



## First report of *Fusarium redolens* causing root rot disease of wheat and barley in Morocco

**Qostal S<sup>1</sup>, Kribel S<sup>1</sup>, Chliyeh M<sup>1</sup>, Mouden N<sup>1</sup>, El Alaoui MA<sup>1</sup>, Serghat S<sup>2</sup>, Ouazzani Touhami A<sup>1</sup> and Douira A<sup>1</sup>**

<sup>1</sup>Laboratoire des Productions Végétales, Animales et Agro-Industrie, Equipe de Botanique, Biotechnologie et Protection des Plantes, Département de Biologie, Faculté des Sciences, Université Ibn Tofail, Kénitra (Morocco)

<sup>2</sup>Faculté des Sciences Ben M'Sik, Université Hassan II, Casablanca (Morocco)

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### Abstract

Field surveys carried out in wheat (durum and soft) and barley fields in April-May 2017 in North-West Morocco resulted in the isolation of a large number of fungal isolates from typical necrotic lesions characterizing the root rot of these cereals. Microscopic observations, based on morphological criteria linked an isolate of *Fusarium oxysporum* with an isolate of *F. solani*. Another *Fusarium* isolate ZF25, showed intermediate morphological characteristics between *F. solani* and *F. oxysporum*. Molecular comparisons showed the ZF25 and *F. redolens* to have a similarity of 99%. The sequence from this isolate was submitted to GenBank (accession number MT758201). Koch's postulate was verified by inoculating these three isolates into varieties of wheat (hard and soft) and barley. All isolates induced necrotic lesions on the roots. Pathogen re-isolations from these lesions were positive. The percentages of the highest severity class S4 can reach 41.6% for wheat and 16.6% for barley. The percentages of incidences and indices of root rot observed in the wheat and barley plants inoculated with the three *Fusarium* isolates vary between 83.6 and 100% and between 58.3 and 70% respectively. These inoculation tests have shown that *F. redolens* has a strong pathogenicity towards wheat and barley. This species has not been reported in Morocco among the fungi associated with roots of barley and wheat and to our knowledge; this is the first report of this pathogen among the *Fusarium* complex responsible for root rot of these cereals.

**Keywords** – Barley – *Fusarium* – Koch's postulate – Lesions – Roots – Wheat

### Introduction

The species of the *Fusarium* and *Bipolaris sorokiniana*, constitute the fungal complex responsible for the disease of root rot (Dyer et al. 2011). These fungal species, the most studied on a global scale (Cook 1980), are known to attack durum wheat (*Triticum durum*), soft wheat (*Triticum aestivum*), barley (*Hordeum vulgare*), triticales (x *Triticosecale Wittmack*) and oats (*Avena sativa*) (Backhouse et al. 2004). *F. graminearum*, *F. nivale*, *F. oxysporum*, *F. poae*, *F. roseum*, *F. solani*, *Fusarium* sp. which were associated with root rot disease affecting wheat and barley cultivations.

Out of all isolates representatives of the *Fusarium*, collected from necrotic lesions of roots of wheat and barley from fields in northwest Morocco, two isolates have been identified, based on

morphological characteristics, as *Fusarium oxysporum* and *F. solani* (Qostal et al. 2019). These latters show intermediate characters with those of other isolates representing the same genus. Thus, aside from pathogenic characteristic study of each isolate, accurate identification using molecular methods are required. In the present work, molecular tools were used to identify isolates of *Fusarium* sp. and their pathogenicity towards wheat and barley has been studied.

## Materials & Methods

### Fungal material

Three isolates of *Fusarium*, isolated from the necrotic lesions of the roots of wheat and barley plants growing in different areas of northwestern Morocco are stored on small slices of filter paper in filter paper bags in the freezer at -20°C (Table 1).

The *Fusarium* isolates were cultured in Petri dishes 90 mm in diameter on PSA medium (Potato Sucrose Agar: 200 g potato, 20 g sucrose, 15 g Agar-agar, 1000 ml distilled water). This agar medium was previously sterilized by autoclaving at 120°C for 30 min. When it cools down and reaches a temperature of about 50° C, the medium was poured into Petri dishes at a rate of 30 to 40 ml per dish added with 100 mg/L of chloramphenicol. Cultures of isolates were incubated in the dark at 28°C and their macroscopic and microscopic characteristics were followed according to the age of the cultures.

The macroscopic examination of the cultures of the fungal species was carried out according to the development of the cultures on PSA medium, and observations were made on the appearance of the cultures, the density of the mycelium, the color, the growth and the production of the spores. The microscopic examination of the cultures focused on the observation under an optical microscope ( $\times 40$ ,  $\times 100$ ,  $\times 400$ ,  $\times 1000$ ) of the nature of the mycelium, the appearance of the conidiophore, shape and size of the conidia, presence of conservation organs, case of chlamydospores. The mounting fluid used for microscopic observations is tap water or cotton blue. The determination of the fungal species of *F. solani*, *F. oxysporum* and ZF25 isolate to *F. redolens* was performed using the Tivoli guide (Tivoli 1988), the manual by Nelson et al. (1983), Gilman's determination keys (Gilman 1957), Messiaen (1959), Ellis (1971), Chidambaram et al. (1974), Booth (1977), Domsch et al. (1980), Nelson et al. (1983), Champion (1997).

