Occurrence, distribution and molecular analysis of *Fusarium* head blight on cereals in Northeast Algeria

Presencia, distribución y análisis molecular de la fusariosis de la espiga en cereales del noreste de Argelia

Ocorrência, distribuição e análise molecular de Fusarium ear blight em cereais no nordeste da Argélia

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Abstract

Fusarium head blight (FHB) affects cereals by reducing yield quality and quantity. This study aimed, on the one hand, to evaluate the prevalence, severity, and incidence of this disease on durum wheat and barley fields in the High Setifian Plains Region Northeast of Algeria and, on the other hand, to identify *Fusarium* species. Durum wheat and barley fields were prospected, and FHB-symptomatic samples were collected during two crop years (2018-2019). The prevalence, incidence, and severity results on durum wheat during the first crop year were 6 %, 12.1 %, and 56.2 %, and on barley, were 23.77 %, 10.22 %, and 49.7 %, respectively. However, in the second crop year, on wheat were 2.5 %, 3 %, and 35 %, and on barley were 35 %, 10.86 %, and 44.29 %, respectively. Morphological characterization was used to select the 102 isolates. They were then identified by molecular analysis by PCR amplification and sequencing of the internal transcribed spacers (ITS) and β-tubulin (tub2) or translation elongation factor 1-alpha (tef1). Thus, seven species of *Fusarium* were identified, namely, *F. equiseti* (27.45 %), *F. graminearum* (25.49 %), *F. acuminatum* (19.6 %), *F. oxysporum* (13.73 %), *F. proliferatum* (9.8 %), *F. avenaceum* (1.96 %), *F. incarnatum* (1.96 %). In addition, *Fusarium proliferatum* and *F. oxysporum* were reported for the first time in Algerian fields.

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Resumen

La fusariosis de la espiga (FHB) afecta a los cereales reduciendo la calidad y cantidad del rendimiento. Este estudio tuvo como objetivo, por un lado, evaluar la prevalencia, la severidad y la incidencia de esta enfermedad en los campos de trigo duro y cebada en la región de las llanuras altas de Setifian, al noreste de Argelia, y, por otro lado, identificar las especies de *Fusarium*. Se prospectaron los campos de trigo duro y cebada y se recogieron muestras sintomáticas de FHB durante dos campañas agrícolas (2018-2019). Los resultados de prevalencia, incidencia y gravedad en el trigo duro durante la primera campaña fueron del 6 %, 12.1 % y 56.2 %, y en la cebada, del 23.77 %, 10.22 % y 49.7 %, respectivamente. Sin embargo, en la segunda campaña, en el trigo fueron del 2.5 %, 3 % y 35 %, y en la cebada del 35 %, 10.86 % y 44.29 %, respectivamente. Se seleccionaron los 102 aislamientos mediante caracterización morfológica, que se identificaron a través del análisis molecular mediante amplificación por PCR y secuenciación de los espaciadores transcritos internos (ITS) y de la β-tubulina (tub2) o del factor de elongación de la traducción 1-alfa (tef1). De esta forma, se identificaron siete especies de *Fusarium*, *F. equiseti* (27.45 %), *F. graminearum* (25.49 %), *F. acuminatum* (19,6 %), *F. oxysporum* (13.73 %), *F. proliferatum* (9.8 %), *F. avenaceum* (1.96 %), *F. incarnatum* (1.96 %). Además, *Fusarium proliferatum* y *F. oxysporum* se reportaron por primera vez en campos argelinos.

Palabras clave: cebada, *Fusarium*, identificación, prospección, trigo duro.

