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VARIATION IN PATHOGENICITY OF *FUSARIUM OXYSPORUM* F. SP. *ALBEDINIS* ON TWO CULTURES ASSOCIATED WITH DATE PALM OF MOROCCAN OASIS

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ABSTRACT

Surveys, conducted in the palm groves of Tafilalet and Zagora, allowed to isolate a large number of *F. oxysporum* f sp. *albedinis* isolates from the roots and wilted palms. In the bibliography, this pathogen is specifically subservient to the date palm, but cannot induce disease to associated species. In this study, the variability of the pathogenicity of the fungus was tested on two unusual hosts, tomatoe and eggplant. In tomato plants, the FA5 isolate of *F. oxysporum* f. sp. *albedinis* has led symptoms such as yellowing followed by necrosis, dryings out and leaves falling. Sometimes, yellowing is followed by blanching. By cons, these symptoms were not observed in inoculated eggplants. Dwarfing indices (DI) and leaf alteration indices (LAI) identified in the inoculated tomato plants are greater, respectively 80% and 0.812 compared to those of eggplant, respectively 56% and 0.689. The root and vegetative masses, the number of flowers, leaf number, number of fruits, are very altered.

The fungus was reisolated from all parts of the inoculated plants (root, hypocotyl and epicotyl). The variability of parasitic specificity of *F. oxysporum* f sp. *albedinis* was discussed in this study.

KEYWORDS: *Fusarium oxysporum f sp. albedinis*, date palm, tomato, eggplant, variation, pathogenicity.

INTRODUCTION

Many species of vascular plants are sensitive to fungi of the genus *Fusarium*, vascular disease agents (Nelson *et al*., 1981). Among the representatives of this kind, *Fusarium* oxysporum (SCH) SN. is the most common parasitic species and the most important cultivated soils fungal microflora (Messaaen and Cassini, 1968).

The study of pathogenicity within the species *Fusarium oxysporum*, has led to the definition of specialized forms theoretically subservient, each to a single host plant; more than 70 specialized forms have been described (Messiaen and Cassini, 1981). These forms are morphologically identical but having sometimes very narrow parasitic specificities (Assigbetse, 1989). *Fusarium. oxysporum* f. sp. *cucumerinum*, for example, infects cucumber and melon; by cons, *F. oxysporum* f. sp. *Ciseri*, *F. oxysporum* f sp. *vasinfectum* and *F. oxysporum* f sp. *albedinis* attack only one species of host plants, respectively banana, cotton and date palm (Fourie *et al*., 2011).

The date palm is the main host of *Fusarium oxysporum* f. sp. *albedinis* (Benzaza *et al*, 1970. Brochard and Dubost, 1970a; 1970b; Fernandez *et al*., 1995). Bayoud disease, is widespread in almost all the Moroccan palm groves and the western and central oasis of Algeria (Benzaza *et al*., 1970. Brochard and Dubost, 1970a; 1970b). It affects different stages of growth of the date palm, attacking mature palms, younger and even rejections. All high quality cultivars of date palm, Majhool, Deglet Nour, Jihel, Boufeggous, etc…, are susceptible to this disease (Pereau-Leroy, 1954; Toutain and Louvet, 1974; Saaidi 1997). The roots of some plants (henna, alfalfa, barley) grown in association with date trees may harbor the fungus without showing any external symptoms (Djerbi *et al*., 1985). These plants are considered healthy carriers but can transmit the disease to susceptible palms (Djerbi *et al*., 1986).

Variability of pathogenicity has been relatively little studied in the species *F. oxysporum* (Henni *et al*., 1994). Bouhot (1981) obtained changes in *F. oxysporum* f. sp. *melonis* races by artificial mutagenesis with nitrosoguanidine. Thus, it was obtained from race 0, mutants belonging to races 1 and 2; similarly, the race 1 provided mutants of races 0, 2 and 1, 2. Follin and Laville (1966) found a wide variability of aggression in *F. oxysporum* f. sp. *cubense* clone progeny; pathogenicity may decrease, then increase and regain its original level. Henni *et al*. (1994) conducted tests in order to verify the stability of pathogenicity in inter and intraclonales progenies of two *Fusarium oxysporum* f. sp. *lycopersici* isolates. These authors did not record any change of race; however, aggressiveness variations were observed.

So, is it logical, not to wonder about the pathogenicity of other special forms of *Fusarium oxysporum*, case of *albedinis* form. This form was isolated for the first time in Morocco by Malençon (1934, 1936) and Mayor (1935), since this time, its pathogenicity has never been tested against plants grown in association with the date palm. All authors agree that these species are healthy carriers of *F. oxysporum* f. sp. *albedinis*, but participate in transmitting the disease to the date palm, the primary host.