**Table 1** Isolates of *Fusarium* sp. and their origins

<i>Fusarium</i> isolate	Sources of isolation	Locality (Country)
<i>Fusarium oxysporum</i> (F1)	durum wheat	Souk Tlat/Morocco
<i>Fusarium solani</i> (F2)	Barley	Ouled Selam/Morocco
<i>Fusarium</i> sp. (ZF25)	Barley	Sidi Allal Tazi/Morocco

### DNA extraction

Young cultures of *Fusarium* sp. (isolate ZF25), 5 days old, were used for molecular analyzes. Fungal genomic DNA was isolated using ISOLATE II Plant DNA kit (Bioline, London, UK, P/N: BIO-52070) referring to the manufacturer's instructions. DNA purity was evaluated using the NanoDrop 8000 spectrophotometer (Thermo Fisher Scientific, USA).

The ITS (Internal Transcript Spacer) region of ribosomal DNA, defined by (White et al. 1990, Gardes & Bruns 1993) was amplified with the universal primers ITS1 and ITS4 (White et al. 1990). This region is non-coding and highly polymorphic.

The reaction volume for each sample is 25 µL, containing 5 µL of 5X buffer (reagents: MyTaq DNA polymerase Bioline kit), 1 µL of dNTP (20 mM), 1 µL of each of the primers (10 µM), 0.2 µL of Taq polymerase (5 U µL-1) and 150 ng of template DNA and ultra-pure water (MiliQ) completing the volume. Negative controls were launched to test for the presence of possible contamination in the reagents and buffers.

The amplifications are carried out with a Veriti thermal cycler (Applied Biosystems). according to the following parameters: A cycle of 1 min at 95°C (initial denaturation), followed by

35 cycles of 15 seconds at 95°C (denaturation), 20 seconds at 57°C (hybridization) and 15 seconds at 72°C (polymerization)], then a last cycle 3 minutes at 72°C (final elongation).

The PCR products were checked by migration on a 1% agarose gel by depositing in each well 10 µl of each PCR product mixed with 0.5 µl of loading buffer (Sigma Aldrich). Migration takes place in 1X TAE and lasts 45 min at 120 V/100 mA and at room temperature. Profiles were visualized under UV light at 254 nm using the G: BOX gel documentation system (Syngene).

The PCR products were enzymatically cleaned using ExoSAP-IT reagent (Affymetrix). Sequencing reactions were performed using BigDye Terminator v3.1 Ready Reaction Cycle Sequencing Kit (P/N: 4337455) with an ABI PRISM 3130XL Genetic Analyzer (Applied Biosystems) using the POP-7 polymer (P/N: 4393708). Data were analyzed by sequencing Analysis Software version 5.3.1 (Applied Biosystems, P/N: 4360967).

### **Submission of sequences to GenBank**

The sequence resulting from the sequencing of the ITS region of ribosomal DNA has been integrated into the GenBank database on the NCBI platform with the code MT758201. Alignment of the sequence generated in this study with other sequences removed from the GenBank database was performed with MEGA 6 software.

### **Plant material**

The apparently healthy grains of durum wheat (Variety: Amjad), soft wheat (Variety: Amal) and barley (Osama) were superficially disinfected by soaking for 2 min in a solution of sodium hypochlorite at 10% followed by six rinses with sterile distilled water. The grains thus disinfected were left to dry on sterile filter paper for 24 hours.

### **Preparation of the inoculum**

The *Fusarium* isolates were subcultured in Petri dishes on PSA medium. After 15 days of incubation, in the dark and at 28°C, the spore-laden surface is scraped aseptically with a metal spatula in the presence of 10 mL of sterile distilled water. The suspension is then stirred for one minute and then filtered through muslin to separate the spores from the mycelial fragments. After counting using a Malassez cell, the spore suspension is adjusted with sterile distilled water to obtain a final concentration of 10<sup>6</sup> spores/ml. The suspension is then added with 0.05% Tween 20 and 0.5% gelatin.

### **Seed inoculation**

The disinfected grains are inoculated by soaking for 24 h in a sporal suspension of 10<sup>6</sup> spores/mL of each of the isolates tested, then left to dry on sterile filter paper. Control grains are treated only with sterile distilled water.

The inoculated and control grains are sown in plastic pots (4 grains/pot) containing a sterile mixture of peat (25%) and Mamora sand (75%). Afterwards, all the pots are brought back to a greenhouse.

The severity of the disease was assessed at the flowering stage and focused on the description of the attack on the entire root system, namely the crown, sub-collar and seed roots. The expression of the severity of the disease in the root system was assessed on a class scale of 0 to 5.

### **Scoring of results**

#### **Assessment of disease severity**

In the laboratory, the root part (system) of the plants was visually examined and the disease severity rating was assessed using the scale proposed by (Greany et al. 1938) who distinguished six classes on the base of types of symptoms observed where S0 no infection; S1, small necrotic lesions scattered around the sub-collar and roots; S2, distinct necrotic lesions on the basal part of the plant, particularly on the sub-collar and roots; S3, large necrotic lesions on crown, sub-collar and roots

with reduced plant vigor; S4, basal rots, plant chlorosis, often dwarfism and wilt and S5, dead plant.

The disease incidence was calculated (according to) using the following formula:  $I = 100 [Nm/Nt]$ , where Nm the number of symptomatic plants and Nt the total number of examined plants percentage of diseased plants, Nm relative to the total number Nt of plants examined. The root rot index was expressed as  $IM = 100 \sum (Ni Si)/(5 Nt)$ , where Ni = number of plants with score i and NT = total number of plants.

The re-isolation of *Fusarium* was performed at the maturation stage from control and inoculated plants. The plants were dug up and freed of their growing medium by washing them thoroughly with running water. Cross sections of 2 cm, at the level of the roots and the crown, were done. Prior to planting, the different segments were placed separately in alcohol at 90°C for 1 to 2 minutes, rinsed thoroughly with sterile water, dried on sterile filter paper and placed on PSA medium.

