USE OF A PCR- BASED ASSAY FOR SPECIFIC IDENTIFICATION OF *Fusarium oxysporum* f. sp. *albedinis* CAUSING BAYOUD DISEASE OF DATE PALM IN MOROCCO

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INTRODUCTION:

Bayoud, is the wilt of Date Palm (*Phoenix dactilyfera*. L.) caused by *Fusarium oxysporum* f. sp. *albedinis*. This disease is the most serious cryptogamic plague in date palm growing areas of Morocco and in the western and central parts of Algeria. The disease continues to advance and constitutes a threat to other date producing areas of the world. It has not caused only the loss of the best cultivars but also accentuated and accelerated the phenomenon of desertification and poses serious problems (ecological, economic and social) (Louvet and Toutain, 1981; Djerbi, 1988; Vanachter, 1989).

Control is achieved by prevention measures including strict phytosanitary rules at borders of date palm growing countries that remain free of Bayoud, to slow down the disease spread. The research for resistant cultivars is also in progress in Morocco and Algeria (Louvet and Toutain, 1973; Djerbi,1988; Saaidi, 1992. Sedra, 1994).

Part of these researches deals with characterization and identification of the pathogen. We have shown that all the *F. o.* f. sp. *albedinis* isolates are genetically similar and belong to a single clonal lineage (Tantaoui *et al.*, 1996). In addition, vegetative compatibility, RFLP of mtDNA and RAPD analysis allowed differentiation between pathogenic isolates of *F. o.* f. sp. *albedinis* and saprophytic strains of *Fusarium oxysporum* or other *formae speciales* (Tantaoui and Boisson, 1991; Tantaoui and Fernandez, 1993; Fernandez and Tantaoui, 1994. Tantaoui, 1994). However, these techniques are time - consuming.

The objective of the current investigation was to test a Polymerase Chain Reaction (PCR) based method for selective identification of *F. o.* f. sp. *albedinis* in Morocco.

MATERIALS AND METHODS:

Fungal isolates: 200 strains of *F. o.* f. sp. *albedinis* isolated in several oases in Morocco (date palm leaves); 35 saprophytic strains of *F. oxysporum* isolated in Morocco (soil and roots) were used in this study.

DNA was extracted as previously described (Tantaoui *et al.*, 1996).

We used PCR primers developed from genomic clones containing a copy of the transposable element Fot1 (Daboussi *et al.*, 1992) which were shown to allow specific amplification of *F. o.* f. sp. *albedinis* DNA (Fernandez *et al.*, 1997). Two primers pairs (Bio 3 / FoA1) and (FOA28 / TL 3) were used to amplify DNA from all isolates in PCR experiments. PCR was performed in 12.5 µl volumes and under amplification conditions as given in Fernandez *et al.*, 1997.

RESULTS

Using primer pair (Bio 3 / FoA 1) PCR amplification resulted in a one band of approximately 200pb from 95% of isolates but no product was generated following amplification of DNA from any of the other isolates tested (fig. 1A). However, following PCR using primer pair (FOA28 / TL3), a single fragment of approximately 400pb (fig. 1B) was amplified from DNA of 99% of isolates of *F. o.* f. sp. *albedinis* including isolates which not identified by the first primer pair. The 1% of isolates of *F. o.* f. sp. *albedinis* not identified by the second primer pair were also produced the band of 200pb. In other to detect all isolates (100%) of *F. o.* f. sp. *albedinis* the two primer pairs must be used.

This specific, sensitive PCR assays represents a new tool for early diagnosis of Bayoud in Morocco.

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REFERENCES:

- DABOUSSI M. J., LANGIN T and BRYGOO Y. 1992: Fot1, a new family of fungal transposable elements. Mol. Gen.- Genet. 232: 12 - 16.
- DJERBI M 1988: Les maladies du Palmier dattier. 127p.
- FERNANDEZ D ., OUINTEN M., TANTAOUI A., CHABANE K., GEIGER J.P.,

DABOUSSI M.J and LANGIN T, 1997: Fot1- specfic insertion in *Fusarium oxysporum* f. sp. *albedinis* genome provide useful PCR targets for detecting the date palm pathogen. Applied Environmental microbiology, soumis.

- FERNANDEZ D and TANTAOUI A 1994: Random Amplified Polymorphic DNA (RAPD) analysis: for rapid characterization of *Fusarium oxyporum* f. sp. *albedinis* isolates? Phytopath. medit. 33, 223 - 229.

- LOUVET J and TOUTAIN G, 1973: Nouvelles observations sur la fusariose du palmier et précisions concernant la lutte. Ann. Phytopath, 5: 35 -52.

- LOUVET J and TOUTAIN G , 1981: Bayoud, *Fusarium* wilt of date palm p: 13- 20 in *Fusarium* diseases Biology and Taxonomy. Edit P. E. NELSON, T. A TOUSSOUN & C. J. COOK.

