Applied **B**iology in **S**aharan **A**reas *Vol. 1, N. 1, p. 13-22, March 2017*
ISSN: 2571- 9823

Test Estimation of Genetic Diversity by Vegetative Compatibility of Fusarium Populations in Relation to Foa (*Fusarium oxysporum* F Sp albedinis) of (Tindouf, Saoura, Gourara, Touati And Tidikelt) Regions

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Abstract – Date palm "bayoud" is a vascular fusariose caused by a soil fungus, *Fusarium oxysporum* F. sp. *albedinis* (FOA) which particularly affects the best varieties of date palms in Algeria and in Morocco. Various techniques of analysis were used in order to consider the isolates genetic diversity of FOA in Algeria. Thanks to the data obtained from survey in the principal areas of Algeria where the disease prevails (Saoura, Ksours of north, Tindouf, Touat, Gourara and Tidikelt) it was possible to collect 39 isolates of FOA starting from various genotypes of palm trees, and 83 saprophytic isolates of soil *Fusarium oxysporum*.

The study of the morphological variation within these stocks reveals the existence of an important morphological variability, three morphotypes were observed; standard cottony, standard downy, and standard mucous short-nap cloth, with predominance of the mucous close-cropped types for pathogenic FOA and downy stocks for the saprophyte isolates. Moreover, the microscopic observations showed the existence of only microconidies, macroconidies and chlamydospores.

The study of the vegetative compatibility of the stocks, by heterocaryosis between the mutants unable to use nitrate, made it possible to determine only one group of vegetative compatibility (GCV) which gathers the stocks of the FOA and three stocks saprophytes *Fusarium oxysporum*. All the isolates of analyzed *FOA* vegetatively proved compatible, and thus belong to only one group (GCV0170). All the results obtained militate in favor of the monoclonal origin of the special *albedinis* form. This result, associated with the historical data of the epidemic, suggests that the populations of Algerian FOA are the result of the dissemination of the same clone, originating in Morocco. The isolates coming from distinct localities have the same genotype; an important genetic diversity classifies the saprophytic isolates into six distinct GCV.

Keywords: Bayoud, *Fusarium oxysporum* F sp *albedinis*, pathogenic, vegetative compatibility, GCV, saprophytes, genetic diversity, variability morphological

1. Introduction

More than a century ago, the date palm has been under the heavy threat of a fungic disease caused by Fusarium oxysporum f sp. albedinis (FOA). More than twelve million palm trees were destroyed in Morocco and in Algeria (Djerbi et al. 1985). The Moroccan clone Mejhoul, of very good quality, almost disappeared and the Algerian clone Deglet-Nour is likely to undergo the same fate if the fungus reaches the zones of intensive cultivation of this clone; namely Tolga and Biskra (Ouiten, 1996).

Various research orientations were developed in order to find solutions to mitigate this plague. The required current objectives are of two types: short-term objectives, including research for resistant clones in the palm plantations devastated by the disease, studies of the date palm genetics and the disease-causing agent; and medium or long term objectives, including selection of resistance and date quality, analyzes relations host-parasite (Ouiten, 1996).

The present work goes within this very general framework. It implements a collection of stocks of F. oxysporum F sp albedinis (FOA) pathogenic and F. oxysporum saprophyte from three areas (Touat, Gourrara and Tidikelt) contaminated by the “bayoud” disease. Our aim is to evaluate the morphological variation of the FOA and Fusarium oxysporum soil isolates, and more largely the genetic diversity existing within the collection based on vegetative compatibility, in order to determine the structures of the populations of this disease-causing agent and soil Fusarium oxysporum.

2. Materials and Methods

2.1 Collection of Fungic strains

The study concentrated on 122 stocks starting from six Algerian areas (Saoura, Ksour of North, Tindouf, Touat, Gourara and Tidikelt) including 39 isolates of FOA isolated starting from various genotypes from palm trees and 83 saprophytic isolates *of Fusarium* soil *oxysporum.*

FOA strains were isolated starting from the spine from palm trees palm by the standardized procedure consisting in sterilizing on the surface of the pieces of faded vascular fabric coming from roots, rachis or stems in ethanol with 50% during one minute, before rinsing them in water and incubating them at 20-25° C on gelosis medium (EPPO, 2003). Each isolate was transferred then preserved on a PDA medium (Djerbi, 1990).

