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Pathogenicity assessment of *Fusarium clavum* associated with wheat head blight in Algeria

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Abstract: Durum wheat (*Triticum durum* Desf) is one of the most important cereal crops in Algeria. During the agricultural season 2021-2022, typical symptoms of *Fusarium* Head Blight were observed in wheat fields in the Setif region. One of the causal agents was identified on the basis of morphological characters and DNA sequences as *Fusarium clavum* J.W. Xia, L. Lombard, Sand.-Den., X.G. Zhang & Crous, a member of the *Fusarium incarnatum-equiseti* species complex. It was isolated from symptomatic wheat glumes. In vitro and in vivo pathogenicity tests were conducted on three Algerian durum wheat varieties to assess the effect of this isolate on the seedling and the mass of durum wheat kernels. The results showed that *Fusarium clavum* caused a significant reduction in the coleoptile (38.9 %) and root length (42 %) and decreased kernels mass by 20.8 %. This study further confirms the presence of *Fusarium clavum* as an agent causing *Fusarium* Head Blight on wheat in Algeria.

Key words: durum wheat, *Fusarium clavum*, pathogenicity, Algeria.

Ocena patogenosti glive *Fusarium clavum*, kot povzročiteljice pšeničnega ožiga v Alžiriji

Izvleček: Trda pšenica (*Triticum durum* Desf) je eno izmed najpomembnejših žit v Alžiriji. V pridelovalnih sezonah 2021-2022 so bili na pšeničnih poljih na območju Setifa opaženi značilni simptomi pšeničnega ožiga, ki ga povzroča gliva iz rodu *Fusarium*. Kot povzročitelj je bila na osnovi morfoloških znakov in DNK zaporedij prepoznana gliva *Fusarium clavum* J.W. Xia, L. Lombard, Sand.-Den., X.G. Zhang & Crous, predstavnica iz kompleksa vrst *Fusarium incarnatum-equiseti*. Izolirana je bila iz simptomatičnih pčeničnih plev. In vitro in in vivo testi patogenosti so bili narejeni na treh alžirskih sortah trde pšenice za oceno učinka tega izolata na sejanke in maso pšeničnih zrn. Rezultati so pokazali, da je gliva *Fusarium clavum* povzročila značilno zmanjšanje dolžine koleoptile (38,9 %) in dolžine korenin (42 %) ter zmanjšala maso zrn za 20,8 %. Raziskava potrjuje prisotnost glive *Fusarium clavum* kot fuzarijskega povzročitelja pšeničnega ožiga v Alžiriji.

Ključne besede: trda pšenica, *Fusarium clavum*, patogenost, Alžirija

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1 INTRODUCTION

Wheat (*Triticum aestivum* L.) is a key cereal crop and a major global source of food for people. Due to its strategic importance for human and animal nutrition, wheat occupies a privileged place in Algerian agriculture. For instance, it is used to make bread and other Algerian food as couscous, which is the country's most popular dish (Kezih et al., 2014). Numerous phytopathogenic *Fusarium* species are found around the world, infecting a variety of crop plants, including cereals such as wheat, maize, oats, and barley (Boutigny et al., 2011). *Fusarium* drastically reduces grain quality and output. More significantly, several species of the *Fusarium* genus produce mycotoxins, which prevent the synthesis of proteins and as a result, exposure to these mycotoxins can lead to several health issues in humans and animals (Pestka, 2010). The wheat head, grain, and occasionally the peduncle are the only areas where *Fusarium* head blight (FHB) symptoms can occur. Usually, when healthy heads are still green, the first discernible indication is the bleaching of a part of or all the spikelets. Spikelets above and below the initial spot of infection may also become bleached as the fungus spreads across the rachis. When examining infected spikelets, pink to orange masses of spores may be seen. It is during rainy, humid conditions that these spore masses are generated. Infected kernels, sometimes known as tombstones, have a shriveled, discolored appearance and are low in mass (Freije & Wise, 2015). There is currently not enough information available in Algeria regarding the causative agent of *Fusarium* head blight of durum wheat (Laraba et al., 2017); besides, *Fusarium clavum* was discovered for the first time in Algeria in a study by Belabed et al. (2025), as a cause of this disease in durum wheat. This study was conducted to isolate and identify *Fusarium clavum* species from infected wheat in the Setif region of Algeria and to evaluate their pathogenicity towards durum wheat varieties. Through morphological characterization, molecular identification, phylogenetic study, and pathogenicity tests conducted in vitro and in vivo, we aim to report *Fusarium clavum* as a head blight agent on durum wheat in Algeria.

