

**BAYOUD OF DATE PALM (*PHOENIX DACTYLIFERA* L.):
POPULATION STRUCTURE AND GENETIC CHARACTERIZATION OF
FUSARIUM OXYSPORUM F. SP. *ALBEDINIS***

D. FERNANDEZ¹, A. TANTAOU², M. OUINTEN¹, T. LANGIN³,
M-J DABOUSSI³ and J-P GEIGER¹.

¹Laboratoire de Phytopathologie Tropicale, ORSTOM, BP 5045, 34032 Montpellier, France.

²Laboratoire de Phytopathologie, INRA, BP 533, Marrakech, Maroc.

³Institut de Génétique et Microbiologie, Université Paris-Sud, Bat 400, 91405 Orsay cedex, France

Bayoud, the *Fusarium* wilt of date palm tree (*Phoenix dactylifera* L.), 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 (Louvet and Toutain, 1981).

Detection and identification of *Fusarium oxysporum* f. sp. *albedinis* remain difficult, mainly because inoculation tests are still required to assess the pathogenicity of the *F. oxysporum* isolates. In addition, the pathogen can be easily disseminated by exchange of some contaminated material, and, at present, strict phytosanitary rules are applied at borders of date palm growing countries that remain free of Bayoud.

Studies on the genetic characterization of the pathogen have been undertaken in order to assess the genetic diversity and the population structure of *F. o. albedinis* and to develop diagnostic tools for its detection. Vegetative compatibility and genomic analyses have allowed characterization of a single clonal lineage within the Algerian and Moroccan *F. o. albedinis* (Tantaoui et al., 1996 ; Fernandez et al., 1997). 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, whatever the host genotype or their geographical origin (Tantaoui et al., 1996 ; Fernandez et al., 1997). Genomic analyses have also shown that the fungus carries repetitive DNA sequences homologous to the DNA transposable element *Fot1*, cloned in *F. oxysporum* f. sp. *melonis* (Daboussi et al., 1992) and widely distributed in *F. oxysporum*. Depending on the *F. o. albedinis* strain, 15-26 *EcoRI*-fragments hybridizing with the *Fot1* sequence were found and several were conserved among the isolates. Use of the *Fot1* element as a dispersed repetitive DNA probe has allowed to characterize more than 64 closely related repetitive patterns (genotypes) among 120 *F. o. albedinis* isolates (Tantaoui et al., 1996; Ouinten, 1996).

These results support the hypothesis that the Algerian and Moroccan isolates of *F. o. albedinis* were probably founded by a single virulent clone that originated from the Moroccan oases where the date palm wilt (Bayoud disease) was first detected (Tantaoui et al., 1996 ; Fernandez et al., 1997). However, some geographic substructuring was apparent in Algeria,

suggesting that local diversification of the pathogen might have occurred. Based on similarity of RAPD patterns and Fot1-genotypes occurring in different oases, and on historical records of the Bayoud disease in Algeria, spread of the pathogen in the different Algerian regions could be partially recounted (Fernandez et al., 1997 ; Ouinten, 1996).

In addition, all molecular patterns differed radically between *F. o. albedinis* isolates and other non-pathogenic *F. oxysporum* isolated from palm-grove soil (Tantaoui, 1994). We have taken advantage of the high genetic relatedness among isolates pathogenic to date palm to develop a sensitive PCR assay (Fernandez et al., sub). Several DNA clones containing a copy of the transposable element Fot1 were isolated from a genomic library of the date palm pathogen. Partial sequencing of the clones was undertaken to obtain the flanking sequences of the Fot1 copies in the genome of the fungus and we designed PCR primers to amplify the DNA regions overlapping each Fot1 insertion site.

When tested on a large sample of *Fusarium* isolates including 286 *F. o. albedinis*, 17 other *formae speciales*, non-pathogenic *Fusarium oxysporum* isolated from palm-grove soils and 8 *Fusarium* species, the primer pair TL3/FOA28 allowed specific amplification of a 400-bp fragment from the *F. o. albedinis* DNA. In addition, sequence analysis showed that one of the Fot1 copies was truncated, lacking 182-bp at its 3' terminus. The primer pair BIO3/FOA1, amplifying 204-bp overlapping the Fot1 truncated-copy and its 3' region of insertion in the *albedinis* genome, enabled identification of 95% of the isolates. Less than 100 pg of purified *albedinis* DNA could be efficiently detected. The primer pairs BIO3/FOA1 and FOA28/TL3 used in PCR assays thus provide a useful diagnostic tool for *F. o. albedinis*.

References:

- Daboussi, M. J., T. Langin, and Y. Brygoo. 1992. Fot1, a new family of fungal transposable elements. *Mol. Gen. Genet.* 232:12-16.
- Fernandez D., Ouinten M., Tantaoui A. and J-P. Geiger. 1997. Molecular records of micro-evolution within the Algerian population of *Fusarium oxysporum* f. sp. *albedinis* during its spread to new oases. *European Journal of Plant Pathology*, *in press*.
- Fernandez D., Ouinten M., Tantaoui A., Geiger J-P., Daboussi M-J and T. Langin. 199-. Fot1-specific insertions in the *Fusarium oxysporum* f. sp. *albedinis* genome provide useful PCR targets for detecting the date palm pathogen. *Applied Environmental Microbiology*, *submitted*.
- Louvet, J., and G. Toutain. 1981. Bayoud, *Fusarium* wilt of date palm, p. 13-20. In P.E. Nelson, T.A. Toussoun and T.A. Cook (eds.), *Fusarium, diseases, biology and taxonomy*. Pennsylvania State Univ. Press.
- Ouinten, M. 1996. Diversité et structure génétiques des populations Algériennes de *Fusarium oxysporum* f. sp. *albedinis*, agent de la fusariose vasculaire (Bayoud) du palmier dattier. PhD thesis. Université Montpellier II, France.
- Tantaoui, A. 1994. Caractérisation moléculaire et diversité génétique chez *Fusarium oxysporum* f. sp. *albedinis*, responsable de la fusariose vasculaire (Bayoud) du palmier dattier. PhD thesis. University of Marrakech, Morocco.
- Tantaoui, A., M. Ouinten, J.-P. Geiger and D. Fernandez. 1996. Characterization of a single clonal lineage of *Fusarium oxysporum* f. sp. *albedinis* causing Bayoud disease of date palm (*Phoenix dactylifera* L.) in Morocco. *Phytopathology* 86:787-792.

Fernandez Diana, Tantaoui A., Quinten Mohamed,
Langin T., Daboussi M.J., Geiger Jean-Paul (1997)

Bayoud of date palm (*Phoenix dactylifera* L.) :
population structure and genetic characterization of
Fusarium oxysporum f. sp. *Albedinis*

In : *10th congress of the mediterranean
phytopathological union*

Montpellier : Société Française de Phytopathologie, p.
199-200 multigr.

Congresss of the Mediterranean Phytopathological
Union, 10., Montpellier (FRA), 1997/06/01-05.