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FUSARIUM BRACHYGIBBOSUM, IDENTIFICATION AND VIRULENCE AS CAUSAL AGENTS OF CROWN ROT ON WHEAT

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Abstract

Description of the subject: *Fusarium* crown rot (FCR) is one of the serious wheat diseases.
Objective: The isolation of *Fusarium brachygibbosum*, which has recently described in the Algerian territory especially for wheat, and evaluation of its pathogenicity and capacity to induce FCR on wheat.
Methods : In this study, one hundred and seventeen *Fusarium* symptomatic samples were collected from the northeast of Algeria. The 34 isolates obtained were identified based on morphological data and confirmed by molecular identification. Isolate FE10, identified as *Fusarium brachygibbosum*, was assigned the accession number MW450596 by NCBI GenBank. Two pathogenicity tests were conducted on 9 cultivars (cv) of bread wheat. The *In vitro* test in the host with the parameters; percentage of germination inhibition (%GI), to determine the impact on the germination of sowing seeds, and the Area Under the Disease Progress Curve (AUDPC) to test the effect on the initial infection of germinated coleoptiles. *In vivo* test in the growth chamber to test the *F. brachygibbosum* ability to induce FCR by the disease severity (DS) parameter.
Results : The results showed that FE10 induced FCR at all tested cultivars with a significant decrease in germination rate and coleoptile emergence with GI% and AUDPC values reaching up to 17.36, and 38.86%, respectively for cv. Nif Encer. In addition *F. brachygibbosum* negatively affected the vegetative system length and fresh weight (RCL% and RCW%) up to 34.49 and 48.43%, respectively with cv. Arz.
Conclusion : The moderate pathogenicity of *F. brachygibbosum* was observed in this study.
Keywords : *Triticum aestivum*; fungus ; phytopathology; wheat; *Fusarium brachygibbosum*.

PREMIER RAPPORT SUR *FUSARIUM BRACHYGIBBOSUM*, IDENTIFICATION ET VIRULENCE EN TANT QU'AGENT CAUSAL DE LA POURRITURE DU COLLET SUR LE BLÉ

Résumé

Description du sujet : La pourriture fusariene du collet (FCR) est l'une des maladies graves du blé.
Objectifs : L'isolement de *Fusarium brachygibbosum*, qui a été décrit récemment dans le territoire algérien, en particulier pour le blé, et l'évaluation de son pouvoir pathogène et de sa capacité à induire la FCR sur le blé.
Méthodes : Dans cette étude, cent dix-sept échantillons symptomatiques de *Fusarium* ont été collectés dans le nord-est de l'Algérie. Les 34 isolats obtenus ont été identifiés sur la base de données morphologiques et confirmés par identification moléculaire. L'isolat FE10, identifié comme *Fusarium brachygibbosum*, le numéro d'accession MW450596 lui attribuer par NCBI GenBank. Deux tests de pathogénicité ont été réalisés sur 9 cultivars (cv) de blé panifiable. Le test *in vitro* sur l'hôte avec les paramètres : pourcentage d'inhibition de la germination (%GI), pour déterminer l'impact sur la germination des graines de semis, et l'Area Under the Disease Progress Curve (AUDPC) pour tester l'effet sur l'infection initiale de coléoptiles germées. Test *in vivo* dans la chambre de culture pour tester la capacité de *F. brachygibbosum* à induire la FCR par le paramètre de sévérité de la maladie (DS).
Résultats : Les résultats ont montré que FE10 a induit la FCR chez tous les cultivars testés avec une diminution significative du taux de germination et de l'émergence des coléoptiles avec des valeurs GI% et AUDPC atteignant jusqu'à 17,36, et 38,86%, respectivement pour cv. Nif Encer. En outre, *F. brachygibbosum* a affecté négativement la longueur du système végétatif et son poids frais (RCL% et RCW%) jusqu'à 34,49 et 48,43%, respectivement avec le cv. Arz.
Conclusion : La pathogénicité modérée de *F. brachygibbosum* a été constatée dans ce travail.
Mots clés : *Triticum aestivum* ; champignon ; phytopathologie ; blé ; *Fusarium brachygibbosum*.

