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ARTICLE

Aggressiveness Assessment of Two *Fusarium* spp. on Durum Wheat Grain Coleoptiles under Controlled Conditions

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ABSTRACT

Fusarium head blight (FHB) is a disease caused by several *Fusarium* species, notably, *F. culmorum* and *F. graminearum*. These pathogens adversely affect the technological and sanitary qualities of cereal grains, particularly durum wheat. Under favorable environmental conditions and in susceptible varieties, these *Fusarium* species can significantly reduce both the quantity and quality of crops. This study evaluated the pathogenicity of the two *Fusarium* species (FC2006 and FG2008) in the growth of durum wheat coleoptiles. The plant material included four commercially grown parental varieties (G9, G10, G11, G12) and eight breeding lines (G1, G2, G3, G4, G5, G6, G7, G8). *In vitro* tests revealed that both *Fusarium* species significantly reduced the coleoptile growth across the studied varieties and lines ($p \leq 0.001$). The control test had an average coleoptile length of 37.87 mm. In contrast, seeds inoculated with FC2006 had an average length of 0.62 mm, and those inoculated with FG2008 had only 0.064 mm. Although there was a slight difference in aggressiveness between the two species, it was not statistically significant ($p > 0.05$). Some variability was also noted in the responses of the durum wheat varieties and lines. The G8 genotype showed remarkable behavior in both isolates, with an average length of 1.83 mm for FC2006 and 0.4 mm for FG2008. The other genotypes showed total inhibition of coleoptile growth (0 mm). These findings highlight the importance of conducting further research on the defense mechanisms of durum wheat against *Fusarium* and assessing the local varieties' pathogenicity to better explore the interactions between these pathogens and durum wheat genotypes under *in vitro* conditions.

KEYWORDS

Fusarium species; aggressiveness; coleoptile growth; durum wheat; *in vitro* test

1 Introduction

Cereal crops are cultivated over an estimated 3.3 million hectares annually, with 1.5 million hectares of durum wheat and 600.000 hectares of soft wheat. The total cereal production in Algeria is approximately

4 million tons, with bread wheat contributing only 1% of the overall harvest [1]. Durum wheat (*Triticum turgidum* var. durum) is the most widely grown cereal crop in the Mediterranean basin, and ranks as the tenth most cultivated species globally [2]. This cereal is a main crop and staple food in certain Mediterranean regions, serving as an essential raw material for finished products such as pasta, couscous, bulgur, and various types of bread, consumed worldwide [3]. In Algeria, wheat has long been the primary dietary staple for consumers [4], and globally, it is the third most harvested crop and the most consumed grain [5].

However, wheat production is affected by severe abiotic factors, including drought, irregular rainfall, frost, extreme temperatures, and increased atmospheric CO_2 levels, all of which can drastically reduce yield and grain quality [6,7]. Additionally, plant diseases caused by pathogenic fungi are prevalent and can lead to significant losses in yield, as well as a decline in grain quality [8,9]. Fusarium head blight (FHB) is one of the most destructive fungal diseases affecting durum wheat, causing yield reductions of up to 61%. FHB is characterized by flower abortion, reduction in grain number and weight, and deterioration of grain quality due to the accumulation of harmful mycotoxins in the grain [10–12]. Consequently, FHB is a major threat to cereal production worldwide with significant economic implications [10].

The *Fusarium* genus is recognized as one of the most pathogenic and aggressive fungal groups, comprising multiple species that infect a wide range of cultivated plants and cereals, many of which are essential for both human and animal nutrition, some of them causing FHB [10]. Key targets of *Fusarium* infection include leaves, roots, stems, heads, and crop residues [13,14]. In recent years, several studies have been conducted in Algeria to identify specific *Fusarium* species involved in FHB in wheat [15–17]. Using morphological and molecular techniques, these studies have confirmed that *F. culmorum* is the dominant species in durum wheat crops affected by FHB in the region [15,17,18]. Additionally, *F. graminearum* and *F. pseudograminearum*, have been identified, with *F. cerealis* being reported for the first time in Algeria [17]. Globally, *F. graminearum* and *F. culmorum* are the two most prevalent species responsible for root, stem, and ear rots, as well as seedling blight in small-grain cereals, including durum wheat [17,19,20].