The combination of all this information has led us to wonder if the parasitic and pathogenic ability of *Fusarium oxysporum* f. sp. *albedinis* has never varied, depending on the cropping system known at the oasis. The absence of notable attack of this special form against the plant species grown in association with the date palm suggests that their genetic immune system cannot be affected by the parasite.

But it has been observed in other plant species (Douira and Lahlou, 1989) that a line of *Verticillium dahliae* native of tomato, after continuous confrontation with chili, unusual host, can give birth to clones of very different aggressiveness levels.

The objective of this study is to show the variability of *F. oxysporum* specialized forms pathogenicity, particularly *F. oxysporum* f. sp. *albedinis* isolated from the roots of a palm tree native of Zagora (south of Morocco) and affected by Fusarium wilt, on two unusual host plants, tomatoes and eggplant.

MATERIAL AND METHODS

Pathogenic agent

The isolate FA5 of *Fusarium oxysporum* f. sp. *albedinis* was obtained from a palm tree affected by Fusarium wilt in Zagora region (Morocco),. It is characterized by hyaline and compartmentalized mycelium and 3 types of spores: microconidia, macroconidia and chlamydospores. Microconidia are unicellular, hyaline spherical or elongated and slightly curved. Macroconidia have 3-5 bulkheads, fusoides to falciforms, pointed at both ends. Chlamydospores are intercalary or terminal, spherical, isolated or in chains combining 2-4 chlamydospores (Fig.1). The isolate FA5 is very aggressive against date palm seedlings of varieties Majhool and Boufegouss (Sghir *et al*., 2015).

F. oxysporum f. sp. *albedinis* cultures are maintained on a potato sucrose agar medium (PSA: 200 g potatoes, 20 g sucrose; Agar-agar 15 g, 1000 ml distilled water) and incubated at 28°C for seven days in the dark. The culture surface is then washed with sterile distilled water and the concentration of conidia suspension was adjusted to 10^5 conidia ml^{-1}.

Host plants

Tomato seeds variety 'Rio Grandy' and eggplant variety 'Black Beauty' are disinfected using sodium hypochlorite at 5%, washed several times with distilled water, then cultivated in alveoli containing peat. The cells were placed in plastic greenhouse at a temperature of 18 to 25°C and watered regularly until the stage of two true leaves.

Inoculation

Young tomato plant and eggplant having reached the stage of two true leaves are dug up; their roots were washed with tap water and then soaked for 30 min in the conidial suspension. Control plants were soaked in water at the same time. The inoculated and controls seedlings are then transplanted into pots containing disinfected Mamora forest soil. Soil sterilization is carried out in an oven at 250°C for 2 hours. Plants were watered on alternate days.

RESULT'S NOTATION

Plant growth: The reduction in size of inoculated plants compared to controls is measured by the dwarfing index (DI) Calculated as follows:

D.I. = (M-X).100 / M

X: Stem height of the inoculated plants.

M: Average size of the control plants for each substrate.

Other parameters were assessed: height and diameter of the plants, leaves number and vegetative and root biomass.

Leaves Alteration

a score is assigned to every leaf using the following scale (Douira and Lahlou, 1989): healthy-looking sheet (0); cotyledonary leaf: wilting or yellowing (1), falls (2); true leaf: wilting or yellowing (3), necrosis (3), fall (5).

The sum of scores related to the number of leaves constitutes the leaf alteration index. An average index is then calculated for each lot of plants.

$L.A.I. = [\sum(i \times x_i)] / (6 \times NtL)$

LAI: Leaf alteration index.

i: Leaves appearance notes 0-4.

x_i: Number of leaves with the note i.

NtF: Total number of leaves.

Measurement of vegetative and root masses: Vegetative and root biomass of control and inoculated plants were weighed by a precision balance.

Presence of *F. oxysporum* f. sp. *albedinis* in inoculated plants: The presence of fungus is searched 30 days after inoculation. Thin sections of roots and stems are placed in alcohol at 90° for 2 minutes, rinsed several times with sterile water, dried quickly on sterile filter paper and then subcultured on the Agar medium (20 g Agar-agar per liter 'distilled water). Observations are made after one week.

The percentage of colonization of different parts of inoculated plants is calculated using the following equation:

$$\%\ Ci = \frac{Ni}{Nt} x100$$

% Ci: Colonization percentage.

Ni: Number of plants having hosted the pathogen in section i.

Nt: Number of the used plants.