Resumo

A giberela (FHB) afeta os cereais reduzindo a qualidade e a quantidade da produção. Este estudo teve como objetivo, por um lado, avaliar a prevalência, a severidade e a incidência desta doença em campos de trigo duro e cevada na região das Planícies Altas de Setifian, a nordeste da Argélia e, por outro lado, identificar espécies de *Fusarium*. Campos de trigo duro e cevada foram prospectados, e amostras sintomáticas de FHB foram coletadas durante dois anos de safra (2018-2019). Os resultados de prevalência, incidência e severidade no trigo duro durante o primeiro ano de safra foram de 6 %, 12,1 % e 56,2 %, e na cevada, foram de 23,77 %, 10,22 % e 49,7 %, respectivamente. No entanto, no segundo ano de safra, o trigo foi de 2,5 %, 3 % e 35 %, e a cevada foi de 35 %, 10,86 % e 44,29 %, respectivamente. A caracterização morfológica foi usada para selecionar os 102 isolados. Eles foram então identificados por análise molecular por amplificação por PCR e sequenciamento dos espaçadores transcritos internos (ITS) e β-tubulina (tub2) ou fator de alongamento de tradução 1-alfa (tef1). Assim, sete espécies de *Fusarium* foram identificadas, *F. equiseti* (27,45 %), *F. graminearum* (25,49 %), *F. acuminatum* (19,6 %), *F. oxysporum* (13,73 %), *F. proliferatum* (9,8 %), *F. avenaceum* (1,96 %), *F. incarnatum* (1,96 %). Além disso, *Fusarium proliferatum* e *F. oxysporum* foram relatados pela primeira vez em campos argelinos.

Palavras-chave: cevada, *Fusarium*, identificação prospecção, trigo duro.

Introduction

Traditionally, cereals are considered the most consumed food in Algeria, in the form of bread, pasta, couscous, and other products of the food industry. The region of High Setifian Plains, northeast of Algeria, has a cereal vocation with 194,520 ha sown in 2020 (MADR, 2020). Unfortunately, many fungal diseases, such as *Fusarium* head blight (FHB), threaten these crops by various *Fusarium* species. Many small grains, including wheat and barley, are considered a target (Moretti, 2009). In addition, FHB is a worldwide pathology causing a significant effect on the quality and the quantity of cereal yields (Parry *et al.,* 1995; Matny, 2015), inducing harvest losses and price discounts (Nganje *et al.*, 2004), the majority impact of these losses appears on wheat and barley production (Wegulo *et al*., 2015). The FHB is becoming an increasing constraint for wheat and barley production worldwide and in Algeria (Yekkour *et al.,* 2015). However, several *Fusarium* species generate mycotoxins on cereals at the growth, harvest, and post-harvest stages (Venkataramana *et al*., 2018). These mycotoxins can cause serious health problems in animals and humans (Parry *et al.,* 1995; Ma *et al.,* 2013). Thus, many *Fusarium* species are associated with FHB disease (Parry *et al.,* 1995), developing under specific conditions, in particular, rainfall, temperature, flowering stage, and other agronomic practices, such as crop rotation and plant genotype (Bottalico and Perrone, 2002). In Algeria, a few studies on FHB disease were published: Yekkour *et al.* (2015), Touati-Hattab *et al.* (2016), Laraba *et al.* (2017a), Bencheikh *et al.* (2018), Bencheikh *et al.* (2020) and Abdallah-Nekache *et al.* (2019). These publications do not exclude the presence of other species that have not yet been reported in Algeria. This study aimed to evaluate the prevalence, severity, and incidence of *Fusarium* head blight (FHB) on durum wheat and barley in the High Setifian Plains Region Northeast of Algeria and to identify *Fusarium* isolates.

Materials and methods

Disease prospection, sampling, and assessment

Fusarium head blight was prospected in 262 durum wheat and barley fields in the high Setifian plains region (Northeast Algeria) between May and July during two agricultural seasons (2018-2019). Thus, symptomatic samples of head blight were randomly collected and put in paper bags. The number of samples was taken depending on the incidence rate from four different directions of the same field, and GPS coordinates were recorded. The prevalence (P), incidence (I), and severity (S) of this fungal disease were estimated visually. The prevalence was determined by the proportion of infected fields in prospected ones (Zahri *et al*., 2014); incidence was determined by averaging infected spikes in each field (Stack and McMullen, 1998). Also, severity was determined by averaging infection in infected spikes (Stack and McMullen, 1998). For statistical analysis, means and standard deviations of incidence and severity were calculated. Maps were created with ArcGIS software using GPS coordinates data to illustrate the distribution of prospected fields and the dispersion of FHB disease. Humidity, precipitation, and temperature during the flowering period were collected to interpret the results from www.historique-meteo.net.

Isolation and molecular identification of FHB disease:

Pathogen isolation

Media culture: PDA (Potato Dextrose Agar) was used as a selective media in 90 mm Petri dishes.