The epidemiological cycle of *Fusarium* begins with the survival of the inoculum in the soil or on plant debris, where it persists as a saprophytic mycelium or thick-walled resting spores. When crops are sown in contaminated soils, diseases such as foot rot and seedling blight, can develop. During flowering, fungal infections can spread to wheat heads through conidia dispersed by rain splash or ascospores carried by the wind [21]. *Fusarium*-contaminated seeds further Cereal crops are cultivated over an estimated 3.3 million hectares annually, with 1.5 million hectares of durum wheat and 600.000 hectares of soft wheat. The total cereal production in Algeria is approximately 4 million tons, with bread wheat contributing only 1% of the overall harvest [1]. Durum wheat (*Triticum turgidum* var. durum) is the most widely grown cereal crop in the Mediterranean basin, and ranks as the tenth most cultivated species globally [2]. This cereal is a main crop and staple food in certain Mediterranean regions, serving as an essential raw material for finished products such as pasta, couscous, bulgur, and various types of bread, consumed worldwide [3]. In Algeria, wheat has long been the primary dietary staple for consumers [4], and globally, it is the third most harvested crop and the most consumed grain [5].

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The present study aimed to evaluate the pathogenicity of *Fusarium culmorum* and *Fusarium graminearum* isolates on durum wheat coleoptile growth while identifying response variations between different varieties and lines. Objectives include measuring the impact of isolates on coleoptile length, comparing the aggressiveness of the two species on the tested genotypes, and analyzing the variability of genotype responses to identify those with increased tolerance or susceptibility. Finally, this research aims to provide useful data for future studies on the management of fusarium head blight under controlled conditions.

2 Material and Methods

2.1 Plant Material

In this study, we used four parental durum wheat varieties: Ardente, Waha, Simeto, and Vitron (Table 1). Additionally, we included eight breeding lines derived from F15 seeds, which were produced through diallel crosses involving five parental varieties: Ardente, Waha, Simeto, Vitron, and Saadi [25]. The seeds of these lines, used as experimental material, were obtained in June 2011 from plots managed by the Higher National School of Agronomy (ENSA), within the Plant Production Department.

2.2 Fungal Material

The pathogenic fungal material that was subjected to *in vitro* test consisted of two *Fusarium* isolates, FG2008 and FC2006, belonging to *F. graminearum* and *F. culmorum* species, respectively. These isolates were identified using a combination of morphological observations of mycelia growth on Potato Dextrose

Agar (PDA) and microscopic examination of conidia, following the method described by Leslie et al. [26]. Species identification was further confirmed by PCR, using species-specific primers [15,17].

Table 1: F15 genealogical lines and parental varieties of durum wheat used in the study

Lines and varieties		Code
Lines	Saadi × Waha 431	G1
	Ardente × Waha 423	G2
	Ardente × Waha 221	G3
	Ardente × Simeto 133	G4
	Ardente × Simeto 151	G5
	Ardente × Simeto 164	G6
	Simeto × Vitron 113	G7
	Simeto × Waha 311	G8
Varieties	Simeto	G9
	Ardente	G10
	Waha	G11
	Vitron	G12

Both isolates were sourced from the mycological collection of the ENSA in El-Harrach, Algiers. The FG2008 isolate was obtained from soft wheat spikelets collected at the ITGC experimental station (Technical Institute of Field Crops) in Algiers, in May 2008. The FC2006 isolate was derived from soft wheat spikelets harvested at the ENSA experimental station in El-Harrach, 2006. Both isolates were obtained from wheat ears displaying typical symptoms of FHB, such as pink to orange discoloration, indicative of significant spore mass formation.