The *Fusarium oxysporum* saprophytic stocks result starts by taking away rhizospheres from the palm palm trees.

2.2 Study of morphological variability

Fusarium oxysporum comprises a great deal of morphologically identical forms, but having very narrow parasitic specificities sometimes. Thus, more than 70 specialized forms were oxysporum described at *F. oxysporum* (Messiaen and Cassini, 1968) among which the *albedinis* form is responsible for the fusariose of the palm trees.

At *Fusarium oxysporum*, the morphology of the thallus is prone to strong variations
(Burgess *et al*, 1989). Variability in mycelial morphology is a common

phenomenon at the specialized forms of *F. oxysporum*. (Burgess *et al*, 1989). The variations relate to farming characters (aspect of the air mycelium, pigmentation of the thallus) (Assigbetse, 1993),

After the purification of fungus on PDA medium, the cloning is carried out by monospore insulation, which allows obtaining a genetically homogeneous material. The morphological variations are observed compared to the farming characters (aspect of the air mycelium, pigmentation of the thallus, the presence of microconidies and chlamydospores, and their characteristics and biometrics.

2.3 Selection, characterization, and confrontations of the Nit mutants

All the Nit mutants were used to evaluate vegetative compatibility among the FOA isolates and saprophytic *Fusarium oxysporum*. All the isolates were cultivated on minimal medium at 22 ° C during 7 days. Following the technique described by Puhalla (1985) the mycelium cuttings of each isolate were transferred in a MMC medium (15 G/L of KClO3, 2g/l NaNO3 and 1,6 G/L of L-asparagine), after incubation at 22 ° C during 8 to 15 days. The mutants presenting thallus of a wild type, on this medium, are regarded as clones are eliminated using nitrate, and the mutants presenting a shaving thallus, without air mycelium, are put in culture on two culture media containing a source of nitrogen, nitrite, or Hypoxanthine.

The isolates present a characteristic growth mean and expansive without air mycelium on the medium MM are considered Nit (mutants). There are three kinds of mutants (Corell et al., 1987): Nit1 (structure gene of nitrate reductase), Nit3 (regulation gene for the nitrate reductase) and Nit M (genes ordering the synthesis of the molybdenum cofactor).
The mutant phenotypes Nit Nit1, Nit3 or NitM are determined by their answer growth on a medium MM where the sodium nitrate was replaced by nitrite of sodium or hypoxanthine.

The aim is to check self-compatibility between Nit1 and NitM mutants of the same stock (Leslie J.F. and Summerall B.A., 2006).

The confrontation of the mutants is made between two different stocks. The crossings Nit1 X NitM were adopted because rate of success is higher than Nit1 X Nit3 or Nit3 X NitM. Anastomosis and the complementation were seen by the formation of dense and abundant air mycelium in the contact zone after 8 to 25 days of incubation.

In this case, the two cross stocks are regarded as pertaining to the same group of compatibility. For the opposite cases (not of complementation connect), the two stocks represent two different groups of vegetative compatibility

The vegetatively compatible stocks are classified in the same group of compatibility (Leslie J.F. and Summerall B.A., 2006).

3. Results and Discussion

3.1 Collection of the fungic strains

After collection of isolates representative of the fusarian populations of the Algerian south-west, several samples of soil were taken by area in order to represent all the epidemiologic situations of the “bayoud” in these areas:

- Old contamination but limited hearths

- Old contamination but generalized hearths
- Recent Contamination but limited hearths
- Generalized Contaminations recent but hearths

The collected samples enabled us to insulate 39 pathogenic stocks resulting from 11 oases, and 17 date palm clones including 1 Khalet (Palm tree exit of spontaneous germination of date core) (**Table 1**) and 83 stocks saprophytic exit 15 oasis starting from rhizosphere of palms trees from 20 cultivars.