Isolation: According to Laraba *et al.* (2017b), with slight modifications from infected wheat and barley ears samples. Grains were rinsed for 10 min in 2 % sodium hypochlorite three times and then washed with distilled and sterilized water. After all, these grains were placed in Petri dishes containing the PDA medium and incubated at 28 °C for 7 d.

Monoconidiale culture: According to Zhang *et al.* (2013), with slight modifications, *Fusarium* isolates were purified using spores' cultures.

Morphologic characterization: After the purification, isolates were cultured in the PDA culture media, Spezieller Nahrstoffarmer Agar (SNA), and Sabouraud Dextrose Agar (SDA) for morphologic identifications according to Nelson *et al.* (1983) and Leslie *et al.* (2006). Based on morphological characterization, *Fusarium* isolates were grouped into clusters, and one isolate was taken for molecular identification from each cluster. Photos of the morphological characters of *Fusarium* species were taken, particularly on Petri dishes and microscopic characters of macroconidias, microconidias, and chlamydospores.

Molecular analysis

DNA extraction: was performed using a commercial NucleoSpin Plant II kit (Macherey-Nagel Germany).

PCR amplification: The polymerase chain reaction (PCR) was amplified at the level of the partial regions ITS, and β-tubulin (just for F05 was amplified translation elongation factor 1 alpha (EF-1 α) using specific primer sequences (table 1) composed of a 25 µL mixture: 0.2 µL of mgcl 2, 5 µL Taq buffer (solisbiodyn), 1 µL of direct starch, 1 µL of reward starch, 0.2 µL of dNTP, 0.2 µL of Taq polymerase (solisbiodyn), 2 µL of genomic DNA and ultra-pure water. The temperature for annealing was 55 °C for ITS (Gardes and Bruns, 1993), 58 °C for EF-1 α (Carbone and Kohn, 1999), and 54°C for β-tubulin (Glass and Donaldson,1995).

PCR product revelation and purification: 10 µL of the PCR product was put in a 1.5 % agarose electrophoresis gel, followed by a bath in 0.5 $\mu g.mL^{-1}$ of Ethidium Brom (Bartlett and Stirling, 2003). The DNA was visualized and photographed under UV (Biorad gel doc systems USA), and then this product was purified with the NucleoSpin® Gel kit and PCR clean-up (Macherey Nagel Germany).

DNA sequencing: The final PCR product was sequenced using the Sanger *et al.* (1977) technique using the BigDye V3.1 Kit (Applied Biosystems). Depending on the quality of the sequencing results, these sequences were edited with BioEdit software. The sequence results were compared with the Blast database. Finally, sequences were submitted with ID numbers in GenBank.

Results and discussion

Prospection: The total number of fields prospected was 262 (table 2), with 142 of barley and 120 of durum wheat. Figure 1 illustrates the geographic distribution of the disease in the study region.

The evaluation of *Fusarium* head blight (FHB) on barley and durum wheat fields in the study region (table 2) showed that the prevalence was 23.77 % and 6 %, respectively, and severity was 47.9 % and 56.2 %, respectively. The incidence was 10.22 % and 12.1 %, respectively, during the first year. However, during the second year, the prevalence was 35 % and 2.5 %, while the severity was 44.29 % and 35 %, respectively. The incidence was 10.86 % and 3 % respectively.

Table 1. Primers used in molecular analysis of F*usarium* species isolated from barley and wheat grains fields in the Setif region of Algeria.

