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First Report of *Fusarium chlamydosporum* Causing Crown Rot and Dumping off on Durum Wheat in Algeria

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Abstract

Several species of *Fusarium* infect durum wheat plants in the eastern part of Algeria. Endophytic fungus from random wheat seeds were sampled from fifty locations in eastern Algeria and shows the existence of *Fusarium chlamydosporum* regarding the macroscopic and microscopic characteristics. Molecular identification using EF1 and ITS1 primers were confirmed the presence of *Fusarium chlamydosporum* for the first time on durum wheat seeds in Algeria. The pathogenicity test was carried out on three durum wheat varieties for determining the effect of this isolate on the coleoptile and basal part of durum wheat. The results show that *F. chlamydosporum* had a negative impact on seed germination of the three varieties tested. Indeed, the reduction in germination varies between 85 and 92% reduction on GTAdur and Waha varieties, respectively. More, *F. chlamydosporum* caused a reduction in coleoptiles length in all varieties ranging from 60.97 to 70.05% in GTAdur and Waha, respectively. On the other hand, it showed a very different impact on plant growth parameters. Thus, the action was much more pronounced on reducing the root system length and the fresh weight of the vegetative system of Waha variety equal to 75.04 and 71.15%, respectively, and it is notable for the fresh weight of the root system of Bousselam variety. These very high levels of infectivity prompt us to reconsider our vision for this species of *Fusarium* in treatment and certification programs.

Key words: *Fusarium chlamydosporum*, Crown Rot, Dumping, Durum Wheat, Algeria

Introduction

Foods derived from wheat play an important role in the diet of the Algerian people. Bread, cakes, pasta and many other Algerian meals as couscous are the most common products made from wheat in Algeria (Kezih *et al.*, 2014). Wheat is grown throughout the different agro-ecological areas of Algeria, but it is essentially concentrated in semi-arid and arid areas (MADRP, 2016). *Fusarium* crown rot (FCR) also

known as (*Fusarium* foot and root rot) and *Fusarium* head blight (FHB) are two different diseases which affect small-grain cereals mainly wheat and barley (Leslie & Summerell, 2006; Scherm *et al.*, 2013).

FCR is an important disease which occurs on wheat worldwide; plants can be attacked in most growth stages. The symptoms of the disease differ according to the time of infection: early stage infections induce pre-or post-emergence mortality of the seedlings with the browning of the coleoptiles; later stage infections induce browning of the first two or three internodes. In serious attack, blighted heads are dispersed in the field, easily perceived when the wheat plants are still green (Fernandez & Jefferson, 2004; Balmas *et al.*, 2015).

FCR is one of high economic impact in different countries and regions of the world such as Australia, North America, South America, Europe, North Africa, South Africa and West Asia (Chakraborty *et al.*, 2006; Scherm *et al.*, 2013; Balmas *et al.*, 2015). FCR caused losses from 18 to 45.5% on wheat in the field in North West of Iran (Saremi *et al.*, 2007). Hollaway *et al.* (2013) have recorded crown rot yield losses of 8 to 36% in bread wheat and 24 to 52% in durum wheat in Australia. In Tunisia losses of 25% were recorded on durum wheat (Chekali *et al.*, 2013).

The severity of FCR is greater in dry soils and in areas with high temperatures (Scherm *et al.*, 2013; Obanor & Chakraborty, 2014; Balmas *et al.*, 2015). These conditions are present in many wheat-growing regions in Algeria. In addition, it has been reported that infection of the stem base of wheat with *Fusarium spp*. leads to the translocation of toxins to the ears and their accumulation in the grain (Covarelli *et al.*, 2012; Beccari *et al.*, 2018).

In Algeria, few studies were published on the *Fusarium* affecting wheat (Touati-Hattab *et al.*, 2016; Laraba *et al.*, 2017). This study was conducted to isolation and identification of some endophytic *Fusarium* species from seeds of durum wheat and to evaluate their pathogenicity.

Material and Motheds

Isolation of endophytes

Random seeds samples were collected from fifty locations of eastern Algeria (one sample from each location), then transferred to the laboratory of applied microbiology (Ferhat Abbas University – Setif) where the isolation experiment was performed. The analysis of a seeds sample was carried out on a test sample corresponding to an approximate volume of 50ml, the analyzed seeds are randomly sampled from the sample received by a vigorous mixing. The seeds were superficially disinfected by using 1.5% of sodium hypochlorite for 10min, and then well drained, spread and placed to dry on sterilized filter paper for 20 to 30min under aseptic condition. A total of 100 seeds were cultured in 15 petri dishes (Between 6 to 7 seeds per dish) containing Potato Sucrose Agar (PSA) and incubated at 28 °C for 5-7 days. Fungi that gave white colour, beige and rose to reddish pigments in PSA and had spores that are appeared similar to those of the genus *Fusarium*, when examined under the microscope, were purified (using the single-spore technique) and subsequently subject to macro and micro-morphological studies (LNPV, 2008).

Macroscopic characteristics

After purification, colonies obtained were conserved on potato dextrose agar (PDA). For macroscopic analysis, the colonies were then subculture on PDA and PSA. The characteristics studied were growth rates, colony appearance and texture, the pigmentation on both the top and reverse plates (Leslie & Summerell, 2006; Seddique *et al.*, 2010).

Microscopic characteristics

The carnation leaf agar (CLA) was used for diagnostic micro-morphological characteristics. Fresh, disease-free carnation leaves were cut into 1cm^2 pieces and oven-dried for 3 to 4h at 110 °C. Water agar (WA) at 15 g/L was prepared and sterilised, when pouring into plates,4 or 5 pieces of carnation leaf fragments were placed, slightly spaced to each other, into the molten agar and the media left to cool. Mycelial tip fragments, of the purified cultures, were deposited onto dishes containing CLA and were incubated at 28 °C. After 10 to 15 days, a fragment of the agar culture was observed using light microscope (Seddique *et al.*, 2010).

The morphological identification was carried out by following that described in Burgess *et al.* (1994) and in Leslie & Summerell (2006), the diagnostic characteristics examined in the identification of species encompass the presence/absence of micro-, meso and macroconidia, their shapes and sizes (if present); the type of phialides (monophialide and/or polyphialide) and the occurrence or absence of chlamydospores, chains of microconidia and sporodochia on CLA.

Molecular identification

To confirm the identity of this fungus, the molecular identification was carried out by BIOfidal laboratory (CEDEX-France).

DNA extraction

DNA was extracted either from mycelium collected by scraping the surface of Petri plate cultures of purified isolate. 100µl lysis buffer (50mM Tris-HCl pH 7.5, 50mM EDTA, 3% SDS and 1% 2-mercaptoethanol) was added and the nucleic acids were isolated according to the microwave mini-prep procedure described by (Goodwin and Lee, 1993). The final DNA pellet was supplemented into 100µl TE buffer (10mM Tris-HCl pH 8.0, 0.1mM EDTA) and stored at −20 °C until used.