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INTRODUCTION

Bread wheat (*Triticum aestivum* L.) is one of the most important staple crops in the world and one of the pillars of global food security [1]. In Algeria, bread wheat is cultivated extensively in the humid and sub-humid provinces of the north of the country, in order to satisfy the growing needs of the agro-food industries and especially the bakeries producing bread widely consumed by the population [2]. *Fusarium* crown rot (FCR) is a serious disease characterized by necrosis and dry rot of the crown, basal stem and root tissue, colonization of vascular tissues by hyphae, abortion of tillers, and formation of white spots with shriveled kernels. The initial infection of seedlings is characterized by brown lesions, which become apparent in the crown, as well as an increased degree of discoloration of the stems and extends to the interior of the leaf sheaths and stems [3]. This disease has become an increasing constraint for wheat production in the world as well as in Algeria [4]. *F. brachygibbosum*, is a filamentous fungus belonging to the group: *Ascomycetes*, Class: *Sordariomycetes*, Subclass: *Hypocreomycetidae*, Order: *Hypocreales*, Family: *Nectriaceae*, Genus: *Fusarium*, Species: *Fusarium brachygibbosum* [5]. This species is considered a soil-borne plant pathogen, with a wide distribution worldwide. It is also responsible for serious economic losses due to reduced yields [6]. The pathogen *F. brachygibbosum* exists in legumes [7], may also have an effect on the growth of nursery almond trees [8], of corn [9], of olive trees [10], of sunflower [11] and the sugar beet [5]. Also causes a lesion and wilting on the leaves of whole watermelon plants [12], and the leaves of oleanders [13]. Spots were observed on cannabis (*Cannabis sativa*) in northern California, and were later recognized as *F. brachygibbosum* [6]. Moreover, Al-Sadi et *al.* and Gashgari et *al.* [14, 15] found isolates of *F. brachygibbosum* in date palms and medicinal plants. *F. brachygibbosum* has a strong ability to infect plant seeds below the soil surface, pods, roots, leaves, stems and plant debris which leads to many symptoms such as vascular wilt, crown rot in the field [16], with a strong potential to infect and reduce the growth and productivity of wheat. It produces symptoms similar to the results obtained with *Fusarium* head blight (FHB) on wheat in South Africa [17].

Recently a first report of *F. brachygibbosum* isolated from a wheat plant in Iraq [18]. In Algeria, new *Fusarium* species continue to appear as causal agents of FCR, such as *Fusarium cerealis* and *Fusarium equiseti* [19-20]. This finding confirms the need for a vigilant monitoring strategy of infected plant material and calls for special attention in future studies of wheat FCR, especially with regard to the prevalence of these pathogens in wheat fields and their effects on crop productivity. The objectives of this study were the following: *(i)* Isolation of *F. brachygibbosum* which has recently described in the Algerian territory especially for wheat, and molecular identification with a phylogenetic study. *(ii)* Evaluation of the pathogenicity of *F. brachygibbosum* on wheat seedlings and its capacity to induce FCR.

MATERIAL AND METHODS

1. Fungal material

For FE10 isolation. One hundred and seventeen samples of symptomatic plants or those suspected of being symptomatic of FCR and FHB were collected during the agricultural season (2018-2019) in several provinces of northeastern Algeria, including: Guelma, Constantine, Oum el Bouaghi, Mila. The infected seeds were peeled from their glumes. While the crowns were cut into fragments of about 10 mm, then disinfected in 2% sodium hypochlorite (NaClO) for 5 min, then rinsed in 3 successive baths of sterile distilled water [21]. Drying between two sheets of sterile paper towels, and then deposited on potato sucrose agar (PSA) medium in sterile Petri dishes (5 fragments per dish), and incubated at 28°C for 7 days. Fungal colonies suspected of belonging to the genus *Fusarium* (a white, beige to brownish white, and pink to reddish color) were transplanted onto the same PSA medium. All isolates were purified by the single-spore technique and stored in 20% glycerol water at -80°C.