Table 1. List of the pathogenic strains isolated from vegetable material infected by Bayoud.

N°	Isolate	Oasis	Date palm clone	N°	Isolate	Oasis	Date palm clone
1	P-IgB10	Igli	Khalet	21	P-Ad10	Adrar/O, Aroussa	Khalet
2	P-IgB11	Igli	Khalet	22	P-Ad11	Adrar/Waina	Tilmessou
3	P-IgB20	Igli	Fegous	23	P-Ad12	Adrar/Waina	Tilmessou
4	P-Igk10	Igli	Fegous	24	P-Ad13	Adrar/Tililene	Tinacor
5	P-Igk12	Igli	Fegous	25	P-Ad14	Adrar/Tililene	Tgaza
6	P-Igk20	Igli	Toumliha	26	P-Ad15	Adrar/Z, Reggani	Tazerzai
7	P-IgM21	Igli	Khalet	27	P-AO10	Aoulef	Tazerzai
8	P-IgM22	Igli	Khalet	28	P-AO11	Aoulef	Degla Baida
9	P-BA10	Béni abbès	Toumliha	29	P-AO12	Aoulef	Tgaza
10	P-BA12	Béni abbès	Toumliha	30	P-AO13	Aoulef	Tilmessou
11	P-BA13	Béni abbès	Toumliha	31	P-AO14	Aoulef	Degla
12	P-BA15	Béni abbès	Toumliha	32	P-AO15	Aoulef	Tgaza
13	P-OT10	L'Ouata	Hemira	33	P-TM10	Timimoun	Tinacor
14	P-OT11	L'Ouata	Hemira	34	P-TM11	Timimoun	Hemira
15	P-OT20	L'Ouata	Kadoussa	35	P-TM12	Timimoun	Adem tinhoul
16	P-OT21	L'Ouata	Kadoussa	36	P-TM13	Timimoun	Rema
17	P-Kr10	Kerzaz	Khalet	37	P-TM14	Timimoun	Sheik
18	P-OKh1	Ouled Khodeir	Aghamou	38	P-TM15	Timimoun	Tinekour
19	P-OKh2	Ouled Khodeir	Hemira	39	P-TM16	Timimoun	Tinacor
20	P-OKh3	Ouled Khodeir	Hartane				

3.2 Study of morphological variability

The study of the macroscopic characters of the saprophytic and pathogenic isolates was done by observation of the morphotypes colonies. Generally the isolates of FOA and saprophytic *F. oxysporum* are characterized by the following morphotypes: downy, close-cropped mucous, cottony, close-cropped senescent. The results obtained show that the morphotypes vary from one stock to another and can advance from one type to another. (**Figure 1** and **2**).

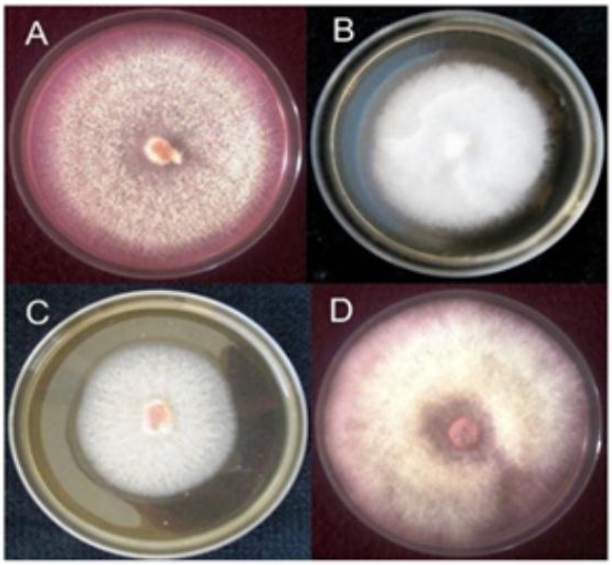

Figure 1. Morphological types of *Fusarium oxysporum* thalli (saprophytic):
Senescent Close-cropped Morphotype (A), Short-nap cloth mucous (B), Downy (C), Cottony (D).