PCR amplifications and gel electrophoresis

Partial sequences of the ITS region and the elongation factor (EF1) were polymerase chain reaction (PCR) amplified using fungal specific primer pair ITS1 (5' TCC GTA GGT GAA CCT GCG G ´3) (White *et al.*, 1990) and EF1-728F (5'CAT CGA GAA GTT CGA GAA GG 3') (Carbone and Kohn, 1999). All amplification reactions were performed in a 50µl reaction volume containing 75mM Tris-HCl pH 9.0, 20 mM $(NH4)_2SO4$, 0.01% (*w*/*v*) Tween 20, 1.5mM $MgCl_2$, 200µM each dNTP, 1 unit of thermo-stable DNA polymerase (GoTaq, Promega®) and 400nM of each relevant oligonucleotide primer. After electrophoresis in 1.2% Agarose gels in 0.5× TAE buffer (20mM Tris- acetate pH 8, 0.5mM EDTA), DNA was visualized by Ethidium bromide staining and UV illumination.

Purification of PCR products ITS1 and EF1

The combined PCR products are purified by mini-column centrifugation (NucleoSpin® Extract II) and the DNA binds in the presence of a chaotropic salt to a silica membrane. The binding mixture is loaded directly onto NucleoSpin® Extract II columns. Contaminations are removed by a simple washing step with ethanolic NT_3 buffer. The pure DNA is finally eluted under low ionic strength conditions with a slightly alkaline NE buffer (5mM Tris-Cl, pH 8.5).

DNA sequencing, alignment and Phylogenetic analysis

The sequences of EF1 and ITS1 regions of the test isolate thus obtained were next edited in order to generate a consensus sequence using the BioEdit Sequence Alignment Editor Software (http/www.bioeditor.sdsc.edu/download.shtml). Next, the most related corresponding sequences of the strain of *F. chlamydosporum* were selected from the GenBank (http://www.ncbi.nlm.nih.gov/BLAST). Multiple sequence alignments were performed in MEGA format by using the Clustal W option in MEGA-X software. Phylogenetic tree and distance matrix were also constructed using MEGA software,

which implemented the Neighbour-Joining (NJ) dendrogram program of Saitou & Nei (1987). The phylogenetic distance was founded on the model of Kimura 2-parameter (K2P) (Kimura, 1980).

Pathogenicity tests

The *Fusarium* isolate representative of the species *F. chlamydosporum* was screened for their pathogenicity. Two inoculation methods were used to assess the aggressiveness of *Fusarium* isolates on wheat cv. Bousselam, Waha and GTAdur the most commonly cultivated in east region of Algeria. The first method was by *in vitro* seed inoculation to study the effect on coleoptile growth compared to the uninoculated control. The second was used to assess aggressiveness on the crown by soil inoculation (Abdallah-Nekache *et al.*, 2019).

Pathogenicity of *Fusarium* on coleoptile emergence and growth

A modified method of Mesterhazy (1983) was adopted, where flasks containing 50ml of potato dextrose broth were inoculated by four plugs of 5mm diameter mycelia of a 7-day-old PDA cultures. The flasks were incubated in an orbital shaker running at 175rpm during 7 days. Mycelium was harvested by centrifugation (5000g for 10min), homogenized, and diluted to 13.3 mg/ml and homogenate with 0.2% of Tween 20 surfactant. Sterilized Whatman No.1 filter paper was placed on fresh PDA plates and 8ml of the homogenate mycelium were positioned on the filter paper; a second filter paper was placed on the top of the inoculum. Twenty-five wheat seeds (cv. Bousselam, Waha and GTAdur) were surface sterilized, dried and placed on each PDA plate and incubated at 25 °C for 4 days. Four replicates were used by *Fusarium* isolate. The coleoptile growth (mm) was measured after 4 days. Results were expressed as percentage of coleoptile emergence inhibition and percentage of coleoptile length reduction compared to uninoculated control seedlings.

Pathogenicity of *Fusarium* isolates on wheat crown

The pathogenicity assay on durum wheat crown was performed according to the protocol established by Khalifa & Matny (2013) with slight modifications. Initially, the *Fusarium* strains were grown on a substrate containing 54g of barley and 22ml of distilled water in 250ml Erlens and sterilized by autoclaving for 20 minutes at 120 °C. Subsequently, each Erlenmeyer flask was inoculated with 5 explants of 6 mm in diameter taken from a 7 days culture old of the *Fusarium* isolate and incubated at 25 °C for 15 days and shaken once every 3 to 4 days. Then each pot was sown with 5 seeds issue from one of the three durum wheat varieties (cv Bousselam, Waha and GTAdur) previously disinfected with sodium hypochlorite (2%). The seeds are sown to a depth of 4 to 5cm in sterilized soil. The artificial infection of the soil was obtained by spreading 2.5g of barley carrying a *Fusarium* strain at a depth of 2cm above the seeds and then covered with a layer of 2cm of sterilized soil. As control pots, three pots were sown only by seeds disinfected on sterile soil. Resulting in an adopted experimental device that is completely randomized with three repetitions where all pots were placed in an illuminated laboratory between 18 and 20 °C and 12h of natural light and 12h of darkness. To keep the plants in good growing conditions, frequent watering was done twice a week.

Plants were examined daily for signs of symptom onset throughout the 45 days of the experiment and the seedling emergence rate, root length, vegetative length, and Fresh weight of the root and vegetative system were measured.

Statistical analysis

Data were subjected to analyses of variance (ANOVA) with SPSS version 25. Significance of mean differences was determined using the Duncan test and responses were judged significant at the 5% level (P=0.05) with a 95% confidence interval.

Result and Discussion

Isolation of endophytic fungi

Close to 60 colonies of endophytic fungal species were obtained from durum wheat seeds, of which only 10 colonies showed reddish pigmentation and 5 other colonies showed beige to brown pigmentation on the PSA with strong indications as being a member of the *Fusarium* genus during preliminary observation.

Macroscopic characteristics

About the *Fusarium* FCh26 isolate, colony development on PDA media was relatively fast; 28 mm diameter colonies developed after only 3 days. The Mycelia are fluffy, compact, fairly dense, off white and turned soft colour with a shade of brown in older portions of the colony followed by golden yellow ring and a yellow-beige peripheral ring. The reverse cultures showed several shades of Persian red colour in the central ring followed by a small yellow-cake ring which is followed by a yellow-beige peripheral ring in the 7 days isolates (Figure 1).