2. Molecular identification and Phylogenetic analysis

The identification of FE10 was performed first on the basis of morphological, cultural and microscopic characteristics on PSA medium. To confirm this identification of FE10. The molecular identification was carried out by the BIOfidal laboratory (CEDEX-France). The DNA Extraction was carried out using a commercial kit NucleoSpin Plant II (Macherey-Nagel Germany).

From the mycelium collected by scraping the surface of the culture on a Petri dish 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 using the microwave mini-preparation procedure described by Goodwin & Lee [22]. The last DNA pellet was completed in a TE 100µl buffer (10mM Tris-HCl pH 8.0, 0.1mM EDTA) and stored at -20 °C until use.
Regions internal transcribed spacer (ITS) of rDNA were amplified using a fungal-specific primer ITS-1 (5' CTT GGT CAT TTA GAG GAA GTA A '3) [23]. All amplification reactions were carried out in a reaction volume 25µl containing the Sample Volume: 14.1 µl of ultra-pure water, 5 µl buffer of Taq Promega, 1.5 µl MgCl2, 0.2 µl dNTP, 1µl of each relevant oligonucleotide primer (F and R primers), 0.2 µl Taq polymerase Promega, 2 µl genomic DNA. The amplification products were revealed after 1.5% agarose gel electrophoresis of a 10µl deposit of PCR products. Migration is followed by staining in an ethidium bromide bath (0.5µg/ml). Afterwards, the DNA was visualized and photographed under UV using the Gel doc system of biorad (USA).

2.1. PCR temperature condition

Initial denaturation 95°C for 5 min, followed by 35 cycles, denaturation 95°C for 30s, hybridization 55°C for 30s, elongation 72°C for 45s, final elongation 72°C for 7min, storage before revelation 10°C until use.

2.2. Purification of PCR products

The PCR products were purified by the NucleoSpin® Gel and PCR Clean-up kit from Macherey-Nagel (Germany) following the protocol described by the supplier.

2.3. Sequencing of PCR products

The isolated and purified PCR products were sequenced using Sanger technique [24] using the Applied Biosystems BigDye v3.1 kit and PCR primers used for the amplification of the fragments of interest. The obtained sequences were analyzed and cleaned by the use of software SnapGene® Viewer 5.2. The final sequences were then compared with those in the GeneBank database by using the program BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi Blast) of NCBI for the identification of the studied Isolates based on % homology with the reference strains.

The ITS region sequences of the resulting isolates were used for multiple sequence alignment in MEGA format using the Clustal W option of the MEGA-X software. The phylogenetic tree and distance matrix were also constructed using MEGA software, which implemented the Neighbour-Joining (NJ) dendrogram from Saitou and Nei [25]. The phylogenetic distance was based on the Kimura 2-parameter (K2P) model [26].

3. Inocula preparation

Fungal discs (13mm diameter) of FE10, used in the tube test, were obtained from 7-day-old young cultures on PSA medium. However, the inoculation suspension was prepared according to the protocol of Bouanaka et *al.* [27]. Strain FE10 was grown on 6 Petri dishes on PSA medium over a period of 12 days. 10ml of sterile distilled water with 0.05% (v/v) Tween-20 were added to the mycelium aerial part on the surface of each dish, and scraped carefully until all the fungus surface part was recovered. The mixture of macroconidia, mycelium and PSA medium was collected in a beaker and shaken well with a magnetic bar to release the macroconidia from the mycelium and homogenize their dispersion, then filtered through a cheesecloth double layer. The macroconidial suspension concentration was adjusted to 7×10^6 conidia/ml by Malassez cell.

4. FCR pathogenicity test

The FE10 isolate was screened for pathogenicity. Two methods (*in vitro* and *in vivo*) were used to evaluate the isolate aggressiveness on 9 local bread wheat cultivars; cv. El Hachimia, Medracen, Nif Encer, Boumerzoug, Arz, Hidhab, Ain Abid, Akhamokh and Bordj Mehis These cultivars were donated by the National Institute of Agronomic Research of Algeria (INRAA).