2 MATERIALS AND METHODS

2.1 FUNGAL MATERIAL

2.1.1 Isolation of *Fusarium clavum*

In this study, symptomatic infected wheat samples were collected randomly during the agricultural season 2021/2022 from several farms located in the Setif region, Algeria.

Diseased samples (seeds, glumes, and crowns) were disinfected in 2 % sodium hypochlorite (NaClO) (commercial bleach, Bref, Henkel, Algeria) for 5 min and rinsed three successive times with sterile distilled water (Benhamou & Chet, 1996). They were then dried between two sheets of sterile paper towels, plated on potato dextrose agar (PDA) medium in sterile Petri dishes, and incubated at 25 °C in the dark for seven days. Resultant colonies resembling *Fusarium* spp. were further sub-cultured on PDA for purification.

2.1.2 Macroscopic and microscopic characterization

A mycelial disc subculture from strain F15B was placed on the PDA for evaluating macroscopic characteristics (Leslie & Summerell, 2006 and Xia et al., 2019). After a 21 - day incubation period in the dark at 25 °C, the morphological characterization was conducted based on the colony's growth, appearance, and texture, and the pigmentation of the Petri dish's face and reverse was examined macroscopically (Leslie & Summerell, 2006 and Xia et al., 2019). Synthetic Nurtrient-poor Agar medium (SNA) (Nirenberg, 1976) was used for the diagnosis of micromorphological characteristics. The strain was observed under a light microscope to describe microscopic characters. The presence or absence of microconidia and macroconidia, their shapes and sizes, and the presence or absence of chlamydospores are diagnostic characteristics used for the identification of *Fusarium* species (Leslie & Summerell, 2006 and Xia et al., 2019).

2.1.3 Molecular identification and phylogenetic analysis

Molecular analysis was performed to validate identification of strain F15B. The mycelium was harvested, and DNA was extracted using Nucleo Spin Plant II kit (Macherey-Nagel Germany). The internal transcribed spacers of ribosomal DNA (ITS) and the transcription elongation factor 1 alpha (TEF1) were amplified using two primers ITS1/ITS4 (CTTGGTCATTTAGAGGAAGTAA/ TCCTCCGCTTATTGATATGC) (White et al., 1990), and EF-728F/EF-2 (CATYGAGAAGTTCGAGAAGG/ GGARGTACCAGT SATCATGTT) (O'Donnell et al., 1998; Carbone & Kohn, 1999). The PCR products were purified using the NucleoSpin® Gel and PCR Clean-up kit from Macherey-Nagel (Germany). Amplicons were sequenced with the Sanger technique

(Sanger et al., 1977). The sequences were edited using MEGA 11 software and compared with sequences in databases by using Blastn (National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov). MEGA 11 was used to phylogenetically place our sample. The tree is based on TEF1 sequences and calculated by using neighbor-joining clustering and bootstrap analyses with 1,000 replications.

2.2 PATHOGENICITY TESTS

2.2.1 Pathogenicity test of F15B isolate towards wheat seedling

A pathogenicity assay was performed following the protocol of Belabed et al. (2023) with some modifications, using strain F15B to determine its pathogenic potential. Durum wheat seeds from each Algerian variety (Guemgoum Rkham, Djnah Khotaifa, and Oued Znati) were surface disinfected for 5 min in 2 % NaClO (commercial bleach, Bref, Henkel, Algeria), rinsed three times in sterile distilled water, and dried. Five healthy wheat seeds from the three varieties were each inoculated with a 5mm diameter fungal disc taken from a 21 day old PDA culture and a sterile PDA disc as a control. Three replicates were set up for all combinations of F15B isolate and wheat variety. The inoculated seeds were placed on sterile double-layer filter paper soaked with Potato Dextrose Broth (PDB) in Petri dishes. Petri dishes were sealed with parafilm and incubated in the dark at 25 °C for five days. The length of the root system, coleoptile, and germination rate were measured to assess the pathogenicity. The reduction rate of coleoptile length (CLr %) and the reduction rate of root length (RLr %) were calculated using the following equation:

$$\text{CLr (\%) or RLr (\%)} = \frac{\text{Length of coleoptile or root (control)} - \text{Length of coleoptile or root (Fusarium-treated)}}{\text{Length of coleoptile or root (control)}} \times 100$$