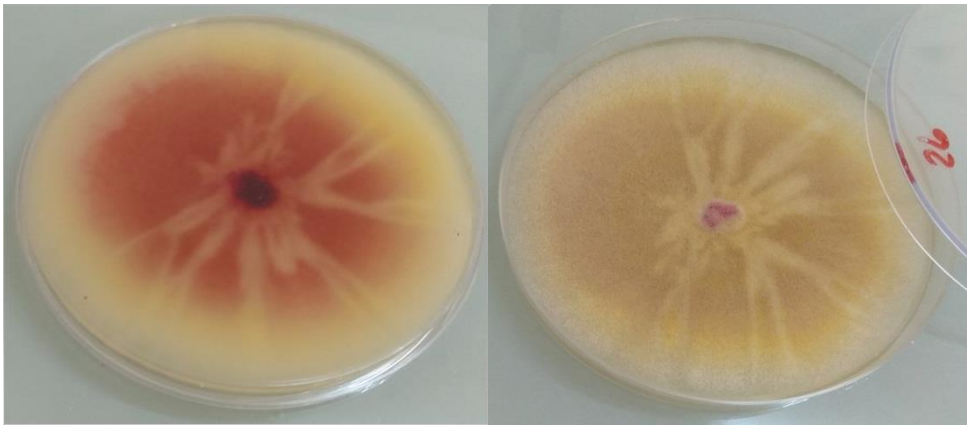

Figure 1. Macroscopic aspect of *F. chlamydosporum* colony cultures on PDA: **A** face and **B** reverse

Unlike the PDA media, the colony development on PSA media was fast with 52 mm diameter colonies developed after 3 days. The Mycelia are fairly dense, lavender and turned hibiscus (or carnation) colour with a shade of yellow in older portions of the colony with not well-defined concentric rings. The reverse cultures showed also not well-defined concentric rings with a greyish rose in peripheral area to burgundy colour in the centre, in the 7 days isolates old (Figure 2).

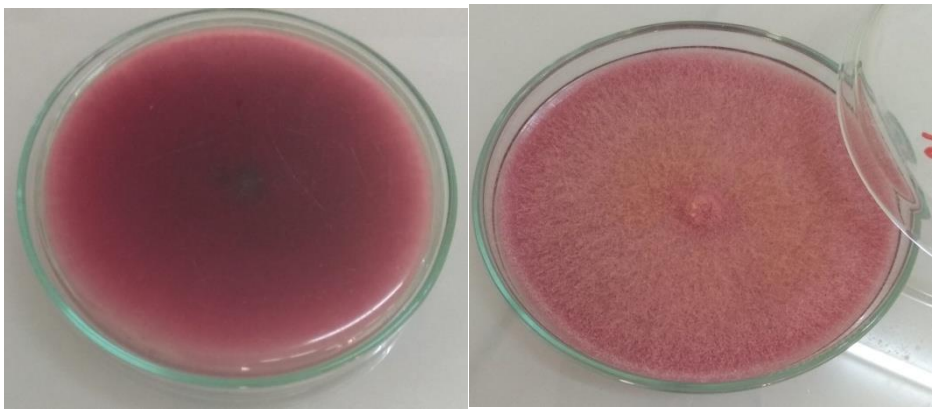

Figure 2. *F. chlamydosporum* colony cultures (observe and reverse) on PSA.

Microscopic characteristics

Generally, microconidia and mesoconidia were abundant, no sporodochia were observed even in 15-day old cultures. Conidiophores in the aerial mycelia were mainly short branched (Figure3). The macroconidia were rare, straight and falcate with 2-3 septa per conidium and microconidia were abundant with oval to obvate forms (Figure4). The isolate also produced primary conidia or mesoconidia with 1-2 septa, chlamydospores were rare and take a long time to be formed (15 days) in single or chain (Figure 5). Conidia were borne from both mono-and polyphialides (Figures 6). Chains of microconidia were absent even in 11-day old colonies.

One from 15 *Fusarium* strains isolated from durum wheat seeds (Waha) of Sidi-Mbarek locality in Bordj Bou Arreridj district were identified morphological putatively as the anamorph *Fusarium chlamydosporum* Wollenweber & Reinking (Leslie & Summerell, 2006).

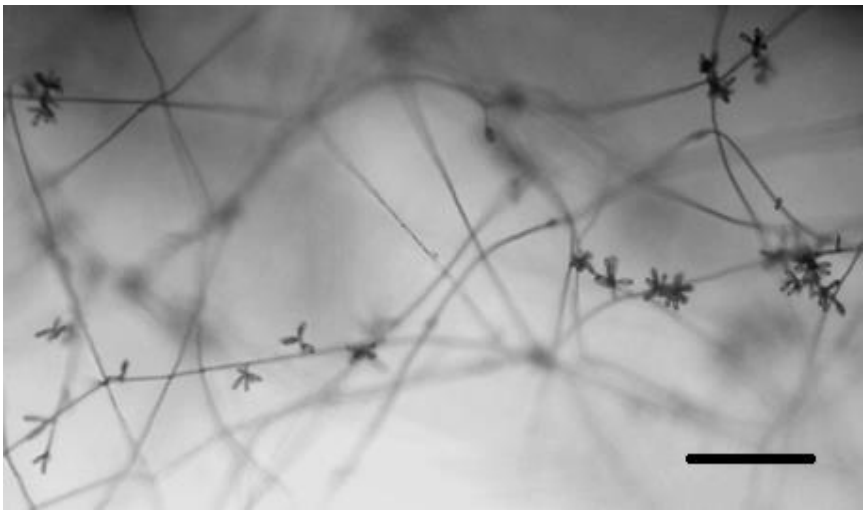

Figure 3. Simple polyphialidic conidiophores of *F. chlamydosporum* aerial mycelium. Scale bar = 50 µm.

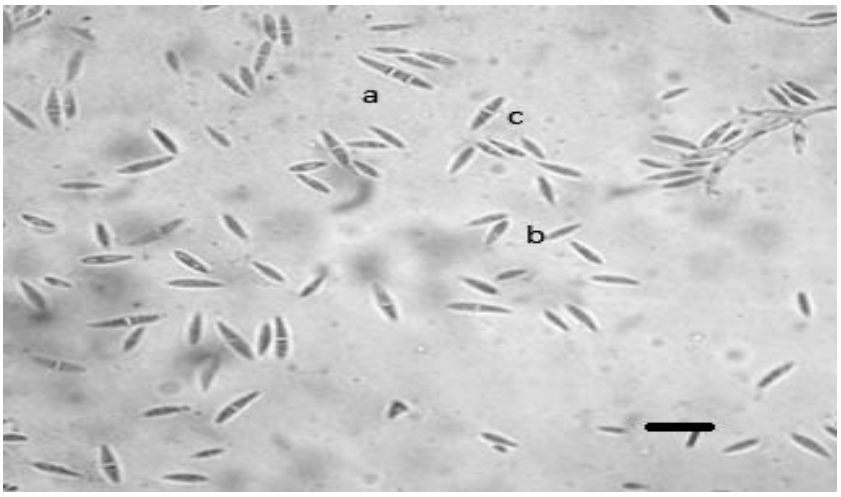

Figure 4. Different fructifications structures of *F. chlamydosporum* macroconidia (a), microconidia (b) and mesoconidia (c). Scale bar = 20 µm.

Molecular identification and Phylogenetic analysis

After the amplification of the rDNA regions with primers EF1 and ITS1, the sequences used for the final phylogenetic analysis were 477 and 646b (Figure 7). After that, the two sequences obtained were compared to the referred sequences collected from the GenBank, similarities values of 97 to 100%. With ITS1 primer, the closest isolate to our isolate is *Fusarium chlamydosporum* isolate (CA1I6F1) with a similarity rate of 527 from 533b which represents a rate of 98.87% (Figure 8). However, with EF1 primer, the closest isolate to our isolate is *Fusarium nelsonii* strain with a similarity rate of 459 from 470b which represents a rate of 97.85%, followed by *Fusarium chlamydosporum* culture (ICMP: 10472) with a similarity rate of 447 from 470b which represents a rate of 97.22% (Figure 9).