### Statistical analysis

Statistical analyzes were performed by analysis of variance by the 5% ANOVA test.

## Results

The morphological characteristics of *Fusarium* isolates (sporulation, mycelial growth rate, macroconidia length and width, phialides, microconidia, chlamydospores and colony growth traits) were evaluated based on cultures grown on PSA culture medium and microscopic observations.

Fungal colony of the isolate F1 of *F. oxysporum* was slow growing. The aerial mycelium was abundant and white in color. When chlamydospores were abundant, the mycelium turned purple or blue in color, but became yellowish-brown when sporodochia were numerous. Its microconidia ( $4.5\text{-}5.5 \mu\text{m} \times 1.5\text{-}2.5 \mu\text{m}$ ) are generally abundant, ellipsoid, borne by simple and short phialids or on a branched conidiophore. They are never formed in chains, generally unicellular, ellipsoid to cylindrical, straight or curved. Macroconidia are variable in size ( $13\text{-}20 \mu\text{m} \times 2\text{-}3 \mu\text{m}$ ), spindle-shaped, slightly curved or arched, pointed at the ends. Basal cells are pedicellate, 3 (5) septa, 27 to  $46 \times 3$  to  $4.5 \mu\text{m}$ . Chlamydospores formed were subglobose, subglobular, were present at the terminal or intercalary positions on the mycelium, hyaline with a smooth or rough wall, and their measurement indicated a size of 5 to 15  $\mu\text{m}$  in diameter. These descriptions are identical to those reported by Meddah et al. (2010), Mouden et al. (2013).

*Fusarium solani* (F2 isolate) is characterized on isolation medium by a ras mycelium, colony with an often-powdery appearance and a homogeneous pigmentation, even on young colonies, dark purple in color. On PSA culture medium, this isolate exhibited a fluffy or cottony colony with white to cream color and a pale reverse. Colony of the isolate was slow, with a regular outline. Microconidia produced were scanty, with a single ovoid cell. The macroconidia had 3 to 5 septa partitioned from 3 to 5 partitions, are elongated, not very curved, with very rounded ends, often in a chain on the mycelium, with very characteristic double walls. Chlamydospores, resistance spores, are in terminal or intercalary position, in singles ( $7.3 \mu\text{m}$ ) or in pairs, measuring 8 to 10.6  $\mu\text{m}$  in length, abundant, with smooth walls. These descriptions are identical to those noted by Chermette et al. (1993), Chliyeh et al. (2017), El Hazzat et al. (2019).

The isolate ZF25 produced a white to pink colony and brown pigment on potato saccharose agar (PSA) (Fig. 1). Microconidia are uni or bicellular (1 septum), measuring 3.18-10.68  $\mu\text{m}$  long, are ovoid; sometimes round, to cylindrical, slightly curved and pointed at one end (Fig. 2a). Macroconidia were 2.58 to 45.9  $\mu\text{m}$  long, with a hooked apical cell and a foot-shaped basal cell. They are multicellular, with 3 to 5 septa (Fig. 2b-c). Chlamydospores are abundant in 4 to 5 weeks old cultures 4 to 5 weeks old, usually present at the ends of mycelial filaments. These resistance spores are isolated or formed in pairs, sometimes in chains of 4 to 5 (Fig. 3). The chlamydospores differentiate from the end to the base of the filaments, the second cell formed at the base of the end (terminal) cell, and the process continues leading sometimes to a string of 4 to 5 chlamydospores

formation. This description was almost consistent with those reported for *F. redolens* by Booth & Waterson (1964), Leslie & Summerell (2006), Bienapfl et al. (2010), Yeğin et al. (2017).

Molecular analysis of the DNA sequence of isolate ZF25, by comparison with sequences of GENBANK, made it possible to relate it to *F. redolens*, with a similarity rate of 99% (Table 2).

After 80 days of inoculation, all tested *Fusarium* were pathogenic to wheat and barley plants inducing variable necrotic lesions on their roots with variable extent translated by an absence, restraint or moderate severity. *F. solani*, *F. redolens* and *F. oxysporum* have induced in wheat plants the highest severity (class S4) of 41.6% which was noted in Amjad variety (Table 3). The response of soft wheat plants to *F. solani* differed significantly showing the severity class S4 of 25% in Wafia variety and 33% in Amal variety while in durum wheat plants were respectively of 25% and 41.6% in Karim and Amjad variety. In barley plants, the severity was lower, reaching 25%.

*Fusarium redolens* have also affected the wheat and barley plants on which the S4 severity class varied between 16.6% (Escourgeons barley) and 41.6% Amjad durum wheat). As for *F. oxysporum*, the severity class S4 was observed in the inoculated wheat and barley plants and was ranged between 16.6% (Psillum barley) and 41.6% (Amjad durum wheat). The S3 severity class noted in the wheat and barley plants inoculated with *Fusarium* isolates varied between 8.33 and 33.3%, whereas the S1 severity class varied between 0 and 25%, those of S2 between 8.33 and 33.3%. In durum wheat plants, *F. redolens* was able to damage more tissue of Amjad variety in which the severity was above that of class S1 (Table 3).