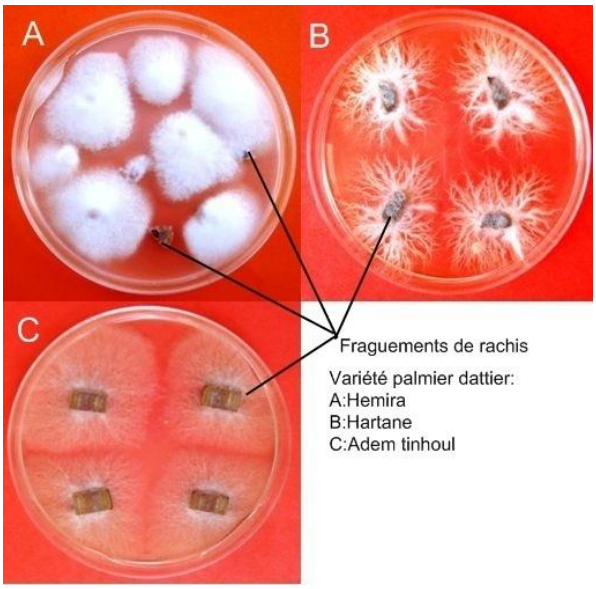

Figure 2. Morphological types of thalli of *Fusarium oxysporum* F sp *albedinis* (Pathogenic):
Cottony Morphotype (A), mucous Short-nap cloth buckled (B), Downy (C).

We noted that the relative frequencies of each morphotype for the pathogenic and saprophytic stocks are different (**Figure 3** and **4**). The mucous close-cropped morphotype is dominating (54%) for the pathogenic stocks, whereas the majority of the saprophytic stocks are of downy type (53%).

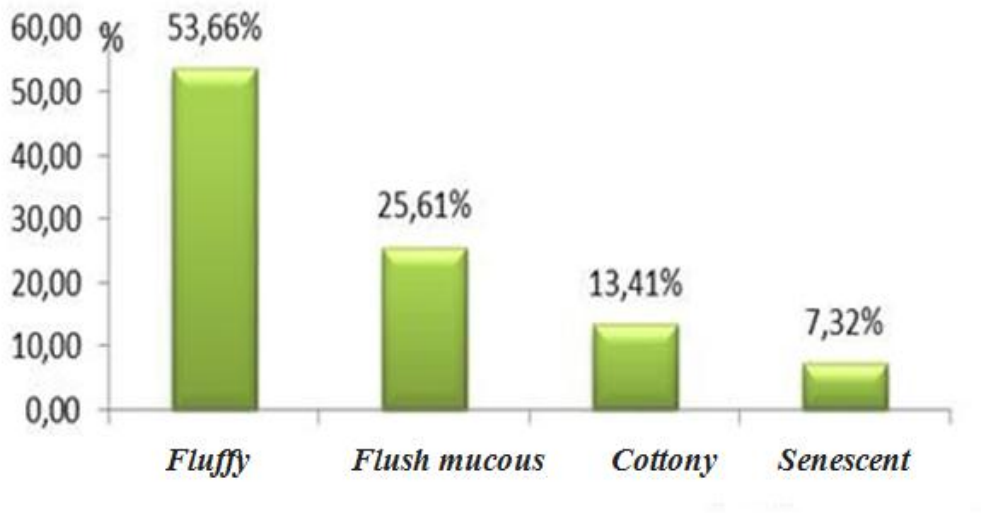

Figure 3. Relative frequencies of morphotypes observed in saprophytic *Fusarium* strains.

