THE PHOENIX SPP. PATHOGENS *FUSARIUM OXYSPORUM* F. SP. *ALBEDINIS* AND *F. OXYSPORUM* F. SP. *CANARIENSIS* ARE DISTINCT GENETIC ENTITIES AS EVIDENCED BY MOLECULAR MARKERS.

D. FERNANDEZ[1], T. R. PLYLER[2] and H. C. KISTLER[2]

1-Laboratoire de Phytopathologie Tropicale, Institut Français de Recherche Scientifique pour le Développement en Coopération (ORSTOM), BP 5045, 34032 Montpellier Cedex 1, France.
2- Department of Plant Pathology, University of Florida, PO Box 110680, Gainesville, Florida 32611-0680, USA.

INTRODUCTION

Fusarium oxysporum Schlechtend.:Fr. f. sp. canariensis (Louvet) W. C. Snyd. & H. N. Hans. (1973), is the causal organism of a vascular wilt of the Canary Island date palm (Phoenix canariensis Hort. ex Chabaud). The disease was first observed in France in 1970 (Mercier and Louvet, 1973), then reported in Italy (1974), Japan (1977), California, USA, (1976), then in Australia (1980) and in 1987 in Morocco and in the Canary Islands. Recently, the disease was also reported in Florida (USA), where it is becoming an important threat to ornamental palm production.

First external symptoms are leaf desiccation, and especially resemble those found in the date palm (Phoenix dactylifera L.) wilt (Bayoud) caused by F. o. albedinis (Killian and Maire) Gordon. The Bayoud has only been reported in Morocco and Algeria, where it occurs since more than a century (Louvet and Toutain, 1981). Studies based on genetical and molecular markers showed that F. o. albedinis isolates belong to a single genetic lineage, evidencing that a unique virulent clone spread throughout the Moroccan and Algerian oases (Fernandez et al. 1997 ; Tantaoui et al., 1996). Because of the earliest record of the Bayoud and the ability of F. o. canariensis to cause disease on P. dactylifera (Mercier and Louvet, 1973 ; Arai and Yamamoto, 1977 ; Feather et al., 1989), it has been hypothesized that canariensis isolates might have evolved from albedinis isolates by acquiring pathogenicity toward Canary Island date palms.

The aims of this study were to assess the genetic relationships between the F. o. canariensis and the F. o. albedinis isolates and to evaluate the amount of genetic diversity in the canariensis special form.

MATERIALS AND METHODS

Restriction fragment length polymorphism (RFLP) experiments were undertaken to investigate a collection of 17 F. o. canariensis isolates of worldwide origin together with 12 representative isolates of F. o. albedinis (Table 1). Isolates were gift from Dr. J. Louvet (INRA, Dijon, France). We used homologous single-copy sequences (nia probe - the nitrate reductase gene, Diolez et al. (1993) - and the randomly cloned probe 6.4.3, T. Langin, pers. com.), the consensus telomeric sequence (TTAGGG)18 contained in clone pLD (Boehm et al., 1994) and the transposable elements Fot1 (Daboussi et al., 1992) and Foret (Julien et al., 1992) as probes on the EcoRI-restricted genomic DNAs prepared as previously described (Fernandez et al., 1994). Genetic distance analyses were based on the Jaccard's coefficient (Jaccard, 1917) which measures proportion of common data (1) between the isolates. A dendrogram was derived from the distance matrix by using the UPGMA algorithm contained in the computer program package Phylip 3.2 (Felsenstein, 1989).

RESULTS

Single-copy DNA RFLP analysis:

When tested with the nia probe, all the isolates displayed a unique hybridizing EcoRI-fragment which size differentiated F. o. canariensis from the F. o. albedinis isolates (7.0- or 5.0-kb, respectively) (Table 1). With probe 6.4.3, three EcoRI-fragments were displayed for each isolate, but only one was common to the canariensis and the albedinis isolates. The F. o. albedinis isolates displayed the same pattern (pattern C) ; polymorphisms (1 band change) were observed among the canariensis isolates (patterns A and B), which differentiated two strains (E2 and E3) originating from the Canary Islands (Table 1).

Cluster analysis was performed by compiling data obtained with the two single-copy probes. The isolates were separated at a genetic distance of 0.43: all the albedinis isolates constituted a single genetic group and the canariensis isolates clustered all together at a distance of 0.2 (Fig.1)