The disease incidence was ranged between 75% and 100% in wheat (soft and hard) and barley plants inoculated with *F. solani* and *F. oxysporum* and between 83.3 and 100% in those inoculated with *F. redolens* (Table 4). Both soft (Amal variety) and durum wheat plants (Amjad variety) recorded a disease incidence of 100% when inoculated with *F. solani*. The highest disease indices were noted in durum wheat plants, Amjad variety, inoculated with *F. redolens* and *F. oxysporum* with respectively 83.3 and 80%, those in barley plants inoculated with *F. redolens* were in the range of 60 and 70% against 58.3 and 83.3% noted in Wafia variety of soft wheat plants and Amjad variety of durum wheat respectively. The disease indices observed in barley and wheat plants (soft and hard) varied respectively between 63.3 (Psillum barley) and 71.6% (soft wheat, Amal variety) inoculated with *F. solani* and between 58.3 (Psillum barley) and 73.3 (soft wheat, Amal variety) inoculated with *F. oxysporum*.

The three *Fusarium* isolates used in the pathogenicity test were re-isolated from lesions of roots of wheat and barley plants, confirming their pathogenicity and complying with the Koch's Postulate (Table 5).

In wheat (soft and hard) and barley plants inoculated with *Fusarium solani*, the re-isolation percentages was in the range of 55 (soft wheat, variety, Wafia) and 100% (soft wheat, Amal variety). In wheat and barley plants inoculated with *F. solani*, the re-isolation percentages fluctuated between 55 (durum wheat, Amjad variety) and 100% (durum wheat, Karim variety). However, the re-isolation percentages of *F. redolens* varied between 75 (Psillum barley) and 100% (durum wheat, Karim variety).

## Discussion & conclusion

The examination of the morphological characters of the cultures and the molecular analysis matched the *Fusarium* sp. (isolate ZF2) to *F. redolens* with an identity of more than 99%. This species presents some morphological characters of *F. oxysporum* and *F. solani* (Booth & Waterson 1964) and has sometimes been attributed to one or the other of these species by various authors (Snyder & Hansen 1940, Gordon 1952, Bilai 1955). In this study, it was noted that the differentiation of chlamydospores on the mycelial filaments of *F. redolens* is identical to that reported in *F. oxysporum* f. sp. *albedinis* by Abdellaoui et al. (2017).

According to Baayen et al. (2000), *F. redolens* has long been considered as *F. oxysporum* (Snyder & Hansen 1940, Nelson et al. 1983) or, at best, a variety of this species, *F. oxysporum* Schlecht.: Fr. var. *redolens* (Wollenw.) (Gordon 1952, Booth 1971, 1977). The morphological

distinction of *F. redolens* from *F. oxysporum* is indeed problematic due to the presence of intermediate forms (Baayen & Gams 1988). An unambiguous distinction between the two species only became possible by molecular means (Waalwijk et al. 1996, O'Donnell et al. 1998).

Koch's postulate was verified by inoculating wheat seeds (hard and soft) with a spore suspension of *F. redolens*. The roots of plants grown from inoculated grains showed necrotic lesions and the percentages of the highest severity class observed in wheat and barley plants varied between 25 and 43.6%. The incidences and indices of root rot observed in wheat and barley plants vary between 83.6 and 100% and between 58.3 and 70%, respectively. These disease estimation parameters are important and sometimes exceed those observed in plants produced from wheat and barley grains inoculated with *F. solani* and *F. oxysporum*. The re-isolations of tested pathogens from necrotic lesions of the roots of wheat and barley plants were positive and the isolation percentages ranged between 55 and 100%. *F. redolens* has never been reported in Morocco among fungi associated with barley and wheat roots and to our knowledge this is the first report of this pathogen among the fusarium complex responsible for the root rot of these cereals.

*Fusarium redolens* [syn: *F. oxysporum* var. *redolens*] is considered to be a pathogen of a large number of host plants. It has been isolated from the necrotic and discolored roots and crown of chickpeas, peas, lentils and durum wheat Bouhadida et al. (2017). These authors noted, after pathogenicity tests, that *F. redolens* is mostly virulent on peas, while *Fusarium graminearum* and *Cochliobolus sativus* were more virulent on durum wheat. *F. redolens* is considered as common species in the Canadian prairies of Saskatchewan and is responsible for reducing crop yields of peas, chickpeas and durum wheat. The species has been reported in Lebanon, Pakistan, Spain (Jiménez-Fernández et al. 2011), Tunisia (Bouhadida et al. 2017) as a causative agent of chickpea wilt and as a pathogen of tomatoes in Algeria (Hamini-Kadar et al. 2010). It is also the causal agent of root and crown rot of asparagus Baayen et al. (2000) as well as wilt and yellowing of *Diplotaxis tenuifolia* plants Taylor et al. (2019). *F. redolens* is also an endophytic fungus of *Fritillaria unibracteata* (Pan Feng et al. 2015).

*Fusarium redolens* isolated for the first time in Morocco was cited among fungal complex associated with root rot disease in wheat and barley Qostal et al. (2019). The pathogenicity tests conducted in greenhouse have shown that this fungal species is endowed with a significant pathogenic power towards wheat and barley showing similar symptoms to those observed in plant roots from infected seeds with *F. solani* and *F. oxysporum*. The three tested pathogens induced in wheat and barley plants the highest severity class S4, very significant incidences and signs of disease. The pathogenicity of *F. redolens* towards wheat and barley sometimes exceeds that of other pathogens of these cereals, such as *F. solani* and *F. oxysporum*.