- SAAIDI M 1992: Comportement au champ de 32 cultivars de palmier dattier vis à vis du Bayoud: 25 années d'observation. Agronomie, 12, 359 - 370.

- SEDRA My M, 1994: Lutte contre le Bayoud, fusariose vasculaire du palmier dattier causée par *Fusarium oxysporum* f. sp. *albedinis*. Sélection de cultivars et clones de qualité, résistants et réceptivité des sols de palmeraies à la maladie. Thèse de Doctorat Es - Sciences. Fact. Scien. Univ. Cadi Ayyad Marrakech. 127p

- TANTAOUI A, 1994: Caractérisation moléculaire et diversité génétique chez *Fusarium oxysporum* f. sp. *albedinis* responsable de la fusariose vasculaire / Bayoud du palmier dattier. Thèse de Doctorat Es - Sciences. Fact. Scien. Univ. Cadi Ayyad Marrakech. 123p.

- 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.

- 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.

- TANTAOUI A, OUINTEN M, GEIGER J.P and FERNANDEZ D, 1996: Characterization of a Single Clonage Lineage of *Fusarium oxysporum* f. sp. *albedinis* causing Bayoud Disease of Date Palm in Morocco. Phytopathology: Vol. 86, No. 7: 787 - 792.

- VANACHTER A, 1989: Strategies for the control of Bayoud disease of the date palm caused by *Fusarium oxysporum* f. sp. albedinis. In vascular wilt diseases of plants. Basic studies and control. Edt. E. C TJAMOS & C. H BECKMAN. Series H: Cell. Biology, Vol. 28. p 500.

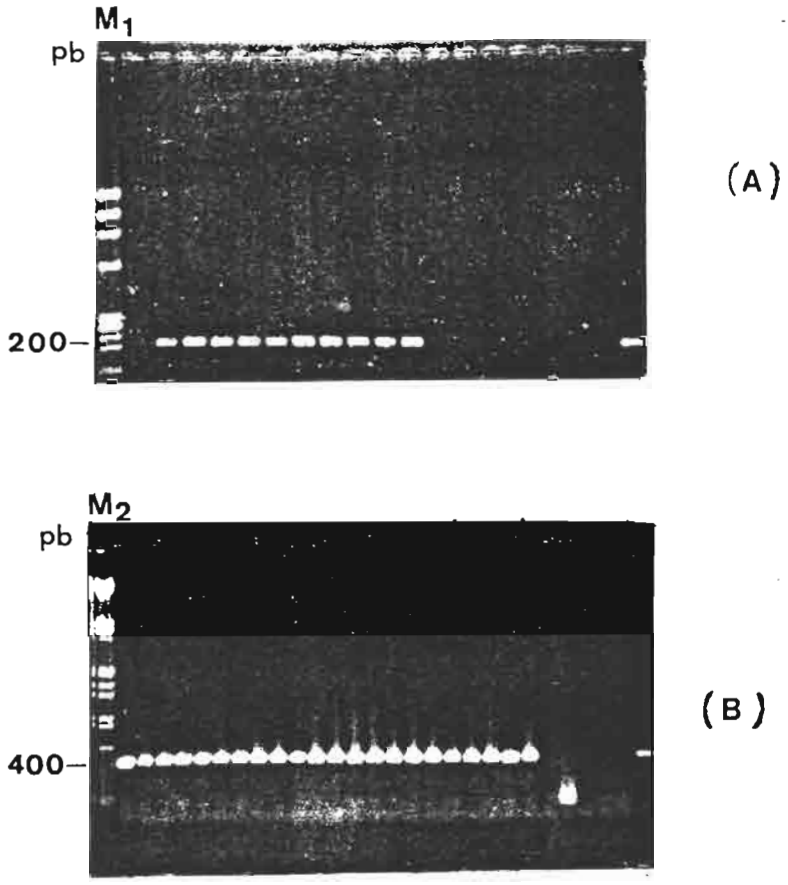

Figure 1: Differentiation of *Fusarium oxysporum* f. sp. *albedinis* by PCR assay: There is an intense band of 200pb with primer pair Bio 3 / FOA1 (A) and 400pb with the second primer pair FOA28 TL3 (B) which were observed only in *F. o.* f. sp. *albedinis* isolates. M1 is a molecular marker, λ DNA digested with *Hind*III / *Eco*RI; M2 is a molecular marker, Φ X 174 / *Hae*III.

Tantaoui A., Ouinten Mohamed, Geiger Jean-Paul, Fernandez Diana (1997)

Use of a PCR-based assay for specific identification of *Fusarium oxysporum* f. sp. *albedinis* causing Bayoud disease of date palm in Morocco

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

Montpellier : Société Française de Phytopathologie, p. 319-322 multigr.

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