2.3 In Vitro Pathogenicity Test on Durum Wheat Grains

The pathogenicity of the fungal isolates was evaluated according to a modified version of the protocol established by Mesterhazy [27] using PDA medium. Conidia, represented by mycelium post-conidial germination, were used to prepare the inocula. Conidial concentration and purity were controlled to ensure a consistent amount of inoculum for both species [9]. Mycelial mass was quantified using the method outlined by Brennan et al. [28].

To produce mycelia, 50 mL of Potato Dextrose Broth (PDB) was prepared. For each isolate, four 5-mm mycelial portions were obtained from 7-day-old cultures on PDA medium. These explants were added to PDB and incubated at 20°C on an orbital shaker at 250 rpm for seven days. After incubation, mycelia were harvested by centrifugation at 5000 × G for 10 min, homogenized, and diluted to a concentration of 13.3 mg/mL in a solution containing 0.2% Tween 20.

Petri dishes (85 mm in diameter) containing the PDA medium were prepared. A sterilized filter paper disk with the same diameter was placed on each PDA medium. Homogenized mycelia, diluted in 0.2% Tween 20, were applied evenly at a rate of 8 mL per dish. A second sterile filter paper disk was placed on the top of the inoculum. For each sample, 10 durum wheat grains were disinfected using 2% sodium hypochlorite for 10 min, rinsed thrice with sterile distilled water, and dried between two sterile blotting

papers. These pre-disinfected grains were placed in Petri dishes. Plates containing grains inoculated with each *Fusarium* isolate were incubated in the dark at 25°C using a randomized design, with three replicates per isolate. Control plates were subjected to identical conditions, except that sterile distilled water was used instead of mycelial inoculum.

The growth of durum wheat coleoptiles was measured on the fourth day of incubation, and the results were expressed as differences in growth compared to the non-inoculated control.

2.4 Statistical Analysis

Statistical analysis of the results was performed using Statgraphics software version 15.1.0. Multiple comparisons of means were conducted using the Least Significant Difference (LSD) test at a 5% significance level to determine homogeneous groups.

3 Results

To assess the impact of the two *Fusarium* isolates, FC2006 from *Fusarium culmorum* and FG2008 from *Fusarium graminearum*, on the growth of coleoptiles in 12 durum wheat genotypes, we developed an *in vitro* evaluation method under controlled laboratory conditions. This experimental approach enabled the precise measurement of the effects of the two isolates on coleoptile growth across different durum wheat varieties and breeding lines. This method provided a clear basis for comparing the differences in coleoptile growth after artificial inoculation with the two *Fusarium* species.

Coleoptile length was measured in millimeters after a 4-day incubation period at 25°C in the dark (Fig. 1). To determine the level of aggressiveness of each *Fusarium* isolate on the different genotypes, coleoptile growth was compared with that of the uninoculated controls. The degree of reduction in coleoptile length was used to quantify the severity of the infection caused by the isolates.

As presented in Fig. 1, coleoptile length was significantly reduced in grains inoculated with either of the *Fusarium* isolates compared to uninoculated controls. This reduction in growth highlights the inhibitory effect of the isolates on durum wheat coleoptiles, consistent with the known pathogenic characteristics of *Fusarium*. The observed inhibition is indicative of diseases, such as grain rot, which are common in infected plants and can lead to substantial economic losses in cereal production.

Statistical analysis of variance revealed that the effects of genotypes, treatments, and the interaction between genotypes and treatments on coleoptile length were highly significant ($p \leq 0.001$). To further compare the mean coleoptile lengths across the three treatments (controls, genotypes inoculated with FC2006, and genotypes inoculated with FG2008), we conducted an LSD test. The test identified three homogeneous groups, with the control group having the longest average coleoptile length of 37.87 mm. By contrast, seeds inoculated with the FC2006 isolate had an average coleoptile length of 0.62 mm, while those inoculated with FG2008 exhibited the shortest average coleoptile length of 0.064 mm (Fig. 2).

The interaction between genotypes and treatments, analyzed using the LSD test, distinguished 10 homogeneous groups (Fig. 3). The results indicated that the coleoptile growth in all genotypes inoculated with *Fusarium* isolates differed significantly from that of the uninoculated controls ($p \leq 0.001$). In the control group, genotype G4 exhibited the longest coleoptile length at 44.50 mm, whereas G1 recorded the shortest length at 32.17 mm after 4 days of incubation.