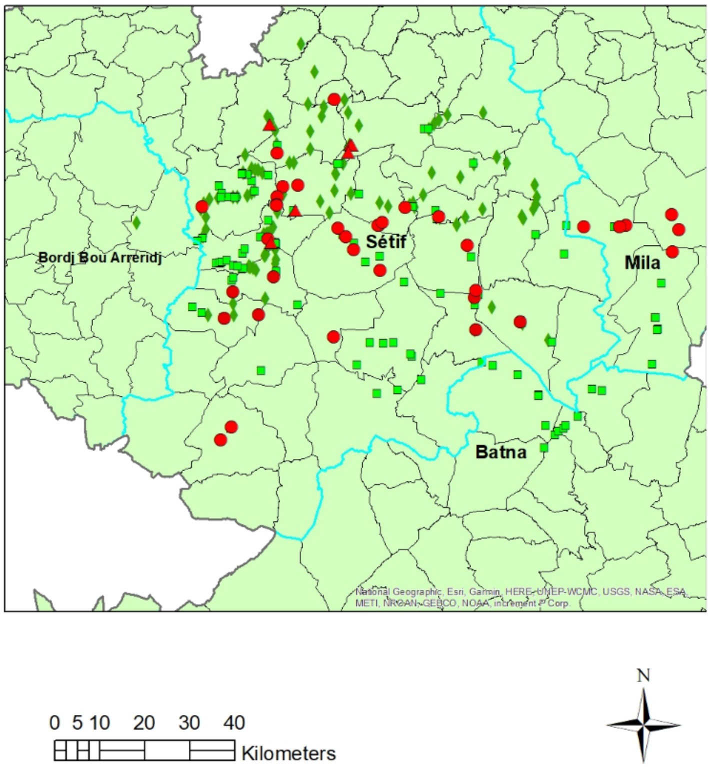
Primers	Sequence 5'-3'	Optimal annealing T (°C)	Product size (pb)	Reference
ITS				
ITS-1F	CAGGAGACTTGTACACGGTCCAG	55	600	Gardes and Bruns (1993)
ITS4-B	CTTGGTCATTTAGAGGAAGTAA			
β-tubulin				
Forward	GGTAACCAAATCGGTGCTGCTTTC	54	495	Glass and Donaldson (1995)
Reverse	ACCCTCAGTGTAGTGACCCTTGGC			
EF-1α				
Forward	CATCGAGAAGTTCGAGAAGG	58	600	Carbone and Kohn (1999)
Reverse	TACTTGAAGGAACCCTTACC			

Table 2. Prospection of *Fusarium* head blight (FHB) during 2 years on durum wheat and barley fields in the Setif region of Algeria.

Cereal Species	Severity %		Incidence %		Prevalence %		Infected fields		Total prospected fields	
	2018	2019	2018	2019	2018	2019	2018	2019	2018	2019
Durum wheat µ	56.2	35	12.1	3	6	2.5	5	1	81	39
σ	19.68	0	3.47	0						
Barley µ	47.9	44.29	10.22	10.86	23.77	35	29	7	122	20
σ	5.23	25.4	5.23	2.89						

µ: mean and σ: standard deviation

Figure 1. Map of *Fusarium* head blight (FHB) results in durum wheat and barley fields in the Setif region of Algeria using ArcGIS. (♦): not infected Durum wheat fields. (▲): infected Durum wheat field. (■): not infected Barley field. (●): infected Barley field.



These results corroborate the climatic conditions during these two agricultural seasons in the study area (table 3); in 2018 the first year of prospection, the number of infected fields was higher than in the second year, caused by a favorable climate for disease infection with *Fusarium* species. Counter wise, the decrease in infected fields with FHB disease was observed in the second year of prospection when the climate was hotter and less humid; these factors were unfavorable for the disease development. According to Osborne and Stein (2007), the environment is the primary factor in the spread of *Fusarium* pathogen. These factors are essentially rain and warm temperature at the period of anthesis with the presence of the *Fusarium* pathogen (Alisaac and Mahlein, 2023).

Table 3. Climatic variables (temperature, precipitation and humidity) of the Setif region of Algeria in 2018 and 2019.

Year	Month	Temperature (°C)	Precipitation (mm)	Humidity (%)
2018	May	18	155	77
	June	17	99	67
2019	May	24	65	61
	June	28	10	43

https://www.historique-meteo.net

Isolation and molecular analysis: The total number of *Fusarium* isolates obtained in two years of prospection was 102 (table 4). In the first year, 93 *Fusarium* isolates were obtained, 76 on barley and 17 on wheat. However, 9 *Fusarium* isolates were obtained from barley and wheat during the second year, seven on barley and two on wheat. The decrease was near 90 % between the 2 years.

Table 4. *Fusarium* species isolated from infected fields in the Setif region of Algeria *F. avenaceum: F. ave., F. acuminatum: F. acu., F. oxysporum: F. oxy., F. proliferatum: F. pro., F. incarnatum: F. inc., F. equiseti: F. equ.* and *F. graminearum: F. gra.*).