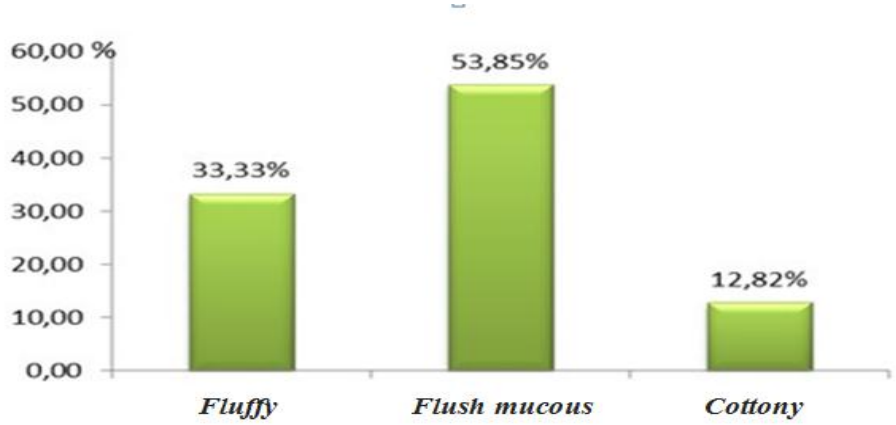

Figure 4. Relative frequencies of morphotypes observed in strains pathogen FAO.

3.3 Selection, characterization of the Nit mutants, and vegetative compatibility grouping

The results obtained show that among the initial batch of the 83 saprophytic stocks of soil *Fusarium oxysporum*, only 31 stocks were classified not user of chlorates on medium MMC. The characterization is done by the use of the mediums nitrates, nitrite and Hypoxanthine, we determined the type of each mutant not nitrate users, according to their frequencies of appearance. All the mutants are classified in **(Figure 5)**:

- The mutants Nit 1=74.53%
- The mutants Nit M=14.78%
- The mutants Nit 3=10.69%

For FOA pathogenic stocks, among the 39 isolates only 21 stocks were classified not user of chlorates on medium MMC, the other mutants are proven as follows (**Table 2**):

- The mutants Nit 1=62.29%
- The mutants Nit M=22.86%
- The mutants Nit 3=14.86%

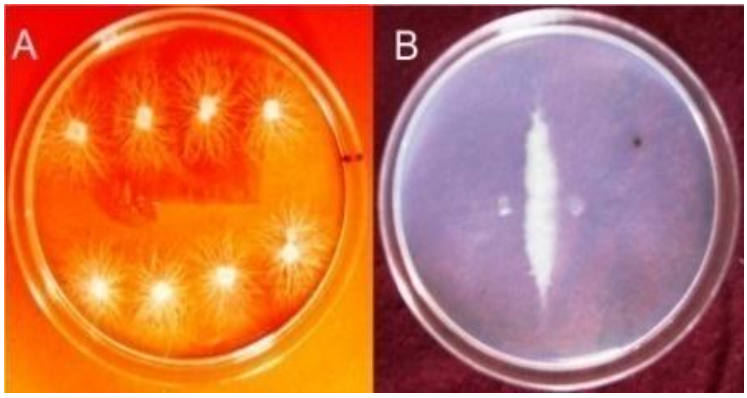

Figure 5. Morphology (A) of the thallus of the resistant chlorate mutant on MMC medium, and (B) Reaction of positive complementation between mutant FOA isolates

Table 2. Type and number of mutants characterized on medium MMC starting from *Fusarium oxysporum* F sp *albedinis* strains.

Isolate	Nit 1	Nit M	Nit 3	Total
P-Igk10	6	3*	1	10
P-Igk20	5	0	1	6
P-IgM21	1	0	3	4
P-BA12	4	1*	4	9
P-BA13	7	0	0	7
P-BA15	8	2*	1	11
P-OT11	9	3*	0	12
P-OKh2	5	4*	0	9
P-OKh3	1	0	1	2
P-Ad10	4	0	0	4
P-Ad11	6	1*	2	9
P-Ad12	6	1*	2	9
P-Ad13	5	4*	3	12
P-Ad14	7	3*	1	11
P-Ad15	4	5*	0	9
P-AO11	3	0	0	3
P-AO12	1	4*	1	6
P-TM10	3	3*	2	8
P-TM12	7	0	0	7
P-TM13	4	0	0	4
P-TM14	5	4*	3	12
P-TM15	8	2*	1	11
Total	109	40	26	175
%	Nit 1=62,29%	Nit M=22,86%	Nit 3=14,86%	100%

We used the vegetative compatibility technique to compare FOA isolates, the special form (albedinis) with the saprophytic isolates of *Fusarium oxysporum* isolated starting from the soils from palm plantations infected by Bayoud, of which we ignore pathogenic capacity. A collection of stock consists of (19 isolates of saprophytic stocks of *Fusarium oxysporum* representing the 6 studied areas and 4 isolates of mutants Nit M (Tester) of FOA accounting for 4 of the 6 studied areas (**Table 3**).