**Table 2** Molecular characteristics of *Fusarium redolens* isolate ZF25 isolated from barley

Code isolate	Accession Number	Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
ZF25	MT758201	<i>Fusarium redolens</i> strain ZF25 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	922	922	1	0	99%	MT446140.1

Importantly, extend the surveys to other regions of Morocco in order to constitute a population of isolates of *F. redolens* and to know the amplitude of the variation in the pathogenicity

of this pathogen via other varieties of wheat and barley widely cultivated in Morocco. Likewise, *F. redolens* is considered in other countries, such as Tunisia, as a pathogen linked to chickpea roots (Bouhadida et al. 2017). In Morocco, cereals and legumes are cultivated in rotation, therefore, *F. redolens* may be among the soil mycoflora of chickpeas and peas. In this sense, it is important to take this pathogen into account in programs to create new resistant varieties of wheat and barley and to monitor its appearance in other regions on other species of host plants.

**Table 3** Percentage of root rot disease severity classes in inoculated wheat (durum and soft) and barley plants

Isolates	Varieties	Severity class %				
		S0	S1	S2	S3	S4
<i>Fusarium solani</i>	<b>Wafia</b>	25 <sup>a</sup>	25 <sup>a</sup>	16.6 <sup>c</sup>	8.33 <sup>d</sup>	25 <sup>c</sup>
	<b>Amal</b>	0 <sup>d</sup>	25 <sup>a</sup>	25 <sup>b</sup>	16.6 <sup>c</sup>	33.3 <sup>b</sup>
	<b>Amjad</b>	0 <sup>d</sup>	8.33 <sup>c</sup>	25 <sup>b</sup>	25 <sup>b</sup>	41.6 <sup>a</sup>
	<b>Karim</b>	16.6 <sup>b</sup>	8.33 <sup>c</sup>	25 <sup>b</sup>	25 <sup>b</sup>	25 <sup>c</sup>
	<b>Escourgeons</b>	8.33 <sup>c</sup>	16.6 <sup>b</sup>	16.6 <sup>c</sup>	25 <sup>b</sup>	25 <sup>c</sup>
	<b>Pisillum</b>	16.6 <sup>b</sup>	25 <sup>a</sup>	8.33 <sup>d</sup>	25 <sup>b</sup>	25 <sup>c</sup>
<i>Fusarium redolens</i>	<b>Wafia</b>	16,6 <sup>b</sup>	16,6 <sup>b</sup>	25 <sup>b</sup>	16,6 <sup>c</sup>	25 <sup>c</sup>
	<b>Amal</b>	0 <sup>d</sup>	8,33 <sup>c</sup>	33,3 <sup>a</sup>	25 <sup>b</sup>	33,3 <sup>b</sup>
	<b>Amjad</b>	0 <sup>d</sup>	0 <sup>d</sup>	25 <sup>b</sup>	33,3 <sup>a</sup>	41,6 <sup>a</sup>
	<b>Karim</b>	16,6 <sup>b</sup>	16,6 <sup>b</sup>	25 <sup>b</sup>	16,6 <sup>c</sup>	25 <sup>c</sup>
	<b>Escourgeons</b>	16,6 <sup>b</sup>	25 <sup>a</sup>	16,6 <sup>c</sup>	25 <sup>b</sup>	16,6 <sup>d</sup>
	<b>Pisillum</b>	8,3 <sup>c</sup>	16,6 <sup>b</sup>	16,6 <sup>c</sup>	33,3 <sup>a</sup>	25 <sup>c</sup>
<i>Fusarium oxysporum</i>	<b>Wafia</b>	16,6 <sup>b</sup>	8,33 <sup>c</sup>	16,6 <sup>c</sup>	25 <sup>b</sup>	33,3 <sup>b</sup>
	<b>Amal</b>	0 <sup>d</sup>	16,6 <sup>b</sup>	25 <sup>b</sup>	33,3 <sup>a</sup>	25 <sup>c</sup>
	<b>Amjad</b>	0 <sup>d</sup>	8,33 <sup>c</sup>	25 <sup>b</sup>	25 <sup>b</sup>	41,6 <sup>a</sup>
	<b>Karim</b>	16,6 <sup>b</sup>	8,33 <sup>c</sup>	25 <sup>b</sup>	25 <sup>b</sup>	25 <sup>c</sup>
	<b>Escourgeons</b>	8,33 <sup>c</sup>	8,33 <sup>c</sup>	25 <sup>b</sup>	33,3 <sup>a</sup>	25 <sup>c</sup>
	<b>Pisillum</b>	25 <sup>a</sup>	16,6 <sup>b</sup>	16,6 <sup>c</sup>	25 <sup>b</sup>	16,6 <sup>d</sup>

\*Two values read on the same column, for each culture, followed by the same letter did not differ significantly at the 5% threshold