Species		F. ave.	F. acu.	F. oxy.	F. pro.	F. inc	F. equ.	F. gra.	Total %
Isolates number	2018	2	20	14	9	0	24	24	93
	2019	0	0	0	1	2	4	2	9
	Total	2	20	14	10	2	28	26	102
Percentage (%)		1.96	19.61	13.73	9.8	1.96	27.45	25.49	100
Durum wheat		01	04	05	03	00	02	06	21
Barley		01	16	09	07	02	26	20	81

Table 4 illustrates the number of *Fusarium* isolates gathered in durum wheat and barley fields. Therefore, *Fusarium* species obtained from barley were 81 isolates (76.9 %) and those obtained from durum wheat were 21 (23.1 %). In addition, *F. incarnatum* has only been isolated from barley fields. This difference between the number of infected durum wheat fields and the number of infected barley fields by FHB disease resulted from their difference in the period of cereals growth stage: inflorescence emergence and anthesis (Zadoks *et al.,* 1974). These two stages are the period when *Fusarium* species infect cereals in a favorable climate. However, in the region of study, these two stages occurred at the beginning of May for barley coincided with a favorable environment for FHB disease development, and the beginning of June for durum wheat coincided with an unfavorable climate for FHB disease development.

The results of the molecular analysis of the ten *Fusarium* isolates (table 5) allowed the identification of seven different species of *Fusarium*: *F. avenaceum* (1.96 %), *F. acuminatum* (19.6 %), *F. oxysporum* (13.73 %), *F. proliferatum* (9.8 %), *F. incarnatum* (1.96 %), *F. equiseti* (27.45 %) and *F. graminearum* (25.49 %).

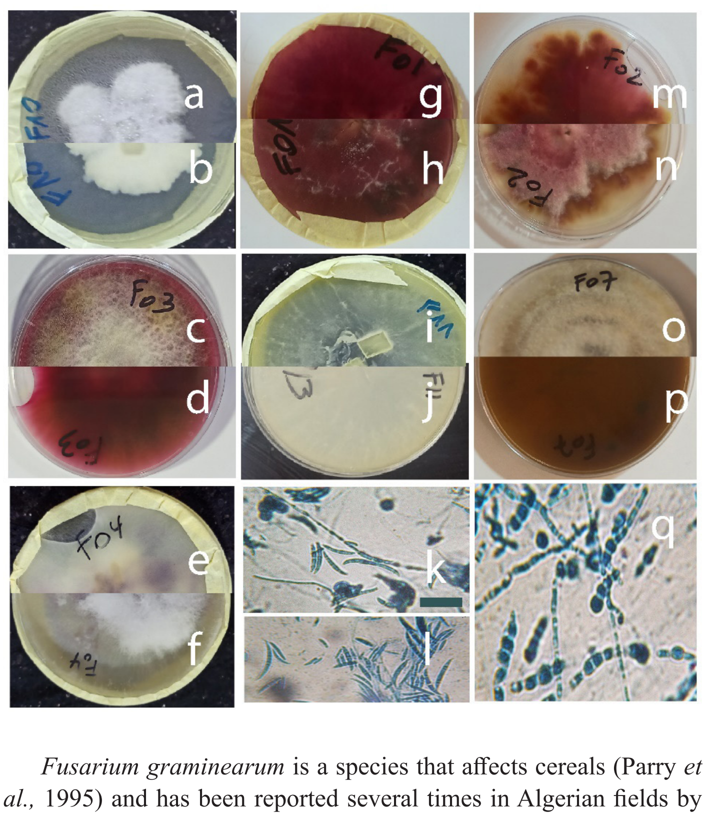
The distribution of *Fusarium* species in the study region was as follows: in the South (*F. oxysporum* and *F. acuminatum*); in the North (*F. graminareaum*, *F. avenaceum*, *F. proliferatum*, *F. equiseti*, and *F. oxysporum*) and the Center (*F. avenaceum*, *F. acuminatum*, *F. oxysporum*, *F. proliferatum*, *F. incarnatum*, *F. equiseti,* and *F. graminearum*). As a result, we noticed that the central region was more infected with *Fusarium* species than the North and the South; this is explained by the different bioclimatic floors in the study region (Tedjari *et al.*, 2014; Djellouli *et al.,* 2020).