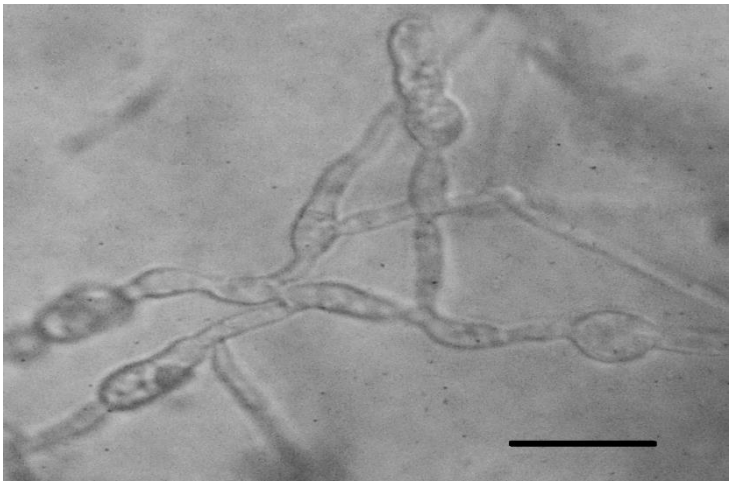

Figure 5. Chlamydospores of *F. chlamydosporum* in the aerial mycelium. Scale bar 20µm.

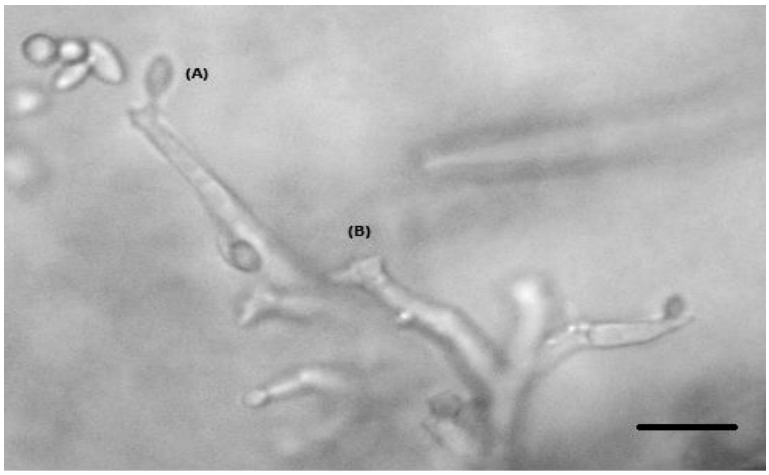

Figure 6. Mono (a) and polyphialidic (b) conidiophores of *F. chlamydosporum* in the aerial mycelium. Scale bar = 20 µm.

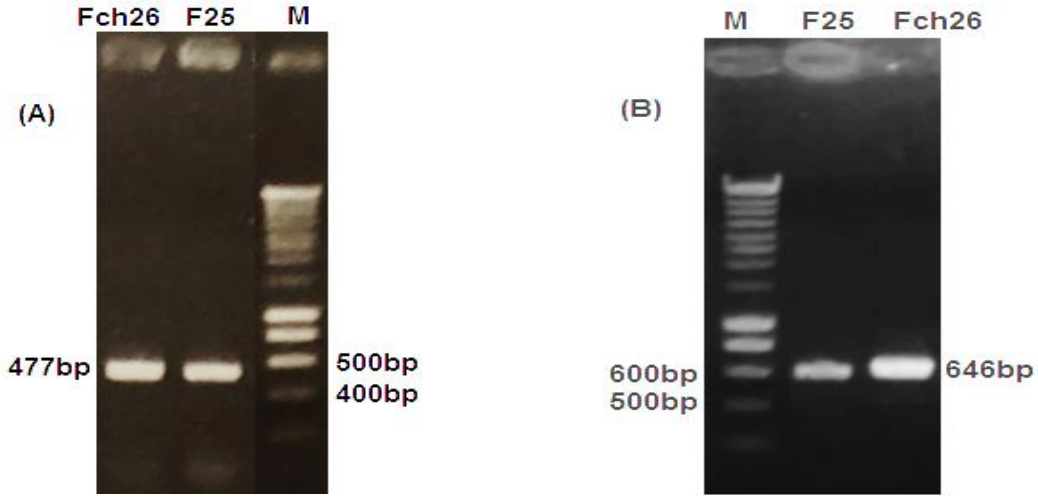

Figure 7. Agarose gel image of PCR-amplified ITS1 (A) and EF1 (B) gene region of *Fusarium* isolates from durum wheat.

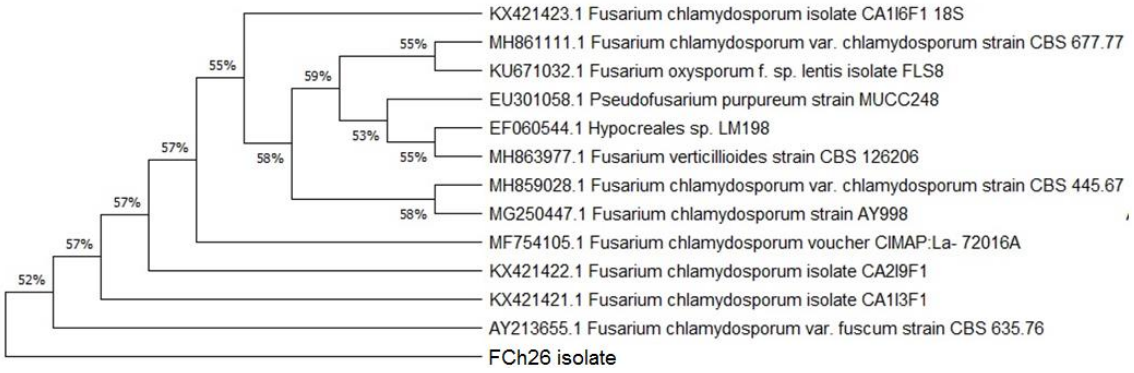

Figure 8. Phylogenetic relationships of *F. chlamydosporum* isolates inferred by Neighbour-Joining (NJ) analysis of ITS sequences.