Among the genotypes inoculated with FC2006, genotype G8 displayed the longest average coleoptile length of 1.83 mm, while genotypes G1, G5, G7, and G11 showed complete inhibition of coleoptile growth, with an average length of 0 mm. Similarly, for the genotypes inoculated with FG2008, genotype G8 again had the highest average coleoptile growth, measuring 0.4 mm, while the other genotypes (G1, G2, G3, G4, G5, G6, G7, G9, G10, and G11) all exhibited total inhibition of coleoptile growth with an average length of 0 mm (Fig. 3). Interestingly, these results revealed that several genotypes exhibited complete inhibition of coleoptile growth following inoculation with both FC2006 and FG2008 isolates.

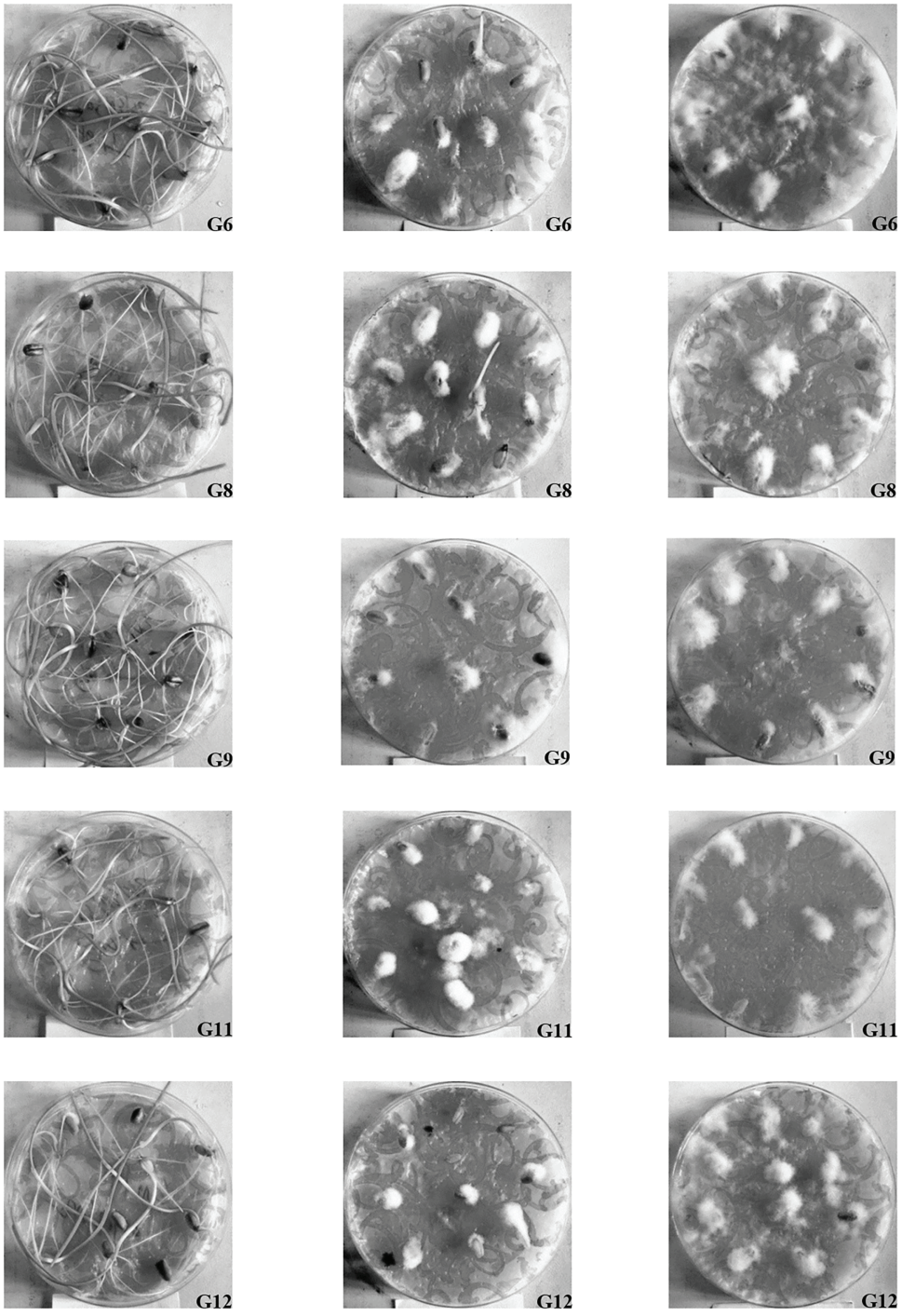

Figure 1: Inhibition of seed germination by both *Fusarium* isolates after 4 days of incubation at 25°C and in darkness, illustrated with some representative images

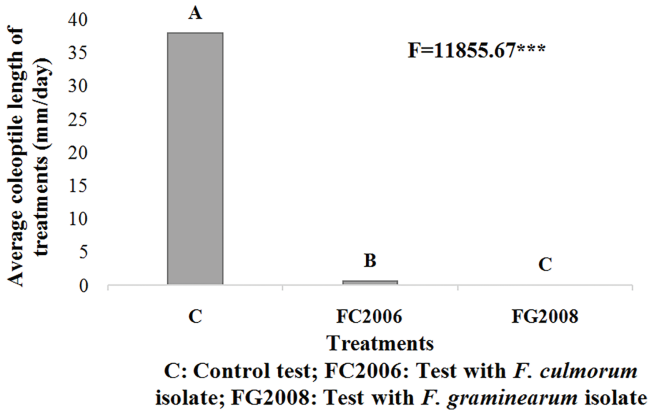

Figure 2: Mean coleoptile length of the three treatments after 4 days of incubation at 25°C and in the dark

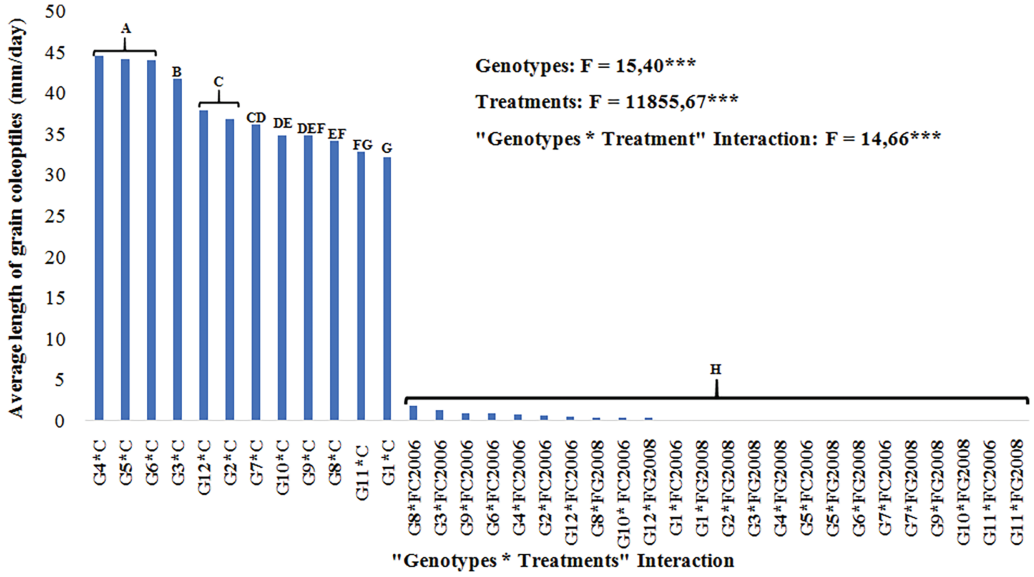

Figure 3: Effect of three treatments on the average coleoptile length after 4 days of incubation at 25°C and in darkness