Statistical analyzes

Statistical analyzes were performed by analysis of variance with one classification criterion anova1 and LSD test (least significant difference).

RESULTS

Three months after inoculation, the most visible manifestations of the *F. oxysporum* f. sp. *albedinis* isolate on the leaves of tomato plants are yellowing followed by necrosis, dryings and leaves falling. Sometimes, yellowing is followed by blanching. In this case, drying out settles after, but always begins at the periphery of whitish areas and then gains the center. The leaves wilting sometimes appears independently of other symptoms (Fig.2). Contrary to tomato plants, foliar symptoms characterizing the disease caused by *Fusarium oxysporum* f. sp. *albedinis*; case of wilting; were not observed in eggplant seedlings inoculated with FA5 isolate, By cons, the drying out and leaf falls were observed in the leaves of inoculated plants of eggplant (Fig.3).

The pathogenicity expressed by the isolate of *F. oxysporum* f. sp. *albedinis* is very important under inoculated tomato and eggplant, judging by the dwarfing index, leaf damage and the disruption of flower formation. Thus, the dwarfing index and leaf alterations index of inoculated tomato and eggplant are 80 and 60% respectively and 0.81 and 0.48. It seems that *F. oxysporum* f. sp. *albedinis* isolate tested did not disturb the leaves fomation of the inoculated tomato plants. By cons, it influences this formation in eggplants (6.1 / 8.2). The number of formed flowers (1.3 / 3), the vegetative mass (10 g / 16.5 g) and root mass (9.1 / 10 g) are altered in tomato plants inoculated compared to those observed in control plants.

For the eggplants, the number of flowers formed does not exhibit any significant difference. But the root and vegetative masses of inoculated plants are very disturbed by *F. oxysporum* f. sp. *albedinis* isolate tested, respectively 8.4 / 12.7 and 16.6 g / 26.1 g (Table1).

The *F. oxysporum* f. sp. *albedinis* isolate studied was detected even in the epicotyls of tomato and eggplant plants, 30 days after inoculation. It is present in roots and hypocotyls of all inoculated plants (100%) and was reisolated from the epicotyl 80% of tomato plants and 70% of the eggplants. It is present in roots and hypocotyls of all inoculated plants (100%) and was reisolated from the epicotyls at 80% of tomato plants and 70% of the eggplants (Table 2), (Fig.4). In the literature, the fungus remains always confined in the roots of unusual species, but never reached the higher levels of the stems. It appears from these results that the *F. oxysporum* f. sp. *albedinis* isolate native of the date palm, is able to infect tomato and eggplant, two unusual hosts to the upper level and affect growth, foliage, flowers and development of the root and vegetative masses.

Figure 4. Culture of *Fusarium oxysporum* f.sp. *albedinis* on PDA.