**Table 4** Incidence (%) and Root Rot Index (%) in inoculated wheat (hard and soft) and barley plants

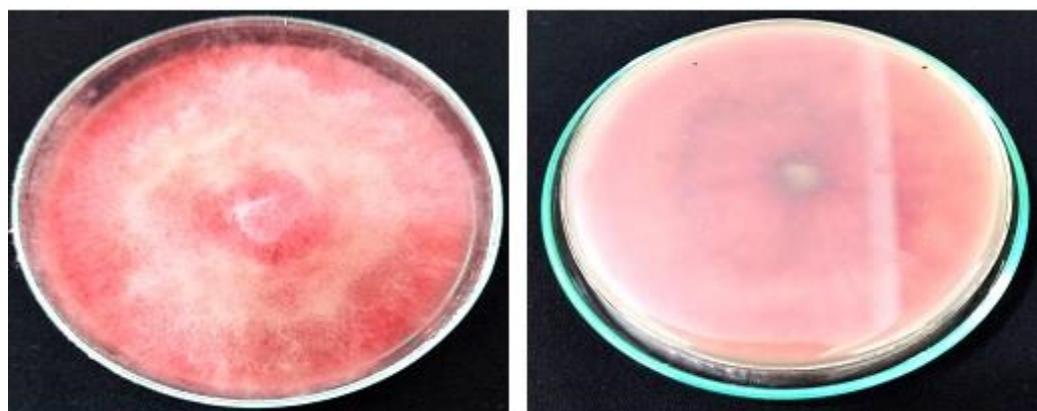
Isolates	Varieties	Incidence	Index
<i>Fusarium solani</i>	<b>Wafia</b>	75 <sup>d</sup>	61.6 <sup>c</sup>
	<b>Amal</b>	100 <sup>a</sup>	71.6 <sup>b</sup>
	<b>Amjad</b>	91.6 <sup>b</sup>	66.6 <sup>c</sup>
	<b>Karim</b>	91.6 <sup>b</sup>	63.3 <sup>c</sup>
	<b>Escourgeons</b>	91.6 <sup>b</sup>	68.3 <sup>c</sup>
	<b>Pisillum</b>	83.3 <sup>c</sup>	63.3 <sup>c</sup>
<i>Fusarium redolens</i>	<b>Wafia</b>	83,3 <sup>c</sup>	58.3 <sup>d</sup>
	<b>Amal</b>	100 <sup>a</sup>	76.6 <sup>b</sup>
	<b>Amjad</b>	100 <sup>a</sup>	83.3 <sup>a</sup>
	<b>Karim</b>	83,3 <sup>c</sup>	63.3 <sup>c</sup>
	<b>Escourgeons</b>	83,3 <sup>c</sup>	60 <sup>cd</sup>
	<b>Pisillum</b>	91,6 <sup>b</sup>	70 <sup>bc</sup>
<i>Fusarium oxysporum</i>	<b>Wafia</b>	83.3 <sup>c</sup>	70 <sup>bc</sup>
	<b>Amal</b>	100 <sup>a</sup>	73.3 <sup>b</sup>
	<b>Amjad</b>	100 <sup>a</sup>	80 <sup>a</sup>
	<b>Karim</b>	83.3 <sup>c</sup>	70 <sup>bc</sup>
	<b>Escourgeons</b>	91.6 <sup>b</sup>	71.6 <sup>b</sup>
	<b>Pisillum</b>	75 <sup>d</sup>	58.3 <sup>d</sup>

\*Two values read on the same column, for each culture, followed by the same letter did not differ significantly at the 5% threshold

**Table 5** Re-isolation percentage of pathogens from wheat and barley roots

Isolates	Varieties	% Re-isolation
<i>Fusarium solani</i>	<b>Wafia</b>	55 <sup>f</sup>
	<b>Amal</b>	100 <sup>a</sup>
	<b>Amjad</b>	60 <sup>e</sup>
	<b>Karim</b>	55 <sup>f</sup>
	<b>Escourgeons</b>	75 <sup>d</sup>
	<b>Pisillum</b>	80 <sup>c</sup>
<i>Fusarium redolens</i>	<b>Wafia</b>	100 <sup>a</sup>
	<b>Amal</b>	95 <sup>b</sup>
	<b>Amjad</b>	75 <sup>d</sup>
	<b>Karim</b>	100 <sup>a</sup>
	<b>Escourgeons</b>	75 <sup>d</sup>
	<b>Pisillum</b>	75 <sup>d</sup>
<i>Fusarium oxysporum</i>	<b>Wafia</b>	60 <sup>e</sup>
	<b>Amal</b>	100 <sup>a</sup>
	<b>Amjad</b>	55 <sup>f</sup>
	<b>Karim</b>	100 <sup>a</sup>
	<b>Escourgeons</b>	75 <sup>d</sup>
	<b>Pisillum</b>	60 <sup>e</sup>