2.2.2 Pathogenicity test of F15B isolate on wheat heads

For the pathogenicity test on Algerian durum wheat heads (Guemgoum Rkham, Djnah Khotaifa, and Oued Znati varieties), the experimental spray inoculation protocol of Mesterhazy (1995) was used. For each variety, three durum wheat seeds were sowed in plastic pots with four replicates distributed randomly. A spore suspension of 4×10^5 spores ml^{-1}, obtained from F15B cultures was prepared using a Malassez hemocytometer. 3 spikes from each replicate were inoculated with spores during the full flowering stage, using glass sprayers, while the control spikes were sprayed with sterile distilled water. The inoculated spikes were covered with a damp polyethylene bag to retain humidity for 24 hours. For comparison purposes, the same experiment was conducted using a known *Fusarium culmorum* isolate (PV123206), which is recognized as pathogenic to wheat heads. This allowed assessing the potential impact of F15B isolate on wheat heads. After harvesting, the ears of each statistical unit were placed in a paper bag. Treated and untreated wheat spikes were harvested and threshed, and the kernels mass was measured. The mass of a thousand kernels (TKM) was then estimated. Subsequently, the reduction rate in the thousand-grain mass (TKMr %) of the *Fusarium culmorum* and *Fusarium clavum*-inoculated spikes was calculated using the following equation:

$$\text{TKMr (\%)} = \frac{\text{Mass of 1000 grains (control)} - \text{Mass of 1000 grains (}\textit{Fusarium}\text{ inoculated)}}{\text{Mass of 1000 grains (control)}} \times 100$$

2.3 STATISTICAL ANALYSIS

Data was subjected to analyses of variance ANOVA using SPSS software (IBM SPSS Statistics version 26) at a 5 % level with a 95 % confidence interval.

3 RESULTS AND DISCUSSION

3.1 MACROSCOPIC AND MICROSCOPIC CHARACTERIZATION

Fungal colonies that resembled *Fusarium* spp. were obtained. Only a single *Fusarium clavum*-like isolate was obtained from durum wheat glumes and assigned as F15B. The morphological characters observed on SNA and PDA (not shown) agreed with the phylogenetic analyses.

3.2 MOLECULAR IDENTIFICATION AND PHYLOGENETIC ANALYSIS

The obtained sequences of F15B isolate were deposited in GeneBank under accession numbers OR900216 (ITS) and PP035912 (TEF). The blastn search of ITS showed 98-99 % identity with several sequences of *Fusarium clavum* (e.g: OR582979, OR123379), while the

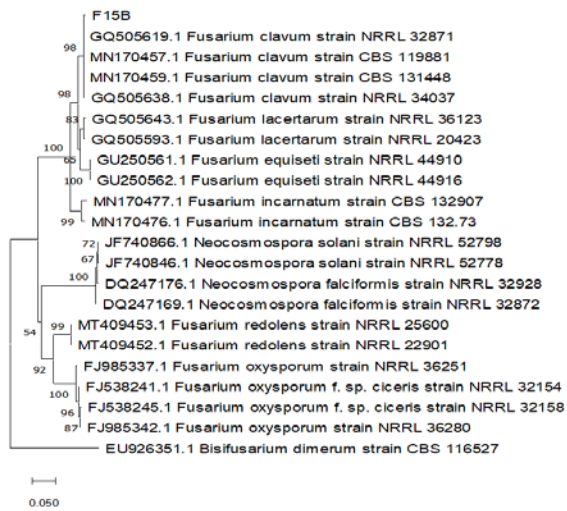

Figure 1: Phylogenetic tree generated from translation elongation factor 1-alpha (TEF1) gene sequences of *Fusarium* spp. Bootstrap values (based on 1,000 replications) are indicated next to the branches. Based on the Neighbor-joining method, isolate F15B clusters with *F. clavum* isolates. The tree is rooted with *Bisifusarium dimerum* (Penz.) L. Lombard & Crous strain CBS 116527

TEF sequence showed 99-100 % with *Fusarium clavum* isolates (e.g: GQ505672, MN170457).