4.1. Pathogenicity dish test on germination (in vitro)

The petri dish test was performed according to the method of Bouanaka et *al.* [28], 200 bread wheat seeds of each cultivar were surface sterilized with 2% NaClO for 8 min, and then rinsed six times with sterile distilled water. For each cultivar, 12 Petri dishes lined with absorbent paper in a triple layer were used, 25 healthy seeds were placed in each Petri dish, 4 dishes were inoculated with 10ml of the FE10 inoculation suspension (7×10^6 conidia/ml), and 4 dishes were used as control (10ml sterile distilled water). All dishes were incubated at 28°C in total darkness (The Host).

Germinated seeds in the 4 inoculated dishes of each cultivar were counted 3 days after inoculation (DAI) (Figure 1.b) and similarly for the 4 control dishes (number considered as 100% germination) (Figure 1.a). The percentage of germination inhibition (%GI) was calculated according to the equation: $\mathbf{\%GI = 100\text{-}\{SG_I\ (\times 100)/SG_C\}}$. Where ***SGI*** = number of seeds that germinated in the inoculated dishes, ***SGC*** = number of seeds that germinated in the control dishes. The ***GI*** value ranges from 0% (non-pathogenic, seeds germinated ≥ the control) to 100% (highly pathogenic, all seeds not germinated by day 3).

The Area Under the Disease Progress Curve (AUDPC) was used to illustrate symptoms on already germinated seeds and thus susceptibility to initial infection by FE10. Ungerminated seeds or seeds with brown spots on the coleoptiles and/or completely covered by mycelium were identified on FE10 inoculated Petri dishes counted each day from 3 to 6 DAI. The kinetics of disease progression in percentage of symptomatic seeds (PSS) was assessed between the first and last readings.

AUDPC was calculated according to the equation:

$$\mathbf{AUDPC} = \left\{\sum_{0}^{m}\left(W_i + W_{i+1}/2\right)\right\}/t\,.$$

Where ***Wi*** is the PSS at the ith score, ***t*** = number of days elapsed between the first and last scores, and m = total number of scores. AUDPCs values are limited from 0 (very resistant, seedlings similar to control) to 100 (very susceptible, all seedlings diseased on day 3).

4.2. Pathogenicity tube test on FCR (in vivo)

This test was performed according to the method of Bouanaka *et al.* [27]. Fifty-four test tubes (14 x 3cm) were filled with 4cm of cotton. 20ml of distilled water was added to each tube and covered with aluminum foil, then sterilized by autoclaving at 180°C for 2 hours. 270 seeds (30 seeds/cultivar) were disinfected and rinsed as described in the previous test. Seeds were placed on the wet cotton swab in each test tube (5 seeds/tube). The tubes were closed with clear caps and placed in the host at 28°C for 4 days, where the seedlings reached the one-leaf stage (Zadoks' GS 11) [29]. Inoculation was carried out as follows. For each cultivar, 3 control tubes and 3 inoculated tubes; 3 per FE10 (13mm disc).