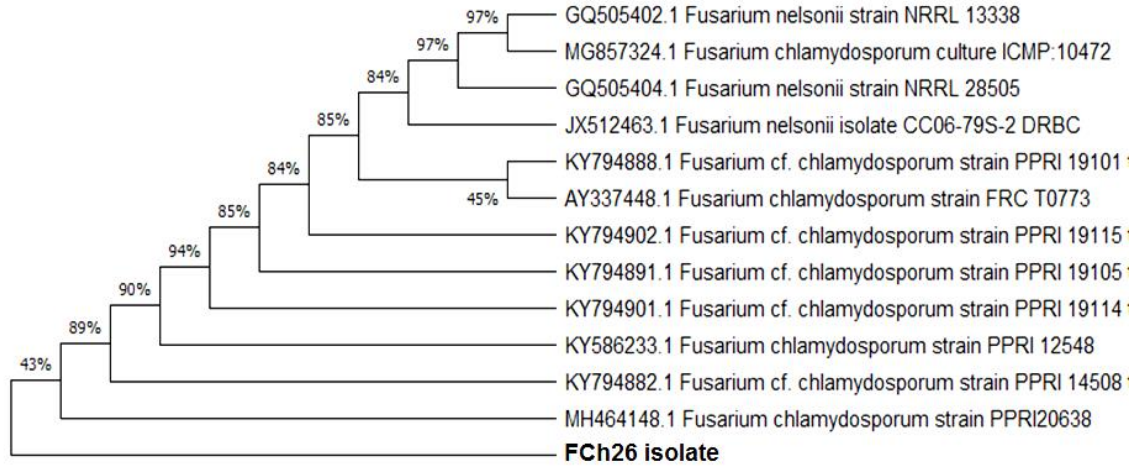

Figure 9. Phylogenetic relationships of *F. chlamydosporum* isolates inferred by Neighbour-Joining (NJ) analysis of EF sequences.

Pathogenicity tests

Effect of *Fusarium* isolates on coleoptile emergence and growth

The results of the *in vitro* test evaluated by the emergence inhibition and the length reduction of coleoptiles, after 4 days of incubation, have shown that the isolate of *F. chlamydosporum* was very aggressive. *F. chlamydosporum* induced 92%, 87% and 85% of coleoptile emergence inhibition on Waha, Bousselam and GTAdur varieties, respectively. However, *F. chlamydosporum* induced 70.47%, 69.45% and 58.01% of coleoptile length reduction on Waha, GTAdur and Bousselam varieties, respectively.

In terms of the behaviour of the three varieties of wheat to infection of *Fusarium* strain, it appears that the Waha variety was the most susceptible to attacks of the *Fusarium* strain with a germination reduction rate of 92%, the other two varieties, namely Bousselam and GTAdur, behave almost in a very similar way to *Fusarium* attacks with germination reduction rates of 87% and 85%, respectively (Table 1). However, the Waha and Bousselam varieties are more sensitive to the infection, with length reduction rate 70.05 and 70.49% respectively, compared to the GTA variety that had a reduction rate of 60,97% (Table 1). Following the obtained results, no significant difference in germination reduction and length reduction is given between the three wheat varieties tested ($p>0,05$). On the other hand, the results illustrate that there is a very highly significant difference ($P<0,001$) between artificially infected plants and uninfected control plants; this proves that our isolate is pathogenic at least on the germination and the length of the coleoptile (Figure 10).

Table 1. Results of the three pathogenicity tests on coleoptile emergence and growth

Wheat Variety	Fungus isolate	Col. length (cm)	Length reduction %	Num.germ_ Seeds	Germination reduction %
Bousselam	F.ch26	1.13	70.49[a]	13	87[a]
	Control	3.82	0	100	0
GTAdur	F.ch26	1.6	60,97[a]	15	85[a]
	Control	3.7	0	100	0
Waha	F.ch26	1.1	70.05[a]	8	92[a]
	Control	3.87	0	100	0

-Means with the same letter in the same column are significantly different at $P < 0.05$ as determined by Duncan test.

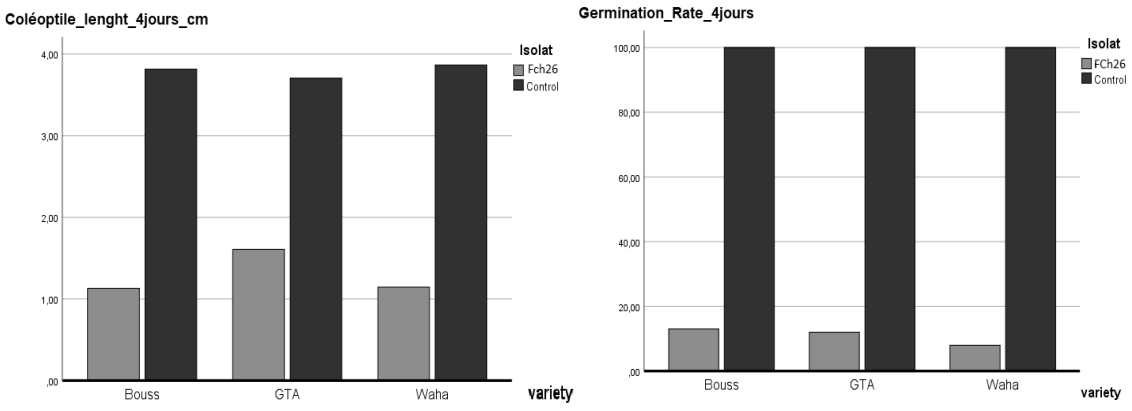

Figure 10. Impact of *F. chlamydosporum* on: (A) coleoptiles length and (B) germination rate after 4 days.

Pathogenicity on wheat crowns

It should be pointed out that artificially infected durum wheat plants with *F. chlamydosporum* isolate exhibit visible crown necrosis. The results indicate that the isolate of *F. chlamydosporum* affect both the seedling emergence rate (60% with the Waha variety), the length of the root and vegetative system (75.04% and 82.58% respectively, with Waha variety) and the fresh weight of the root and vegetative system (36.67% and 71.15% with Bousselam and Waha varieties, respectively) (Table 2).

Statistically, the results showed a very highly significant difference ($P<0.001$) between the three varieties of durum wheat tested when it regards to the root system length. Indeed, the average length of the three varieties Bousselam, GTAdur and Waha varies from 11.75, 13.65 to 2.75cm respectively. The results showed also a highly significant difference ($p<0.01$) between the three varieties of durum wheat tested when it regards to the length of the vegetative system. Indeed, the average of the length of the three varieties Bousselam, GTAdur and Waha varies from 14.33, 15.85 to 2.63cm respectively (Figure 11).

Otherwise, the results indicated a significant difference ($p<0.05$) between the three durum varieties tested when it regards to the fresh weight of the vegetative system. Indeed, the average of the fresh weight of the three varieties Bousselam, GTAdur and Waha varies from 0.035, 0.042 and 0.015g respectively. This indicates a variable behavioral of durum wheat varieties when they are infected or not by the *F. chlamydosporum* (Table 2).

Finally, the results showed no significant difference ($p>0.05$) between the three varieties of durum wheat tested when it comes to the fresh weight of the root system. Indeed, the average fresh weight of the three varieties Bousselam, GTAdur and Waha varies from 0.0195, 0.019 to 0.032g respectively. It is concluded that the three varieties of durum wheat used have a very similar behaviour, whether infected or not by this pathogenic fungus (Figure 11).

Table 2. Results of the pathogenicity test on wheat crown

Variety	Treatment	FWRS	% FWRS reduction	FWVS	% FWVS reduction	LRS	% LRS reduction	LVS	% LVS reduction
Bousselam	F.ch26	0.019[a]	36.67	0.035[a]	46.97	11.75[a]	20.38	14.33[a]	4.82
	Control	0.030	0.00	0.066	0.000	14.75	0.000	15.05	0.000
GTAdur	F.ch26	0.019[a]	17.39	0.042[a]	26.32	13.65[a]	10.77	15.85[a]	2.18
	Control	0.023	0.00	0.057	0.000	15.3	0.000	16.2	0.000
Waha	F.ch26	0.032[a]	3.03	0.015[b]	71.15	2.75[b]	75.04	2.63[b]	82.58
	Control	0.033	0.00	0.052	0.000	11.00	0.000	15.11	0.000

FWRS: Fresh weight root system; **FWVS**: Fresh weight vegetative system; **LRS**: Length root system**; LVS**: Length of vegetative system; **LVS:** Length of vegetative system. Means with the same letter in the same column are significantly different at $P < 0.05$ as determined by Duncan test.