3.3 PATHOGENICITY TESTS

3.3.1 Pathogenicity test of F15B isolate towards wheat seedling

In vitro, the isolate F15B was responsible for 29,9 %, 60,4 %, and 26,3 % of coleoptile length reduction on Guemgoum Rkham, Djnah Khotaifa, and Oued Znati varieties. Furthermore, it induced 25,7 %, 64,3 %, and 35,9 % of root length reduction on Guemgoum Rkham, Djnah Khotaifa, and Oued Znati varieties (Tab. 1).

After 5 days of incubation, it appears that 'Djnah Khotaifa' was more sensitive to *F. clavum* as coleoptile length and root length reduction were highest compared to vars. Guemgoum Rkham and Oued Znati. Also, it appears that var. Djnah khotaifa was most susceptible to attacks of F15B isolate with a germination rate of 93 % compared to vars. Guemgoum Rkham (100 % germination rate) and Oued Znati (95,3 %) (Tab. 1).

There was no statistically significant difference between coleoptile length reduction rate ($p = 0.33 > 0.05$), root length reduction rate ($p = 0.31 > 0.05$), and seed germination rate ($p = 0.39 > 0.05$) of the controls of the tested durum wheat varieties. On the contrary, the results showed that there was a significant difference ($p < 0.05$) between the infected plants and the non-infected control plants. This result shows that *Fusarium clavum* F15B isolate was pathogenic and significantly affected coleoptile and root lengths (Tab.1).

3.3.2 Pathogenicity test of F15B isolate on wheat heads

Twenty-six days after inoculation, typical *Fusarium* head blight symptoms appeared on the inoculated spikes, while the control spikes remained free of symptoms. After harvest, inoculated grains exhibited deformities compared to the negative controls. Re-isolation from the infected grains and glumes was conducted to satisfy Koch's postulates. The results of TKM after means comparing (Tab. 1) showed that *Fusarium clavum* reduced TKM from 55,9 g to 44,8 g in 'Guemgoum Rkham', and from 45,3 g to 26,5 g in 'Djnah Khotaifa', compared to the negative controls. On the other hand, no TKM reduction was observed in 'Oued Znati'. Concerning the rate of TKM reduction (TKMr %) by the isolate F15B, the results were 21,1 % for 'Guemgoum Rkham' and -7,1 % for 'Oued Znati'. However, it appears that 'Djnah Khotaifa' was the most susceptible to attacks of the isolate with a TKMr % of 41,3 %. The pathogenic isolate *Fusarium culmorum* (PV123206) exhibited a higher pathogenic potential, causing severe disease symptoms in the tested wheat varieties. This was reflected in the TKM, recorded as 29,9 g for var. Goumgoum Rkham, 16,5 g for var. Djenah Khotifa, and 29,1 g for var. Oued Zenati. Compared to the non-infected plants, this represents a TKMr % of 44,7 % for var. Goumgoum Rkham, 63 % for var. Djenah Khotifa, and 36,3 % for var. Oued Zenati. These reductions were higher than those caused by *Fusarium clavum* isolate.

Statistical analysis showed a significant difference ($p = 0.01 < 0.05$) between the inoculated and uninoculated control spikes. The reduction in TKM caused by the F15B isolate is directly associated with yield and quality loss, confirming its pathogenic impact on wheat heads.

The most serious diseases affecting root, stem, and spike of wheat and at all stages of growth are caused by *Fusarium* species. In Algeria, some studies have been carried out on *F. culmorum* the main *Fusarium* Head Blight agent focusing on its occurrence, pathogenicity, and diversity. Other pathogenic *Fusarium* species have not yet been studied well in Algeria (Touati-Hattab et al., 2016; Laraba et al., 2017; Abdallah-Nekache et al., 2019).