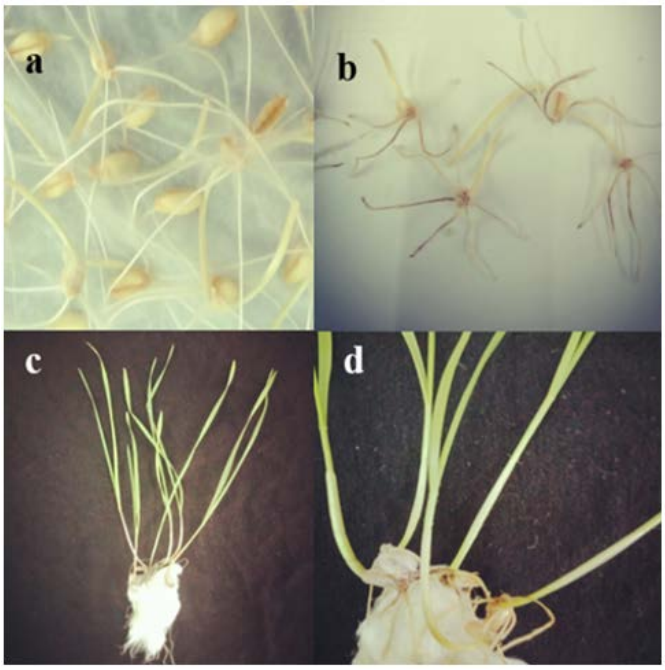

Figure 1. Photos illustrate, on top Petri dish test (**a**) Healthy seeds control. (**b**) Seeds infected by FE10. On bottom tube test, (**c**) Healthy crown control, (**d**) Crown infected with FE10.

After inoculation, the tubes were transferred to the growth chamber (25/19°C day/night temperature, 16/8 h light/dark cycle) for 10 days, they were arranged in a complete randomization scheme. When seedlings reached the two-leaf stage (Zadoks' GS 12) [29], they were carefully removed from the cotton.

FCR severity classes for crown discoloration were assigned in the laboratory on a scale of 0 to 4: Where Class 0 = healthy crown (Figure 1.c); 1 = light crown browning; 2 = half crown browning; 3 = complete crown browning (Figure 1.d); and 4 = seedling death.

Disease severity (DS) of inoculated tubes was calculated using the McKinney index [30], which expresses the percentage of disease severity (i.e. 100) according to the formula: $\mathbf{DS} = \{\sum(\mathbf{d\ x\ f})/\mathbf{m\ x\ M}\}\ \mathbf{x\ 100.}$ Where **d** = disease class, **f** = frequency, **m** = number of observations, and **M** = the highest value of the adopted empirical scale (class 4).

The percentage reduction in length and weight of the coleoptile were calculated as follows: **%RCL=100-(Infected CL x 100/Control CL); %RCW=100-(Infected CW x 100/Control CW)**

All tests were repeated twice in a row. Koch's postulates were satisfied by re-isolating the FE10 pathogen from the infected cowns.

5. Statistical analysis

Analyses of the data, graphs, tables and descriptive statistics (mean, standard deviation), were carried out using SPSS software (IBM SPSS Statistics version 25), comparisons between groups (Inoculated FE10 and control) were determined by Paired-Samples T-Test. Correlations between the different parameters were determined by Pearson correlation.

Means of the different treatments were compared using Tukey's honestly significant difference test. Results were considered significant at the 5% level ($p \leq 0.05$) and highly significant at the 1% level ($p \leq 0.01$).

RESULTS

1. Morphological and Molecular identification and phylogenetic analysis

Isolate FE10 was studied on PSA medium, the culture grew in 10 days and developed abundant powdery, sandy, pale yellow to shrimp orange mycelium. The center of the colony is white also the periphery is irregular white and dark around the medium (Fig. 2. a).The growth is quite slow, not even half of the 9cm Petri dish in 10 days (Figure 2. a). The reverse side of the culture shows an orange-brown gradient from the middle to the edges until it becomes white (Figure 3. b). The appearance of macroconidia is very slow with a number of chlamydospore (Figure 4. a,b). Macroconidia are robust, thick-walled, apical and basal, curved cells, usually with 4 septa (Figure 4. c,d). No microconidia were produced.