The results showed a very highly significant difference between the fungus tested and the control when it regards to the length of the vegetative ($p<0.001$) and a highly significant difference when it regards to the fresh weight of vegetative and root systems ($p<0.05$). On the other hand, the results showed no significant effect ($p>0.05$) between the fungus tested and the control when it comes to the fresh weight of the root system. This proves that the fungus is pathogenic, at least along the length of the root and vegetative system and the fresh weight of the vegetative system but not on the fresh weight of the root system (Figure 11).

For the symptoms observed are either observed in the vegetative system that are necrosis (observed at the collar) and yellowing that usually end in the mortality of the plants, or symptoms observed in the

root system that are a necrosis on several parts of the roots and a less developed root system with weak branching related with short roots (short length) compared to root length of controls (Figure 11).

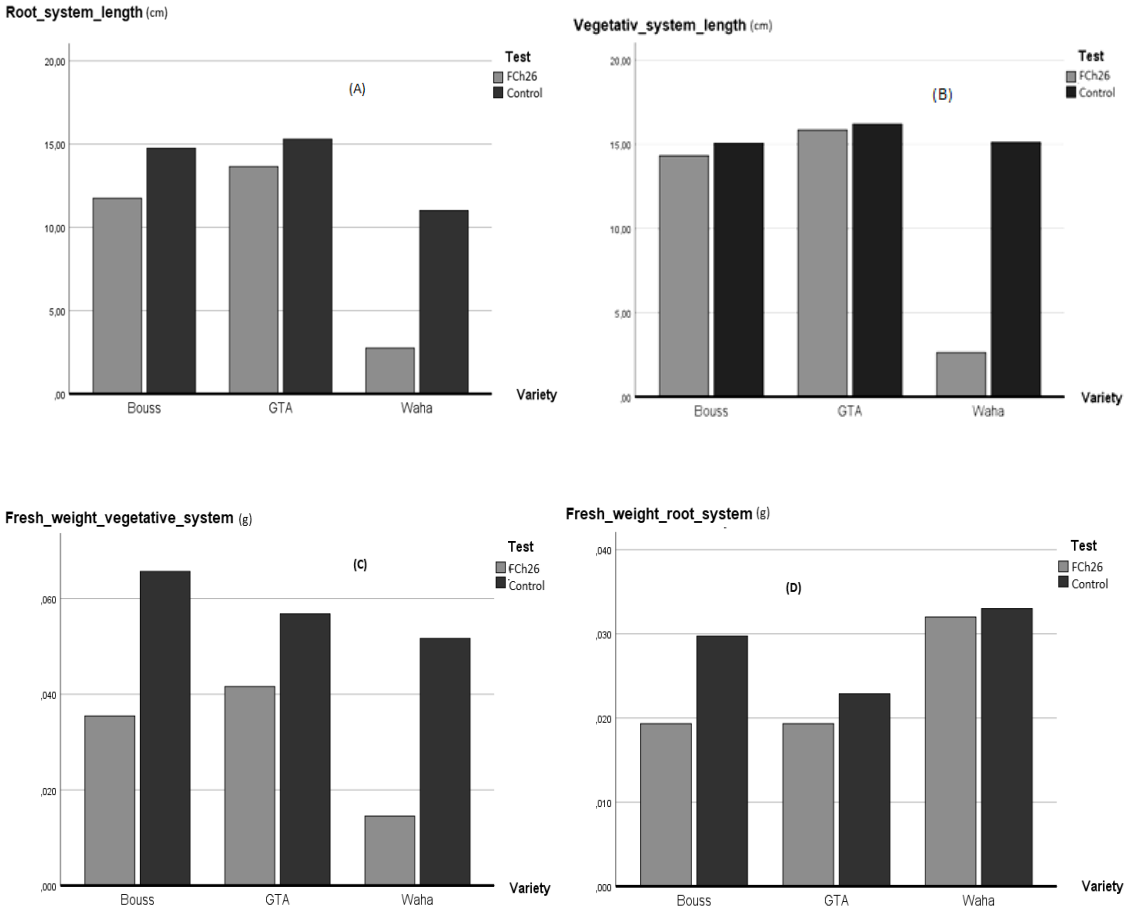

Figure 11. Impact of *F.chlamydosporium* on: (A) root system length, (B) vegetative system length, (C) vegetative system fresh weight and (D) root system fresh weight.

The anamorphic genus *Fusarium* is a phytopathogenic fungus with a worldwide distribution, able of infecting a large range of agricultural crops like orchid, maize or barley and wheat plants, and also decrease quality and yield of the grain (Seddique *et al.*, 2010).

Many species of the genus *Fusarium* are causing the most serious diseases affecting root and head of wheat plant and in all growing stages, still has not been adequate investigated in Algeria (Abdellah-Nekkach *et al.*, 2019).

Traditionally, species of *Fusarium* have been separated and described based on morphological and cultural characteristics, such as pigment production, presence colour of mycelia, phialides, macro-, micro and meso-conidia shape-size, sporodochia and chlamydospores (Burgess *et al.*, 1994; Lesli & Summerell, 2006). Cultural methods coupled with morphological details from microscopy, are among the techniques used and assign one to detect exactly which taxon is present (identification). These methodologies are easy, fast and consistent in the given putative species name, however, have limitations which impede a proper assessment.

In this study, a *Fusarium* isolate previously identified on the basis of morphological characteristics as *F. chlamydosporum* was confirmed by molecular sequencing using the gene sequences of the ITS1 and EF1 regions of the rDNA.

The phylogenetic analysis, showed that this isolate gave the same gene sequence to each other (97-99% of similarity) and to the referred *F. chlamydosporum* strains (AY213655 and AY337448, Lesli & Summerell, 2006) (88,74 and 84,98% of similarity) from NCBI.

Few researches are conducted on the pathogenicity of *F. chlamydosporum* (especially on durum wheat) which makes it difficult to compare our results with other researches. However, *F.chlamydosporium* can affect other plants species like guava (*Psidium guajava* L.) (Gupta & Misra, 2012) and *Coleus forskohlii* (Singh *et al.*, 2009). Lazreg *et al*. (2014) reported that *F. chlamydosporum* can affect the Aleppo-pine seedlings and cause a root growth inhibition, germination reduction and vegetative system length reduction with 51.94%, 52% and 47.24% respectively. Those results are considered smaller than ours obtained with wheat waha variety, and greater only for vegetative system length comparing to GTAdur and Bousselem varieties. Njeru *et al*. (2016), reported that *F. chlamydosporum* was the most prevalent in crop residues sampled from wheat fields (24%).