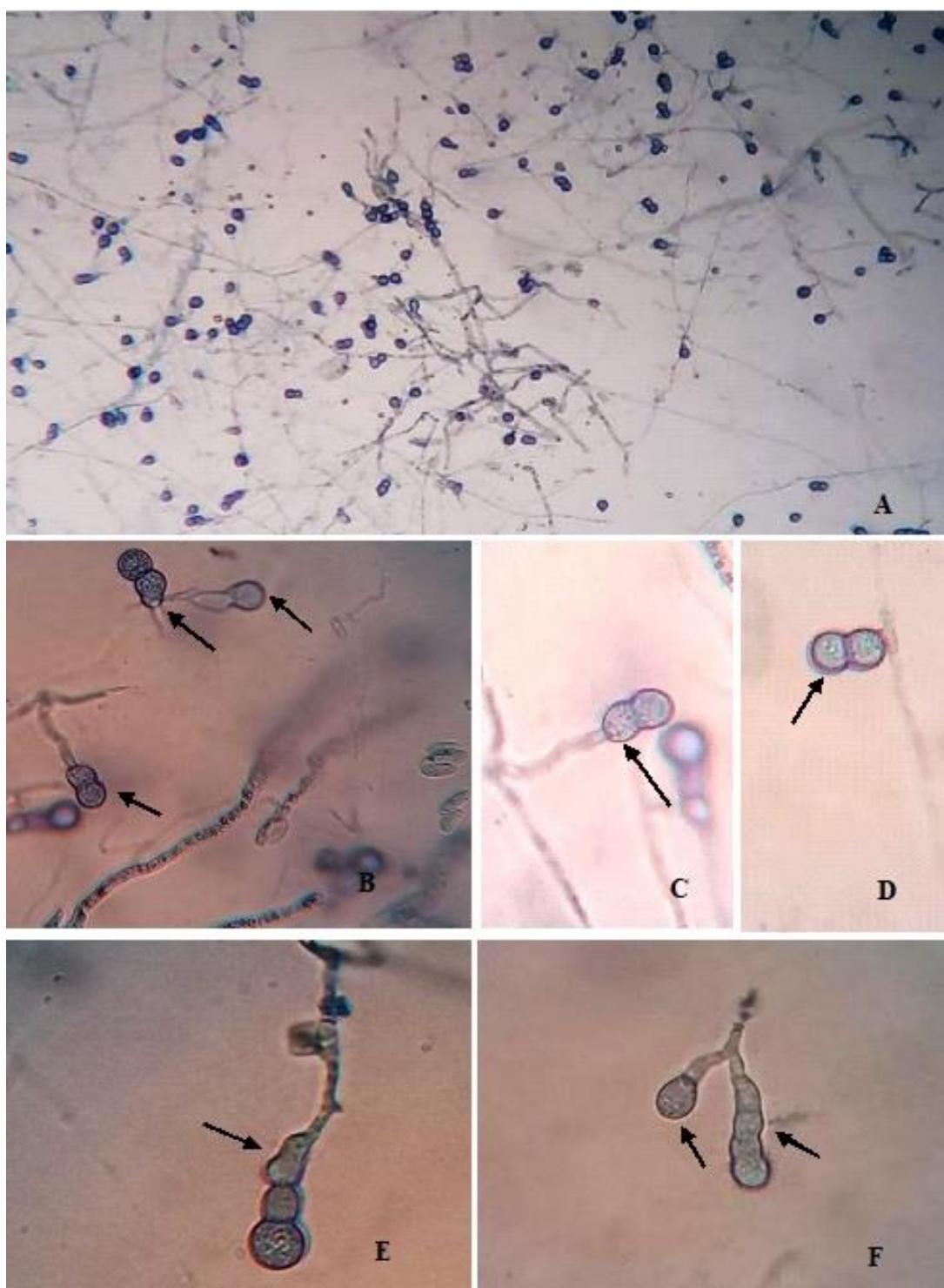
\*Two values read on the same column, for each culture, followed by the same letter are not significantly at the 5% threshold



**Fig. 1** – Colony aspect of *Fusarium redolens* on PSA culture medium.



**Fig. 2** – Microconidia (a) and macroconidia (b and c) of *Fusarium redolens* isolate ( $\times 400$ ).



**Fig. 3** – Chlamydospores of *Fusarium redolens* formed in singles, in pairs, and in chains (A, B, C, D, E and F) obtained in PSA medium at (x100) (a) and (x400)

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## References

- Abdellaoui S, El Aissami A, Benkhemmar O, Ouazzani Touhami A et al. 2017 – Study of the differentiation of *Fusarium oxysporum* f. sp. *albedinis* chlamydospores on different culture media. Annual Research & Review in Biology 18, 1–9.
- Baayen R, van den Boogert P, Bonants P. 2000 –*Fusarium redolens* f.sp *asparagi*, causal Agent of *Asparagus* root rot, crown rot and spear rot. European Journal of Plant Pathology 106, 907–912.
- Baayen RP, Gams W. 1988 – The Elegans fusaria causing wilt disease of carnation. I. Taxonomy. Netherlands Journal of Plant Pathology 94, 273–288.
- Backhouse D, Abubakar AA, Burgess LW, Dennis JI et al. 2004 – Survey of Fusarium species associated with crown rot of wheat and barley in eastern Australia. Australasian Plant Pathology 33, 12.
- Bienapfl JC, Malvick DK, Percich JA. 2010 – First report of *Fusarium redolens* causing root rot of Soybean in Minnesota. The American Phytopathological Society (APS) Publications 94, 1069–1069.
- Bilai VI. 1955 – The fusaria (biology and systematics). Akademia Naukwe Ukrainskii, SSR, Kiev 129–141.
- Booth C. 1971 – The genus Fusarium. C.M.1. Kew. Surrey, England 237.
- Booth C. 1977 – Fusarium. Laboratory guide to the identification of the major species. C.M.1. Kew. Surrey, England 58.
- Booth C, Waterson JM. 1964 – *Fusarium solani*. C. M. I. Description of pathogenic fungi and bacteria. No. 29, Commonwealth Mycological Institute, Ferry lane, Kew, Surrey, London, U.K.
- Bouhadida M, Jendoubi W, Gargouri S, Beji M et al. 2017 – First report of *Fusarium redolens* causing Fusarium yellowing and wilt of chickpea in Tunisia. The American Phytopathological Society (APS) Publications 101, 6 1038–1038.
- Champion R. 1997 – Identifier les champignons transmis par les semences. INRA, Paris 398.
- Chermette R, Bussieras J, Mialot M, Raynal PC. 1993 – Subcutaneous *Taenia crassiceps* cysticercosis in a dog. J Am Vet Med Assoc 203, 253–255.
- Chidambaram P, Mathur SB, Neergaard P. 1974 – Identification of seed-borne Drechslera species. Handbook on Seed Health Testing 3, 165–207.
- Chliyeh M, MSSayri S, Ouazzani Touhami A, Benkirane R, Douira A. 2017 – Detection of *Fusarium solani* as a Pathogen Causing Root Rot and Wilt Diseases of Young Olive Trees in Morocco. Annual Research & Review in Biology 13, 1–7.
- Cook ET. 1980 – *Fusarium* foot rot of wheat and its control in the Pacific Northwest. *Plant Dis.* 64, 1066.
- Domsch KH, Gams W, Anderson TH. 1980 – Compendium of soil fungi. Academic Press, London 1, 859.
- Dyer AT, Rew EA, Jacobsen LJ, Moya-Elizondo BJ, Hogg AC. 2011 – Distribution and Prevalence of *Fusarium* Crown Rot and Common Root Rot Pathogens of Wheat in Montana. *Plant Dis.* 95, 1099–1108.
- El Hazzat N, Ouazzani Touhami A, Chliyeh M, Errifi A et al. 2019 – Effect of a composite endomycorrhizal inoculum on the manifestation of chickpea wilt, caused by *Fusarium solani*. Plant Cell Biotechnology and Molecular Biology 20, 486–500.
- Ellis MB. 1971 – Dematiaceous Hyphomycetes. Commonwealth Mycological Institute Kew, Surrey, England 608.
- Gardes M, Bruns TD. 1993 – ITS primers with enhanced specificity for Basidiomycetes – applications to the identification of mycorrhizae and rusts. Molecular Ecology 2, 113–118.
- Gilman CJ. 1957 – A manual of soil fungi, Second Edition. The Iowa State College Press-Ames, Iowa, U.S.A. 452.