4 Discussion

Coleoptiles are conical structures that emerge from seeds and develop into leaves when exposed to light and moisture, making their length a critical indicator for early growth, wheat breeding, and plant development [29,30]. In this study, inoculation of durum wheat grains with *Fusarium* isolates was aimed at evaluating the aggressiveness of the fungi in inhibiting coleoptile growth in different durum wheat varieties andbreeding lines. The observed growth inhibition confirms that both *F. culmorum* and *F. graminearum* fungi have detrimental effects on seed germination and subsequent coleoptile development. Notably, a slight difference in aggressiveness was detected between the two isolates, with *F. graminearum* (FG2008) exhibiting slightly higher aggressiveness than *F. culmorum* (FC2006).

Our findings align with those of previous research, which identified *F. culmorum* and *F. graminearum* as among the most aggressive pathogens affecting the coleoptile growth of wheat and barley [28,31]. Reduced germination rates and shorter coleoptile lengths are commonly used as key indicators of fungal

aggressiveness, particularly in *F. graminearum* [24]. Brennan et al. [28] similarly reportedreport that both *F. culmorum* and *F. graminearum* exhibit optimal pathogenicity at temperatures between 20°C and 25°C, reducing coleoptile growth by over 89.3% compared to uninoculated controls. Their study further concluded that *F. graminearum* is a more aggressive species, reducing coleoptile growth by as much as 96% at 25°C, which is consistent with the findings of the present study.

Additional studies have reported that *F. culmorum* can reduce coleoptile length by up to 91.32%, whereas *F. graminearum* can cause a 78.32% reduction in growth [31]. Furthermore, *in vitro* seed inoculation test revealed that *F. culmorum* isolates can be highly aggressive, with some strains completely inhibiting coleoptile growth [16]. This extreme sensitivity to the initial infection was especially pronounced in durum wheat genotypes, where *F. culmorum* caused a significant reduction in germination rates. For example, the "Hogar" durum wheat variety experienced a 52.17% reduction in germination after just three days of inoculation [32].

Therefore, both *F. culmorum* and *F. graminearum* are regarded as highly aggressive pathogens, although their relative degrees of aggressiveness may vary depending on several factors. These include the type of host plant, environmental conditions, interactions with other microorganisms, and the genetic characteristics of the specific fungal isolates.

Other studies reported other *Fusarium* species (*F. equiseti*, *F. chlamydosporum*, *F. poae*, *Microdochium nivale*, *F. verticillioides* and *F. solani*) having negative effect on seed germination and coleoptile length in durum wheat, but with coleoptile length reduction variable depending on the fungal species and isolates [12,28,33,34].

5 Conclusion

This study examined the effects of two *Fusarium* isolates, *F. culmorum* (FC2006) and *F. graminearum* (FG2008), on coleoptile growth in different durum wheat genotypes. The results demonstrated that both isolates significantly inhibited coleoptile development across all genotypes tested, with wheat coleoptiles exposed to the fungi exhibiting markedly reduced growth compared to the controls. Moreover, there was a slight but notable difference in aggressiveness between the two isolates, with *F. graminearum* showing a marginally stronger inhibitory effect on coleoptile growth than *F. culmorum*.

These findings offer valuable insights for farmers and breeders in selecting wheat varieties that are more resistant to *Fusarium* infections. It is possible to mitigate the effect of *Fusarium* on crop yields by identifying varieties with increased resistance to these aggressive fungal species, ultimately enhancing the sustainability of wheat production in regions vulnerable to these pathogens.

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Author Contributions: The authors confirm contribution to the paper as follows: study conception and design: Salah Hadjout, Fathi Abdellatif Belhouadjeb, Houcine Bougrine, Abdeldjalil Belkendil; data collection: Salah Hadjout, Mohamed Zouidi, Amer Zeghmar; analysis and interpretation of results: Salah Hadjout, Mohamed Zouidi, Walid Ouaret; draft manuscript preparation: Salah Hadjout, Mohamed Zouidi, Fathi Abdellatif Belhouadjeb, Walid Soufan. All authors reviewed the results and approved the final version of the manuscript.

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