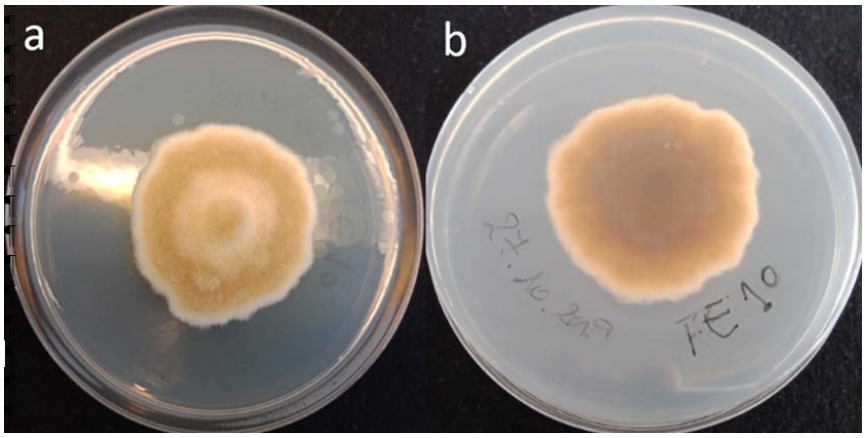

Figure 2. Macroscopic appearance of *F. brachygibbosum* (FE10) after 10 days of incubation on PSA medium, (**a**) the face and (**b**) the reverse.

The ITS1-2 sequence of FE10 was compared with reference sequences collected from GenBank (Table 1). The FE10 isolate was identified as *F. brachygibbosum* and an accession number (MK299139) was assigned by NCBI GenBank. The phylogenetic analysis deduced by Neighbour-Joining (NJ) analysis of the ITS region groups the isolates into 4 distinct clades (Fig. 3). The third clade is notable for the single *F. brachygibbosum* isolate LWU_40. The fourth clade groups our strain FE10 with strains AV7 (elephant foot yam) and S2590 (sandy beach soil) isolated from India and Malaysia, respectively (Table 1).

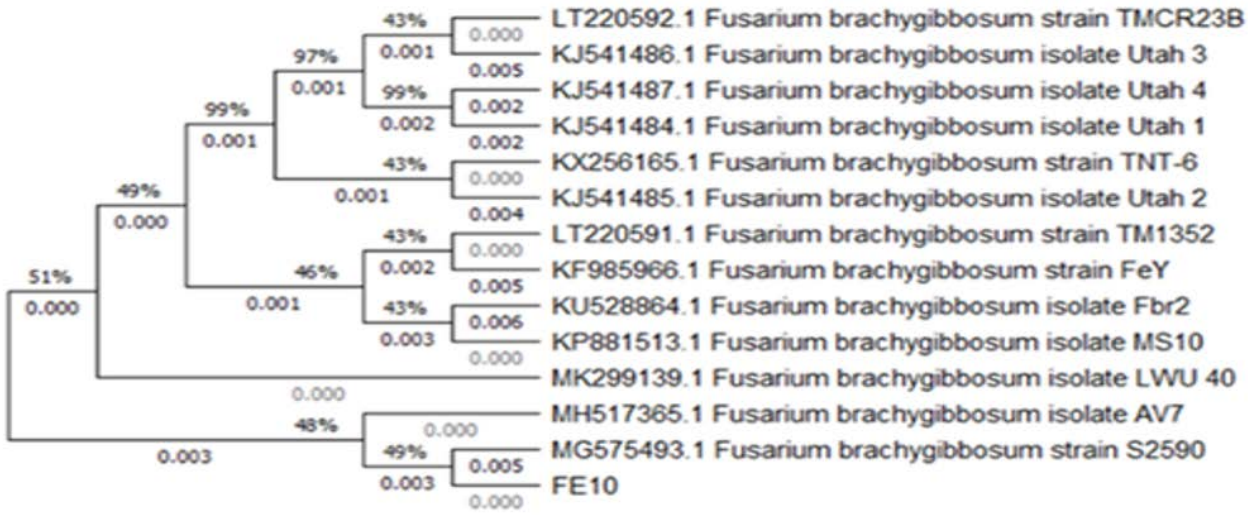

Figure 3. Phylogenetic relationships of *F. brachygibbosum* isolates (FE10) inferred by Neighbour-Joining (NJ) analysis of ITS sequences.