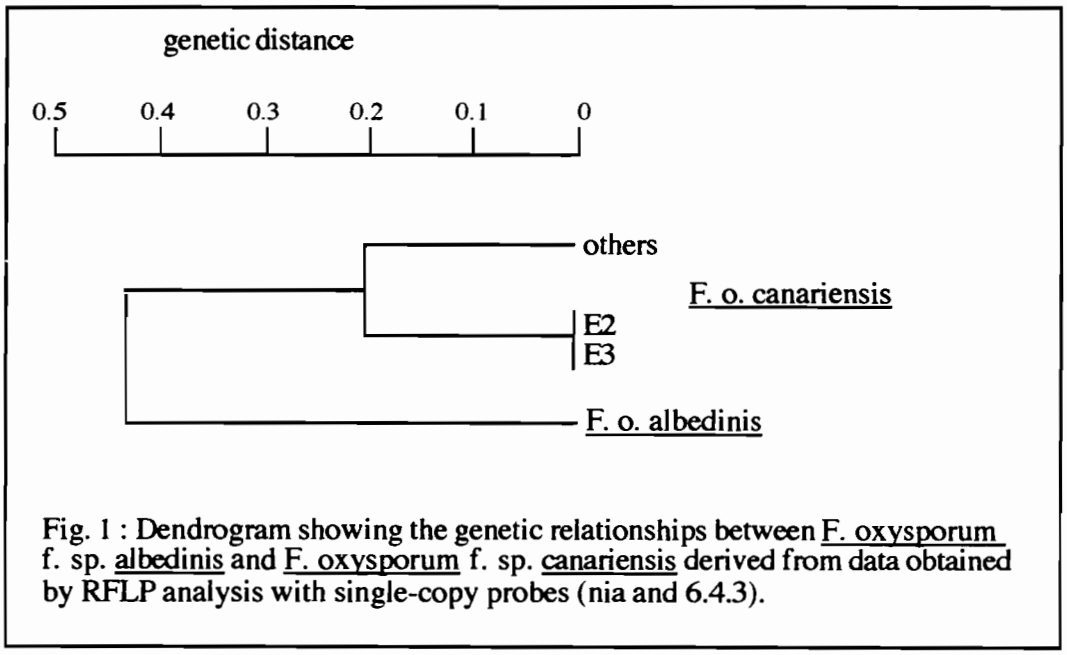

Fig. 1 : Dendrogram showing the genetic relationships between F. oxysporum f. sp. albedinis and F. oxysporum f. sp. canariensis derived from data obtained by RFLP analysis with single-copy probes (nia and 6.4.3).

Telomeric sequence RFLP analysis:
For each isolate, one to three EcoRI-bands hybridized with the consensus telomeric sequence. Unique hybridization patterns differentiated F. o. canariensis from the F. o. albedinis isolates. Four patterns were observed among the canariensis isolates (patterns A, B, C and D), unrelated with their geographic origin (Table 1): RFLPs were displayed between isolates from the Canary Islands, France, Japan and USA. The F. o. albedinis isolates displayed a 9-kb single-band pattern (E) and only one strain (M1) exhibited a two-band pattern (F) (Table 1).

Research of transposable elements:
The F. o. albedinis isolates displayed a repetitive pattern (18-26 EcoRI fragments) of hybridization with the Fot1, in accordance with results previously obtained on a larger collection of isolates (Tantaoui et al., 1996). In contrast, the F. o. canariensis isolates did not show any hybridizing fragments evidencing that their genomes were devoid of any copy of Fot1.
With the Foret probe, a single 10-band pattern (F) of varying intensity was found for the F. o. albedinis isolates, whereas the canariensis isolates exhibited 3-8-band patterns of hybridization (A-E) (Table 1). No common EcoRI-fragment was found between the two special forms.

CONCLUSIONS
F. o. canariensis and F. o. albedinis isolates were differentiated with all the probes used in RFLP experiments. These results clearly indicate that the albedinis and canariensis isolates have distinct phylogenetic origins and that the canariensis isolates do not derive from the albedinis special form. In addition, genetic diversity was found between the canariensis isolates, but there was no direct relation with the geographic origin of the isolates. Distribution of the genetic diversity in the distinct locations suggests (i) local emergence and spread of new canariensis isolates in each region and (ii) movement of specific fungal isolates between locations. This latter point is exemplified with the two canariensis isolates UC1 and F1, from California (USA) and France, respectively, which displayed the same restriction patterns with all the probes tested.

Table 1 : F. oxysporum isolates tested and hybridization results obtained with several DNA probes in RFLP experiments.

F. oxysporum isolates	geographic origin	nia (kb)	6.4.3 pattern no.	pLD pattern no.	Fot1 presence*	Foret pattern no.
f. sp. canariensis						
F1	France	7.0	A	D	-	D
F2-F4	France	7.0	A	A	-	A
F5	France	7.0	A	B	-	A
J1	Japan	7.0	A	C	-	B
J2-J3	Japan	7.0	A	B	-	C
I1-I2	Italy	7.0	A	C	-	C
E1	Canaries Isl. (Spain)	7.0	A	C	-	B
E2-E3	Canaries Isl. (Spain)	7.0	B	C	-	B
UC1	California (USA)	7.0	A	D	-	D
UF1-UF2	Florida (USA)	7.0	A	D	-	B
UF3	Florida (USA)	7.0	A	C	-	E
f. sp. albedinis						
M1	Morocco	5.0	C	F	+	F
M2-M9	Morocco	5.0	C	E	+	F
A1-A3	Algeria	5.0	C	E	+	F

* - : no hybridization ; +: 15-26 hybridizing fragments

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