Our results showed that *F. chlamydosporum* was very pathogenic on wheat plant, especially if it was compared with *F.graminearum* and *F.culmorum* which they are considered the responsible of crown rot and FHB of wheat plant (Wu *et al.*, 2005; Abdellah-Nekkach *et al.*, 2019). Hudec (2007), reported that *F. culmorum* and *F.graminearum* were the most severe pathogens towards coleoptile development of wheat and barley, the coleoptile length reduction was 91,32% with *F.culmorum* and 78.32% with *F.graminearum*, these results are not far from ours (coleoptile length reduction), which confirm the high pathogenicity of *F.chlamydosporum*.

In research on the mycotoxins produced by *F. chlamydosporum*, Savard *et al*. (1990) reported that the latter could produce a new mycotoxin in addition to the mycotoxin listed in other researches, such as trichothecene and neosolaniol monoacetate, isolated from a single-spore culture of *F. chlamydosporum* by Lansden and his colleagues in 1978, he named this new mycotoxin the Chlamydosporol. O'Donnell *et al*. (2018) has been reviewed that the *F. chlamydosporum* can produce a several mycotoxine like moniliformin (MON), beauvericin (BEA) and butenolide (BUT).

These mycotoxins can affect the severity of affection on both coleoptile emergence and length, root system length and weight and finally vegetative system length and weight, this may be due to the fact that the mycotoxin inhibit production of plant protein and enzymes responsible for stimulating host plant defences (Khalifah & Matny, 2013), Studies have also shown that increasing the amount of the fungal toxin, increases the pathogenesis of the fungus, either by its effect on protein synthesis within plant cells or by stimulating the plant to produce hydrogen peroxide which stimulates programmed cell death (Burlakoti *et al.* 2007; Aleandri *et al.* 2007; Desmond *et al.* 2008).

This study identified *Fusarium chlalydosporum* for the first time on durum wheat in Algeria and highlighted its pathogenicity on the three varieties of durum wheat, namely Waha, Bousselam and GTAdur. This pathogenicity was materialized through the drastic reduction of seeds germination and coleoptile length of durum wheat. It has also affected negatively some growth parameters like the length and fresh weight of root and vegetative systems.

References

Abdallah-Nekache, N., Laraba, I., Ducos, C., Barreau, C., Bouznad, Z. & Boureghda, H. 2019. Occurrence of *Fusarium* head blight and *Fusarium* crown rot in Algerian wheat: identification of associated species and assessment of aggressiveness. *European Journal of Plant Pathology*, 154: 499–512.

Aleandri, M. P., Magro, P. & Chilosi, G. 2007. Modulation of host pH during the wheat *Fusarium culmorum* interaction and its influence on the production and activity of pectolytic enzymes. *Plant Patholology*, 56: 517-525.

Balmas, V., Scherm, B., Marcello, A., Beyer, M., Hoffmann, L., Migheli, Q. & Pasquali, M. 2015. *Fusarium* species and chemotypes associated with *Fusarium* head blight and *Fusarium* root rot on wheat in Sardinia. *Plant Pathology*, 64: 972-979.

Beccari, G., Prodi, A., Pisi, A., Nipoti, P., Onofri, A., Nicholson, P., Pfohl, K., Karlovsky, P., Gardiner, D. M. & Covarelli, L. 2018. Development of three *Fusarium* crown rot causal agents and systemic translocation of deoxynivalenol following stem base infection of soft wheat. *Plant Pathology*, 67: 1055 –1065.

Burlakoti, R. R., Estrada, R.J., Rivera, V.V., Boddeda, A., Secor, G.A. & Adhikari, T.B. 2007. Real-time PCR quantification and mycotoxin production of *Fusarium graminearum* in wheat inoculated with isolates collected from potato, sugar beet, and wheat. *Phytopathology*, 97: 835- 841.

Burgess, L. W., Summerell, B. A., Bullock, S., Gott, K. P. & Backhouse, D. 1994. *Laboratory Manual for Fusarium Research*, 3rd ed. Department of Crop Science, University of Sydney, Sydney.

Carbone, I. & Kohn, L.M. 1999. A method for designing primer sets for speciation studies in filamentous ascomycetes. *Mycologia*, 91: 553-556.

Chakraborty, S., Liu, C. J., Mitter, V., Scott, J., Akinsanmi, O. A., Ali, S., D ill-Macky, R., Nicol, J., Back house, D. & Simpfendorfer, S. 2006. Pathogen population structure and epidemiology are keys to wheat crown rot and *Fusarium* head blight management. *Australasian Plant Pathology*, 35: 643 – 655.

Chekali, S., Gargouri, S., Berraies, S., Nicol, M. J. & Nasraoui, B. 2013. Impact of *Fusarium* foot and root rot on yield of cereals in Tunisia. *Tunisian Journal of Plant Protection*, 8: 75–86.

Covarelli, L., Beccari, G., Steed, A., and Nicholson, P. 2012. Colonization of soft wheat following infection of the stem base by *Fusarium culmorum* and translocation of deoxynivalenol to the head. *Plant Pathology*, 61: 1121-1129.

Desmond, O.J., Manners, J.M., Stephens, A.E., Maclean, D.J., Schenk, P.M., Gardiner, D.M., Munn, A.L. & Kazan, K. 2008. The *Fusarium* mycotoxin deoxynivalenol elicits hydrogen peroxide production, programmed cell death and defense responses in wheat. *Molecular Plant Pathology*, 9: 435-445.

Fernandez, M. R. & Jefferson, G. 2004. Fungal populations in roots and crowns of common and durum wheat in Saskatchewan. *Canadian Journal of Plant Pathology*, 26: 325-334.

Goodwin, D.C. Lee, S.B. 1993. Microwave mini prep of total genomic DNA from fungi, plants, protists and animals for PCR. *Biotechniques*, 15: 438-444.

Gupta, V.K. & Misra, A.K. 2012. *Fusarium chlamydosporum*, causing wilt disease of guava (*Psidium guajava* L.) in India, *Archives of Phytopathology and Plant Protection*, 45: 2425-2428.

Hollaway, G. J., Evans, M. L., Wallwork, H., Dyson, C. B., and Mc Kay, A. C. 2013. Yield loss in cereals, caused by *Fusarium culmorum* and *F. pseudograminearum*, is related to fungal DNA in soil prior to planting, rainfall, and cereal type. *Plant Disease*, 97: 977-982.

Hudec, K. 2007. Pathogenicity of fungi associated with wheat and barley seedling emergence and fungicide efficacy of seed treatment. *Biologia, Bratislava*, 62: 287-291.

Kezih, R., Bekhouche, F. & Merazka, A. 2014. Some traditional Algerian products from durum wheat. *African Journal of Food Science*, 8: 30–34.

Khalifah, M. & Matny, O. 2013. Pathogenicity evaluation of *Fusarium spp* isolates, causal agent of crown rot disease in wheat. *The Iraqi Journal of Agricultural Sciences*, 44: 480-489.

Kimura, M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Appl. Environ. Microbiol*, 16: 11-120.

Lansden, J. A., Cole, R. J., Dorner, J. W., Cox, R. H., Cutler, H. G. & Clark, J. D. 1978. A new trichothecene mycotoxin isolated from *Fusarium tricinctum*. *J Agric Food Chem*, 26: 246-249.