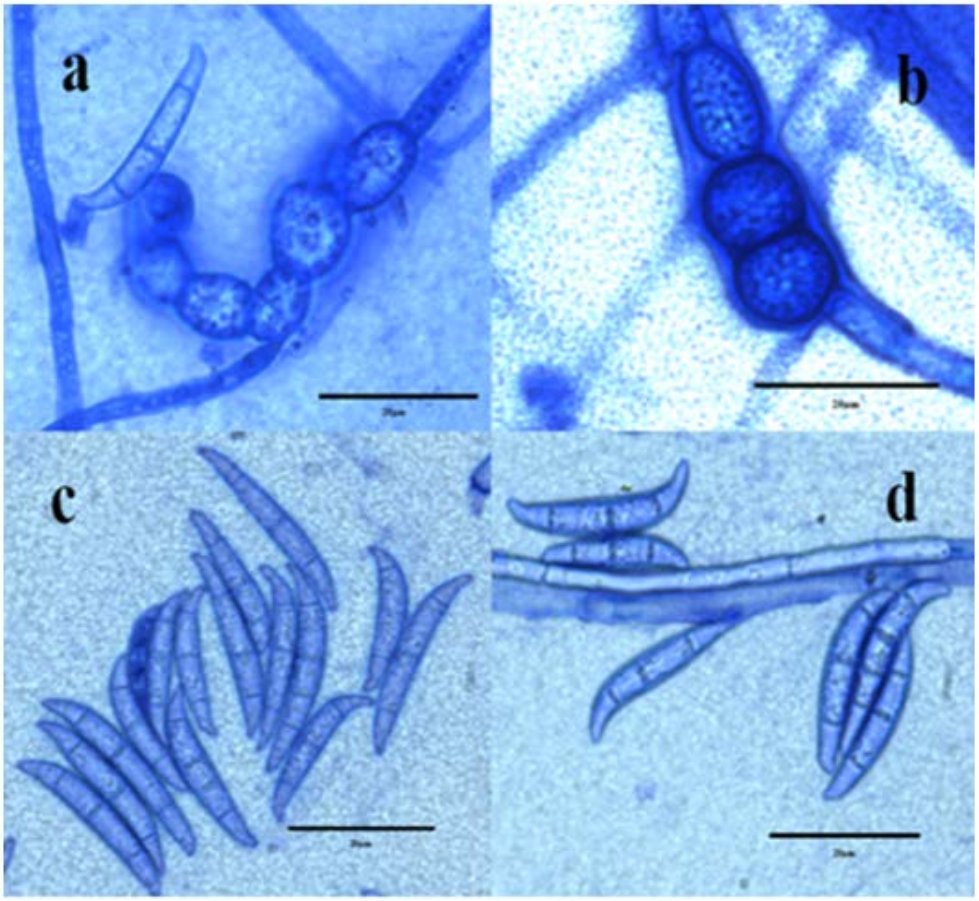

Figure 4. Microscopic aspects of *F. brachygibbosum* FE10, (**a**) and (**b**): macroconidia. (**c**) and (**d**): chlamydospore. Scale bar 20µm.

Table 1. The comparison of the DNA sequence of FE10 isolate (obtained with ITS1-2) to the referred sequences collected from the Genbank (NCBI).