This study showed this region's diversity of *Fusarium* species (table 4 and figure 2). This diversity is dominated by *F. equiseti* (27.45 %), followed by *F. graminearum* (25.49 %), and finally *F. acuminatum* (19.61 %). Moreover, this study allowed us to identify and characterize for the first time two species, namely *F. oxysporum* (isolate F03, submitted with an identification number OR228993 and PQ279785 in GenBank for ITS partial regions and β-tubulin respectively) and *Fusarium proliferatum* (isolate F04, submitted with an accession number OR594284 and PQ301480 in GenBank for ITS partial regions and β-tubulin respectively).

Table 5. *Fusarium* sequences submitted in NCBI Gen bank and their accession number.

Fusarium species	Host	Primers	Accession number GenBank
- F01.alasetif Isolate :	Durum	Partials regions ITS	OR225820
F. avenaceum	Wheat head	β-tubulin	OR267009
- F02.alasetif isolate :	Barley's	Partials regions ITS	OR228995
F. acuminatum	head	β-tubulin	OR608511
- F03.alasetif isolate :	Barley's	Partials regions ITS	OR228993
F. oxysporum	head	β-tubulin	PQ279785
- F04.alasetif isolate:	Barley's	Partials regions ITS	OR594284
F. proliferatum	head	β-tubulin	PQ301480
- F05.alasetif isolate:	Barley's	Partials regions ITS	OR228992
F. acuminatum	head	Elongation factor 1-α	PQ332999
- F07.alasetif isolate :	Barley's	Partials regions ITS	OR228997
F. incarnatum	head	β-tubulin	PQ301481
- F08.alasetif isolate :	Barley's	Partials regions ITS	OR228998
F. equiseti	head	β-tubulin	PQ316047
- F10.alasetif isolate :	Barley's	Partials regions ITS	OR228990
F. graminearum	head	β-tubulin	PQ305980
- F11 alasetif isolate :	Barley's	Partials regions ITS	OR228994
F. equiseti	head	β-tubulin	PQ333000
- F13.alasetif isolate :	Barley's	Partials regions ITS	OR228996
F. equiseti	head	β-tubulin	PQ360734

Figure 2. Morphologic characteristics of isolated *Fusarium* species (a) and (b): *F. graminearum*, (c), and (d): *F. oxysporum*, and (f): *F. proliferatum*, (g), and (h): *F. avenaceum*, (i) and (j): *F. equiseti*, (m) and (n): *F. acuminatum*, (o) and (p): *F. incarnatum* and (k), (l): macroconidias, and (q): chlamydospores.



Fusarium graminearum is a species that affects cereals (Parry *et al.,* 1995) and has been reported several times in Algerian fields by Hadjout and Zouidi (2022), and Djaaboub *et al*. (2020). *Fusarium acuminatum* and *Fusarium equiseti* are very common in Spain and southern Europe (Marín *et al.,* 2012); this last species was reported for the first time in Algeria by Bencheikh *et al.* (2020) on durum wheat. As well as *F. incarnatum* by Bouanaka *et al.* (2023). *F. oxysporum* has been reported several times worldwide, in the United States, Bulgaria, Hungary, and Japan (Parry *et al.,* 1995).

Conclusion

The results of the prospection of *Fusarium* head blight (FHB) at the level of 142 and 120 barley and durum wheat fields, respectively, during the two agricultural campaigns in the study region, made it possible to map the spatial distribution of the disease and to evaluate the prevalence, severity, and incidence of this disease. Additionally, the results of the molecular analysis identify and characterize seven *Fusarium* species with different proportions and classified in descending order: *F. equiseti* (27.45 %), *F. graminearum* (25.49 %), *F. acuminatum* (19.6 %), *F. oxysporum* (13.73 %), *F. proliferatum* (9.8 %) and finally *F. avenaceum* and *F*. *incarnatum* with 1.96 %. Finally, the identification of *F. oxysporum* (accession number OR594284 and PQ279785) and *Fusarium proliferatum* (accession number OR228993 and PQ301480) in Algerian cereal fields (barley and durum wheat) has been reported for the first time. It was observed that *Fusarium* head blight (FHB) affects barley more than durum wheat because, for barley, the conditions are favorable between fusarium head blight, the vegetative stage (flowering), and the climatic conditions of the study region during two agricultural seasons in 2018-2019. It would be desirable to expand the fields of prospection for this disease in all areas of Eastern Algeria, to identify and characterize the species of *Fusarium* spp., and to evaluate their pathogenicity on the most cultivated varieties in these regions. Finally, identify and assess the toxigenic potential of these fusarium species.

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