Once the self-compatibility checked for all the isolates, we carried out confrontation according to any possible combination; all the results allowed us to highlight 6 GCV of the (saprophytic) soil isolates. The isolates of each group are vegetatively compatible with one another, and not with the isolates of the other groups. We noted that 3 isolates of soil (Mas3, AO31 and Okh11) vegetatively complemented with the isolates of special form FOA being thus assigned to the same group GCV of the FOA **(Figure 5)**.

The GCV 1 is represented by two isolates of the same locality (Beni-ounif), the GCV 2 is represented by 2 isolates of (Tindouf and Gourrara), the GCV 5 is represented by 2 isolates of the same locality (Touat), the GCV 3 and 6 are represented respectively by 4 isolates, the first of Saoura and the second of (Touat and Tidikelt).

As a conclusion of the results obtained, all the isolates of pathogenic FOA belong to the group of vegetative compatibility GCV 0170, the same group to which all the identified FOA belong (Tantaoui et al., 1996). The saprophytic isolates of Fusarium oxysporum are classified in 6 distinct GCV, whereas 3 saprophytic isolates of *Fusarium oxysporum* are compatible with the isolates of FOA. One notes a strong genetic diversity at the isolates of *Fusarium oxysporum* of the soil.

Table 3. Confrontations of the FOA with the saprophytic strains of Fusarium of oasis soil infested by Bayoud.

Regions	Isolat	BO 11	BO 12	T17	IgM3	Igk3	BA10	OT22	Kr3	OKh11	Ad32	Ad46	Reg32	AO11	AO17	AO19	AO31	Kal10	Mas3	AB10	P-BA15	P-Ad12	P-AO12	P-TM15
K N	BO 11	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	BO 12		+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Tindouf	T17			+	-	-	-	-	-	-	-	-	-	-	-	-	-	FR	+	-	-	-	-	-
Saoura	IgM3				+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	IgK3					+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	BA10						+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	OT22							+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Kr3								+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	OKh11									+	-	-	-	-	-	-	-	-	-	-	+	+	+	+
Touat	Ad32										+	+	-	-	-	-	-	-	-	-				-
	Ad46											+	-	-	-	-	-	FR	-	-	-	-	-	-
	Reg32												+	+	+	+	-	-	-	-	-	-	-	-
Tidikelt	AO11													+	+	+	-	-	-	-	-	-	-	-
	AO17														+	+	-	-	-	-	-	-	-	-
	AO19															+	-	-	-	-	-	-	-	-
	AO31																+	-	-	-	+	+	FR	+
Gourrara	Kal10																	+	-	-	-	-	-	-
	Mas3																		+	-	+	+	+	+
	AB10																			FR	-	-	-	-
*Saoura	P-BA15																				+	+	+	+
*Touat	P-Ad12																					+	+	+
*Tidikelt	P-AO12																						+	+
*Gourrara	P-TM15																							+

4. Conclusion

Our results of compatibility studies have identified a single vegetative compatibility group for isolates from FOA revealing genetic identity; related genes that control vegetative compatibility. The study identified six VCGs saprophytic strains of Fusarium oxysporum of soil, and a high genetic diversity. Studies in other regions have shown a greater number of GCV (Ouinten, 1996) and (Benlarbi, 2009).

The technique of vegetative compatibility remains a simple and effective method for differentiating Fusarium oxysporum strains and their grouping into different genetic entities (special forms). The coupling of this technique with molecular (RADP, RFLP, PCR, ...) permits the determination of other factors such as geographic origin, pathogenicity, infectivity, ...

Finally, the characterization of the genetic structure of FOA populations must be

accompanied by a study of the genetic diversity of palm trees. The comparison of population structure of the FOA and the host are needed to better understand the dynamics of their interactions and to explore effective control strategies.

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