were specific of the Touat population (Correspondence between patterns and haplotypes are A/P1, C/P3, D/P4 and D/P5, respectively). Asexually reproducing fungi may evolve primarily by accumulating point mutations in their genome, and by genomic rearrangements due to the presence of transposable elements and the occurrence of chromosomal recombination during mitosis (Kistler and Miao, 1992; Daboussi and Langin, 1994; Zolan, 1995). The genome of *F. oxysporum* f. sp. *albedinis* contains several copies of the Fot1 transposable element (Daboussi et al., 1992; Tantaoui et al., 1996) and copies of other transposons have also been detected (D. Fernandez, unpubl.). It is likely that some copies might have retained activity for transposition and would contribute to the genomic evolution of the pathogen. Use of the Fot1 element as a dispersed repetitive DNA probe has allowed to characterize 23 distinct repetitive patterns (genotypes) among 44 *F. oxysporum* f. sp. *albedinis* isolates in Morocco (Tantaoui et al., 1996). Identifying the *F. oxysporum* f. sp. *albedinis* genotypes with Fot1 in the Algerian oases will allow confirmation of specific ways of dissemination of the pathogen and would be useful to detect genetic changes in the populations overtime.

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