Laraba, I., Boureghda, H., Abdallah, N., Bouaicha, O., Obanor, F., Moretti, A., Geiser, D. M., Kim, H. S., McCormick, S. P., Proctor, R. H., Kelly, A. C., Ward, T. J. & O'Donnell, K. 2017. Fungal population genetic structure and mycotoxin potential of the wheat crown rot and head blight pathogen *Fusarium culmorum* in Algeria. *Fungal Genetics and Biology*, 103, 34–41.

Lazreg, F., Belabid, L., Sanchez, J., Gallego, E. & Bayaa, B. 2014. Pathogenicity of Fusarium spp. associated with diseases of Aleppo-pine seedlings in Algerian forest nurseries. *Journal of Forest Science*, 60: 115–120.

Leslie, J. F. & Summerell, A. B. 2006. *The Fusarium laboratory manual*. Blackwell Publishing. Ames, USA.

LNPV: Laboratoire National de la Protection des Végétaux. 2008. Toutes céréales, détection et identification des espèces de *Fusarium spp* et *Microdochium nivale* sur grains de céréales par isolement mycologique semi-sélectif et étude microbiologique. Réf. MH-03.16 : version B.

MADRP. 2016. Statistiques agricoles. Ministère de l'agriculture et du développement durable et de la pêche. Alger, Algérie.

Mesterhazy, A. 1983. Breeding wheat for resistance to *Fusarium graminearum* and *F.culmorum*. *Zeitschrift für Pflan zenzüchtung*, 91:295 –311.

Njeru, N.K., Muthomi, J.W., Mutegi, C.K. & Wagacha, J.M. 2016. Effect of cropping systems on accumulation of *Fusarium* head blight of wheat inocula in crop residues and soils. *Journal of Plant Sciences*, 11: 12-21.

Obanor, F. & Chakraborty, S. 2014. Aetiology and toxigenicity of *Fusarium graminearuman* and *F.pseudograminearum* causing crown rot and head blight in Australia under natural and artificial infection. *Plant Pathology*, 63: 1218–1229.

O'Donnell, K., McCormick, S.P., Busman, M., Proctor, R.H., Ward, T.J., Doehring, G., Geiser, D.M., Alberts, J.F. & Rheeder, J.P. 2018. Marasas *et al.* 1984 "Toxigenic *Fusarium* Species: Identity and Mycotoxicology" revisited. *Mycologia*, 6: 1058-1080.

Saitou, N. & Nei, M. 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution*, 4: 406-425.

Saremi, H., Ammarellou, A. & Jafary, H. 2007. Incidence of crown rot disease of wheat caused by *Fusarium pseudograminearum* a new soil born fungal species in North West Iran. *Pakistan Journal Biological Sciences*, 10: 3606 –3612.

Savard, M.E., Marc, E., Miller, D.J., Salleh, B. & Strange, R. 1990. Chlamydosporol, a new metabolite from *Fusarium chlamydosporum*. *Mycopathologia*, 110: 177-181.

Scherm, B., Balmas, V., Spanu, F., Pani, G., Delogu, G., Pasquali, M. & Migheli, Q. 2013. *Fusarium culmorum*: causal agent of foot and root rot and head blight on wheat. *Molecular Plant Pathology*, 14: 323 –341.

Seddique, S., Yusuf, U. & Zainudi, N. 2010. Morphological and molecular detection of *Fusarium chlamydosporum* from root endophytes of *Dendrobium crumenatum. African Journal of Biotechnology*, 9: 4081-4090.

Singh, R., Parameswaran, T.N., Prakasa Rao, E.V.S., Puttanna, K., Kalra, A., Srinivas, K.V.N.S., Bagyaraj D.J. & Divya, S. 2009. Effect of *arbuscular mycorrhizal* fungi and *Pseudomonas fluorescens* on root-rot and wilt, growth and yield of *Coleus forskohlii*. *Biocontrol Science and Technology*, 19: 835-841.

Touati-Hattab, S., Barreau, C., Verd al-Bonnin, M. N., Chereau, S., Richard-Forget, F., Hadjout, S., Mekliche, L., and Bouznad, Z. 2016. Pathogenicity and trichothecenes production of *Fusarium culmorum* strains causing head blight on wheat and evaluation of resistance of the varieties cultivated in Algeria. *European Journal of Plant Pathology*, 145: 797-814.

White, T. J., Bruns, T., Lee, S. & Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. pp. 315-322, In: Innis, M. A., Gelfand, D. H., Sninsky, J. J. & White, T. J. (Eds) *PCR Protocols: a guide to methods and applications*. Academic Press, San Diego.

Wu, A.B., Li, H.P., Zhao, C.S. & Liao, Y.C. 2005. Comparative pathogenicity of *Fusarium graminearum* isolates from China revealed by wheat coleoptile and floret inoculations. *Mycopathologia*, 160: 75-83.

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اولین گزارش از *Fusarium chlamydosporum* عامل پوسیدگی طوقه و مرگ گیاهچه گندم دوروم در الجزایر

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چکیده

در قسمت شرقی الجزایر گندم دوروم را چندین گونه از *Fusarium* آلوده می‌کنند. در ۵۰ نقطه در شرق الجزایر به طور تصادفی از قارچ‌های اندوفیت بذور گندم نمونه‌برداری شد و بر اساس ویژگی‌های ماکروسکوپی و میکروسکوپی وجود گونه *F. chlamydosporum* اثبات گردید. بررسی ملکولی با استفاده از آغازگرهای EF1 و ITS1 وجود قارچ *F. chlamydosporum* را برای اولین بار در بذور گندم دوروم در الجزایر ثابت کرد. آزمون بیماریزایی به منظور بررسی اثر این جدایه بر روی کولئوپتیل و بخش پایینی در سه رقم گندم دوروم انجام شد. نتایج نشان داد که قارچ *F. chlamydosporum* بر روی جوانه زنی بذر این سه رقم تحت بررسی اثر منفی داشت. در واقع، کاهش جوانه زنی بذور دوروم بین ۸۵ و ۹۲ درصد در ارقام GTAdur و Waha متغیر بود. به علاوه، قارچ *F. chlamydosporum* به ترتیب باعث کاهش طول ۶۰/۹۷ تا ۷۰/۰۵ درصدی در طول کولئوپتیل رقم‌های GTAdur و Waha شد. از طرف دیگر، تأثیر بسیار متفاوتی در پارامترهای رشد گیاه مشاهده گردید. بدین ترتیب که اثر قارچ بر کاهش طول ریشه و وزن اندام رویشی سبز رقم Waha به ترتیب ۷۵/۰۴ و ۷۱/۱۵ درصد بود و در مورد وزن تر ریشه رقم Bousselam مقدار قابل توجهی بود. این سطوح بالای آلودگی موجب می‌شود تا در برنامه‌های تیماری و صدور گواهی بذور در مورد این گونه از فوزاریوم تجدید نظر گردد.

واژه‌های کلیدی: *Fusarium chlamydosporum*، پوسیدگی طوقه، گندم دوروم، الجزایر