Fusarium clavum is a species of the *Fusarium incarnatum-equiseti* species complex (FIESC) which is a

Table 1: Observed effects of pathogenicity tests and thousand kernels mass reduction

Varieties	Treatment	Coleoptile length (cm)	CLr (%)	Germination (%)	Root length (cm)	RLr (%)	TKM (g)	TKMr (%)
Guemgoum Rkham	Control	5,03	0	100	5,5	0	55,87	0
	F15B	3,36	29,93	100	4,03	25,72	44,79	21,12
Djnah Khotaifa	Control	5,3	0	100	2,53	0	45,26	0
	F15B	2	60,38	93	0,7	64,25	26,48	41,28
Oued Znati	Control	3,86	0	100	4,76	0	45,84	0
	F15B	2,86	26,29	95,33	3,03	35,86	49,08	-7,06

*Each number in the table represents the average of the replicates in each parameter (F15B: inoculated by F15B isolate)

phylogenetically species-rich complex that includes over 30 recognized phylogenetic species (Xia et al., 2019). *Fusarium clavum* was reported for the first time in Algeria in the study of Belabed et al. (2025) as a causal agent of wheat head blight. The F15B isolate was morphologically identified as *F. clavum*. It was isolated from durum wheat glumes in Algeria's Setif area in the North. The results of macroscopic and microscopic characteristics were similar to those described by Manganiello et al. (2021) and Belabed et al. (2025). The micro-morphological and plate-culturing characteristics of F15B isolate were also similar to those of *Fusarium clavum* (Wang et al., 2019; Xia et al., 2019). The phylogenetic tree, constructed using the Neighbor-Joining (NJ) method based on partial TEF1-α sequences, showed that the F15B isolate clustered with *Fusarium clavum*, indicating its genetic relatedness to this species and confirming its classification within it. This study reports the presence of an *F. clavum* isolate in Algerian durum wheat fields. In contrast, Belabed et al. (2025) identified multiple isolates of this species, further supporting its occurrence in the region.

Fusarium clavum is a plant pathogen occurring worldwide as it can infect a wide range of plant hosts, such as *Cucumis melo* L. (Meshram et al., 2023), *Solanum lycopersicum* L. (Gilardi et al., 2021), *Beta vulgaris* L. (Khan et al., 2024), *Rosa* spp (Manganiello et al., 2021) and *Phoenix dactylifera* L. (Rabaaoui et al., 2021).

Few research was conducted on the pathogenicity of *F. clavum* on wheat. *Fusarium clavum* has been identified as a pathogenic species responsible for *Fusarium* head blight in wheat in Mexico (Leyva-Mir et al., 2022). Azil et al. (2021) investigated the *Fusarium incarnatum-equiseti* species complex associated with tuber dry rot and wilt of potatoes but did not report *F. clavum*. This highlights the need for further studies on the occurrence of *F. clavum* in different crops.

The obtained results showed that strain F15B had a moderate aggressiveness toward wheat coleoptile length, root length, and seed germination, which concord with the finding of Belabed et al. (2023). They concluded that members of the *F. incarnatum-equiseti* species complex are moderate or weak pathogens. The F15B isolate had a moderate effect on wheat TKM (20.8 % TKM reduction), especially if compared with *F. culmorum* (Wm.G.Sm.) Sacc. one of the major FHB agents of wheat plants (48 % TKM reduction) (Abdallah-Nekache et al., 2019). The significantly greater virulence of *F. culmorum* aligns with its well-documented ability to induce severe disease symptoms in wheat, leading to substantial yield losses. This strong impact can be attributed to its aggressive infection mechanisms, including rapid colonization, toxin production, and disruption of host physiological processes. Additionally, the reduction rate of TKM caused by *F. culmorum* and *F. clavum* strains may be linked to the susceptibility of the wheat genotypes used in the experiment, highlighting differences in host responses to fungal infection. This parameter plays a very important role and impacts the results. In this study, var. Djnah Khotaifa was the most sensible, comparing to vars. Guemgoum Rkham and Oued Znati. This is an important information benefiting breeding programs that aim at reducing susceptibilies of cultivars against *Fusarium* head blight agents.

4 CONCLUSIONS

In Algeria, *Fusarium clavum* has been identified on wheat, and its pathogenic effects have been confirmed across three durum wheat varieties. Observed impacts included reduced seed germination, decreased coleoptile and root lengths, and reduced thousand kernel mass. Our findings highlight the importance of expanding stu-

dies focusing on determining the distribution, prevalence, and toxigenic potential of *Fusarium* species associated with wheat diseases in Algeria.

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