- Gordon WL. 1952 – The occurrence of *Fusarium* species in Canada. 2. prevalence and taxonomy of *Fusarium* species in cereal seed. *Can J Bot* 30, 209–251.
- Greany FJ, Machacek JE, Johnston CL. 1938 – Varietal resistance of wheat and oats to root rot caused by *Fusarium culmorum* and *Helminthosporium sativum*. *Sci. Agric.*, 18, 500–523.
- Hamini-Kadar N, Edel-Hermann V, Gautheron N, Steinberg C. 2010 – First report of *Fusarium commune* and *Fusarium redolens* causing crown and root rot on tomato in Algeria. *New Disease Reports* 22, 3.
- Jiménez-Fernández D, Montes-Borrego M, Jiménez-Díaz RM, Navas-Cortés JA, Landa BB. 2011 – In planta and soil quantification of *Fusarium oxysporum* f. sp. *Ciceris* and evaluation of *Fusarium* wilt resistance in chickpea with a newly developed quantitative polymerase chain reaction assay. *Phytopathology* 101, 250–262.
- Leslie JF, Summerell BA. 2006 – The *Fusarium* Laboratory Manual. Blackwell Publishing. Ames IA, Oxford, UK 1–2.
- Meddah N, Ouazzani TA, Douira A. 2010 – Mycoflore associée au bananier (*Musa acuminate L.*), variété Grande naine, cultivé sous serre dans la région du Gharb (Maroc). *Bulletin de l’Institut Scientifique, Rabat, section Sciences de la Vie*, 32, 1–1.
- Messiaen CM. 1959 – La systématique du genre *Fusarium* selon Snyder et Hansen. *Rev. Pathol. vég. Entomol. agric. Fr.*, 38, 253–266.
- Mouden N, Benkirane R, Ouazzani TA, Douira A. 2013 – Mycoflore de quelques variétés du fraisier (*Fragaria ananassa L.*), cultivées dans la région du Gharb et le Loukkos (Maroc). *Journal of Applied Biosciences* 61, 4490–4514.
- Nelson PE, Tousson TA, Marasas WFO. 1983 – *Fusarium* Species: An Illustrated Manual for Identification. Pennsylvania State University, University Park.
- O'Donnell K, Kistler HC, Cigelnik E, Ploetz RC. 1998 – Multiple evolutionary origins of the fungus causing Panama disease of banana: concordant evidence from nuclear and mitochondrial gene genealogies. *Proc Natl Acad Sci USA* 95, 2044–2049.
- Pan Feng B, Su X, Hu B, Yang N et al. 2015 – *Fusarium redolens* 6WBY3, an endophytic fungus isolated from *Fritillaria unibracteata* var. *wabuensis*, produces peimisine and imperialine-3 $\beta$ -d-glucoside. *Fitoterapia* 103, 213–221.
- Qostal S, Kribel S, Chliyeh M, Serghat S et al. 2019 – Study of the fungal complex responsible for root rot of wheat and barley in the north-west of Morocco. *Plant Archives* 19, 2143–2157.
- Snyder WC, Hansen HN. 1940 – The species concept in *Fusarium*. *American Journal of Botany* 27, 64–67.
- Taylor A, Barnes A, Jackson AC, Clarkson JP. 2019 – First report of *Fusarium oxysporum* and *Fusarium redolens* causing wilting and yellowing of wild rocket (*Diplotaxis tenuifolia*) in the UK. *Plant Disease* 103, 1428.
- Tivoli B. 1988 – Guide d'identification des différentes espèces ou variétés de *Fusarium* rencontrées en France sur la pomme de terre et dans son environnement. *Agronomie, EDP Sciences* 8, 211–222.
- Waalwijk C, de Koning JRA, Baayen RP, Gams W. 1996 – Discordant groupings of *Fusarium* spp. from sections Elegans, Liseola and Dlaminia based on ribosomal ITS1 and ITS2 sequences. *Mycologia* 88, 361–368.
- White TJ, Bruns TD, Lee S, Taylor JW. 1990 – Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Gelfand MAIDH, Sninsky JJ and White TJ (eds.) *PCR Protocols: A Guide to Methods and Applications*. Academic Press, New York 315–322.
- Yeğin NZ, Ünal F, Tekiner FN, Dolar S. 2017 – First report of *Fusarium redolens* causing root and crown rot of Barley (*Hordeum vulgare*) in Turkey. *J. Turk. Phytopath* 46, 101–105.