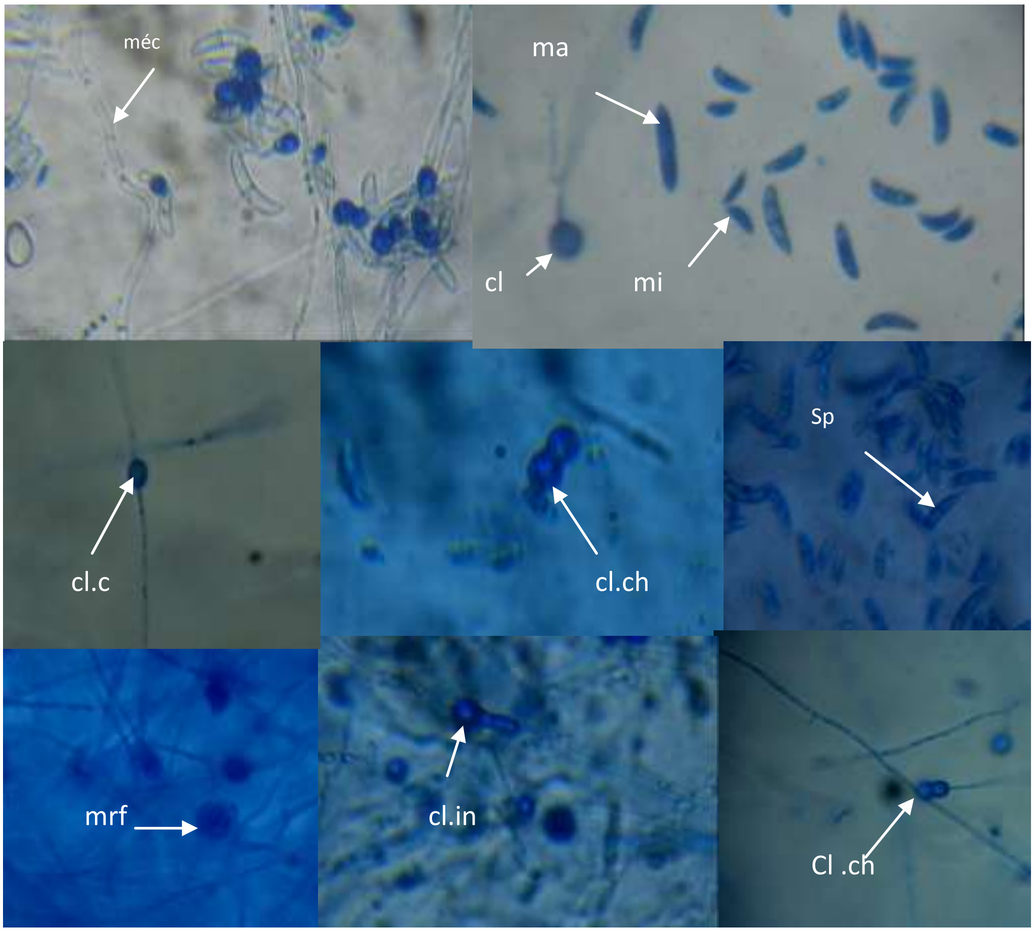

Figure 1: Microscopic characteristics of *Fusaruim oxysporum f. sp. albedinis*: mi microconidia; ma: macroconidia; cl: clamydospores; méc: compartmentalized mycelium; Sp: spore, Mrf: microphialides; Cl.ch: clamydospores in chain; cl.c: Short clamydospores, cl.in: clamydospore interlayer.

Table 1: Effect of *Fusarium oxysporum* f. sp. *albedinis* on tomato and eggplant plants.

	Tomato	Eggplant
DI	80a	60a
LAI	0 ,812d	0 .489e
Leave's number	3.2c	6.1c
Flower's number	1.3d	2.2d
A.F.W. (g)	10b	16.6b
R.F.W. (g)	9.1b	8.4c

DI: dwarfing index; **LAI**: Leaf alteration index. **P.F.A**: Aerial fresh weight; **R.F.W.**: Root fresh weight.

The averages of the same column affected with the same letter are not significantly different from 5% threshold.

Table 2. Percentage of re- isolation of *Fusarium oxysporum* f. sp. *albedinis* from different parts of tomato and eggplant, three months after inoculation.

	Tomato	Eggplant
Root	100%a	100%a
hypocotyl	100%a	100%a
Epicotyl	80%b	70 %b

The averages of the same column affected with the same letter are not significantly different from 5% threshold.

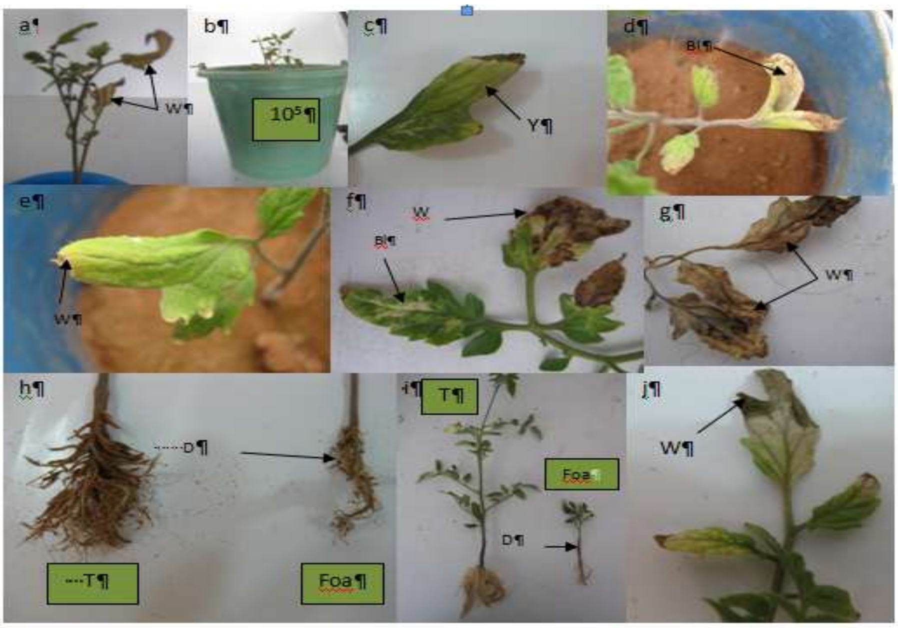

Figure 2. Symptoms induced by *Fusarium oxysporum f. sp. albedinis* on tomato.
Various symptoms observed on leaves (a, b, c, d, e, f, g, j) roots (h), root and aerial part (i). W: wilting; D: dwarfing; Y: yellowing; Bl: blanching of the leaves.

Figure 3: Effect of *Fusarium oxysporum* f. sp. *albedinis* on the development of aerial and root part of the eggplant. D: root system reduction

DISCUSSION AND CONCLUSION

The variation in pathogenicity in *F. oxysporum* f. sp. *albedinis* is little studied in the literature. Louvet and Mercier (1973) and Sedra and Djerbi (1986) proposed to study the stability of the strains aggressiveness of this parasite after their passages on secondary hosts as the canary palm. Others (Bullit *et al.*, 1967; Djerbi *et al*., 1985b) proposed the passage of these strains on healthy carriers like henna and alfalfa. All these authors, according to Sedra (1993) have not responded to their proposals.

Djerbi *et al*. (1985b) and Sedra and Djerbi (1986), reported that it is apparently easy to identify *F. oxysporum* f. sp. *albedinis* by morphological characteristics. However, according Tantawi and Drink (1991), large variations are often observed in the aggressiveness of the "typical" isolates specialized *albedinis* form derived from firstly infected palms, and from *F. oxysporum* isolates originating from soil or other healthy carriers. The first ones are usually very aggressive, but the seconds are not aggressive or pathogenic. According to Tantawi and Boisson (1991), it must then consider the seconds, as *F. oxysporum* f. sp. *albedinis* of low aggressiveness or as saprophytes without any apparent link with the specialized form responsible of bayoud. Djerbi and Ben Brader (1990) and Tantawi and Drink (1991) have shown, also, different vegetative compatibility group in *F. oxysporum* f. sp. *albedinis* without demonstrating the existence of different virulence of the parasite.

Sedra (1993) studied the effect of certain factors on the aggressiveness stability of dozens *F. oxysporum* f. sp. *albedinis* isolates, taken from palms affected by Bayoud disease and from different origins: successive subcultures of the fungus on artificial media (4 subcultures), change in colony morphology and relationship between morphology and power. The results showed that all these factors generally, did not alter the aggressiveness on date palm seedlings.

All these authors have never studied the pathogenicity of *F. oxysporum* f. sp. *albedinis* isolated from palm, through cruciate inoculations on unusual host species grown in association with the date palm. Djerbi *et al*. (1986a) reported that these plants can harbor and multiply *F. oxysporum* f. sp. *albedinis*, without showing any external symptoms, and transmit the disease to susceptible palms (Djerbi *et al*., 1986b). Many points remain to be clarified concerning this finding. Is that fungus which is capable of multiplying on unusual hosts, never induces symptoms in these plants? Will *F. oxysporum* f. sp. *albedinis* continued always, over time, to multiply and induce date palm disease? Is that unusual host species will always be considered as a living support for multiplication of this special form? Are the populations of conidia formed on the usual host, the date palm, and on unusual host species, called healthy carriers will remain stable over time and continued to infect only their preferential host? Are these formed populations are not homogenous and can develop with time variable parasitic and pathogenic aptitudes depending on the host they will encounter.

The FA5 isolate of *Fusarium oxysporum* f. sp. *albedinis* isolated from the roots of date palm affected by Fusarium wilt, selected among others, able to attack tomato and eggplant, presented a large amplitude variations that affected his parasitic ability and pathogenicity towards tomato and eggplant, unusual hosts of this special form. Parasitic skills concerned the penetration of the fungus on the roots of inoculated plants and its progression in the conducting vessels to higher levels. Pathogenicity was demonstrated by the ability of the fungus to induce different types of symptoms in inoculated plants: stunting, leaf alteration, formation of leaves and flowers and reduction of vegetative and root mass.

This study allowed us to note the possibility of the presence of *F. oxysporum* f. sp. *albedinis* able to attack the date palm and species grown in association. Further studies will be extended to other crops such as tomato, eggplant and to other isolates of *F. oxysporum* f. sp. *albedinis*. Cruciate inoculations will be used to check whether the isolates of *F. oxysporum* f. sp. *albedinis* isolated from palm will be pathogenic on other plant species. Similarly, isolates collected from unusual species called healthy carriers, will be inoculated to seedlings of different date palm varieties. In this study, we compared only one isolate originated from date palm to two plant species, tomato and eggplant, often found in oasis with date palms. These studies may contribute to a better understanding of the amplitude of the *F. oxysporum* f. sp. *albedinis* change in the aggressiveness, and its ecology, necessary to complete the evaluation of varietal resistance.

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