Description	Query Length	Total score	Query cover	E value	Per. ident	Host	Country	Accession
Fusarium brachygibbosum strain FE10 internal transcribed spacer 1	484 bp					Wheat	Algeria	MW450596
Fusarium brachygibbosum isolate LWU_40 internal transcribed spacer 1	494 bp	894	100%	0.0	100%	Medicinal plant	India	MK299139
Fusarium brachygibbosum strain S2590 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1	585 bp	894	100%	0.0	100%	sandy beach soil	Malaysia	MG575493
Fusarium brachygibbosum isolate AV7 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1	550 bp	894	100%	0.0	100%	elephant foot yam	India	MH517365
Fusarium brachygibbosum strain TNT-6 internal transcribed spacer 1	492 bp	894	100%	0.0	100%	root	China	KX256165
Fusarium brachygibbosum genomic DNA sequence contains ITS1, 5.8S rRNA gene, ITS2, strain TMCR23B	494 bp	894	100%	0.0	100%	Tenebrio molitor	Portugal:Douro Wine Region	LT220592
Fusarium brachygibbosum genomic DNA sequence contains ITS1, 5.8S rRNA gene, ITS2, strain TM1352	494 bp	894	100%	0.0	100%	Tenebrio molitor	Portugal:Douro Wine Region	LT220591
Fusarium brachygibbosum isolate Fbr2 internal transcribed spacer 1	527 bp	894	100%	0.0	100%	Olea europaea	Tunisia	KU528864
Fusarium brachygibbosum isolate MS10 internal transcribed spacer 1	494 bp	894	100%	0.0	100%	pigeonpea stem	India	KP881513
Fusarium brachygibbosum isolate Utah 4 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1	1131 bp	894	100%	0.0	100%	Nicotiana attenuata	USA	KJ541487
Fusarium brachygibbosum isolate Utah 3 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1	1113 bp	894	100%	0.0	100%	Nicotiana attenuata	USA	KJ541486
Fusarium brachygibbosum isolate Utah 2 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1	1129 bp	894	100%	0.0	100%	Nicotiana attenuata	USA	KJ541485
Fusarium brachygibbosum isolate Utah 1 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1	1134 bp	894	100%	0.0	100%	Nicotiana attenuata	USA	KJ541484
Fusarium brachygibbosum strain FeY 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1	557 bp	894	100%	0.0	100%	oleander	Iran	KF985966

2. In vitro pathogenicity on germination

The pathogenicity test was performed on 9 bread wheat varieties to determine the impact of this isolate on germination and initial seed infection, as well as infection of the wheat basal part (crown). The low pathogenicity of FE10 on germination of Ain Abid and Boumerzoug cultivars with very low GI values (00.00 and 4.07%) lasted for the next 3 days, with similarly low AUDPC values (5.32 and 8.13%), respectively (Table 2). All our cultivars showed FE10 sensitivity from the 3 DAI, with a decrease in the germinated seeds number, each variety in comparison with its own control. Ain Abid the genotype that showed the most important germination resistance (GI=00.00 %), also with the germinated seeds initial infection (Table 2).

Table 2. The percentage of germination inhibition %GI and the AUDPC obtained by the Petri dish test, conducted on 9 bread wheat cultivars.

Parameters	Germination Inhibition GI%	AUDPC
Grain / Cultivar	*F. brachygibbosum* (FE10)	*F. brachygibbosum* (FE10)
Medracen	10.94±2.48^{bc}	16.32±4.03^{bc}
El Hachimia	14.18±3.77^{c}	19.88±3.56^{c}
Boumerzoug	4.07±1.47^{ab}	8.13±1.41^{ab}
Akhamokh	13.81±2.76^{c}	18.39±2.54^{c}
Nif Encer	17.36±3.00^{c}	38.86±4.22^{d}
Arz	11.20±1.96^{bc}	20.97±2.53^{c}
Ain Abid	00.00±00.00^{a}	5.32±1.21^{a}
Bordj Mehis	16.53±2.89^{c}	32.21±3.46^{d}
Hidhab	11.27±2.28^{c}	18.77±1.76^{c}

For each column, the values (mean± Std. Deviation) with different letters denote the statistical significance determined by ANOVA followed by Tukey test ($P < 0.05$)

3. In vivo pathogenicity on FCR

FE10 induced FCR to all tested bread wheat cultivars ($DS_{brachygibbosum}$ > 10%). The FE10 pathogenicity was also manifested by a significant reduction in wheat coleoptile length (%RCL). The cultivars Arz, Nif Encer and Ain Abid had the highest reduction rates with %RCL= (34.49, 29.58 and 29.41%), respectively. The same three cultivars recorded the highest reduction rates with %RCW = (48.43, 49.09 and 34.59%), respectively (Table 3).

The cv. Akhamokh showed a high tolerance to FE10 with the lowest whole collection DS=10.71%, despite this tolerance a clearer disease pattern on the vegetative system length and weight of the seedling (coleoptiles) (Table 3). The values of the coleoptiles RCL and RCW were positive except for cv. El Hachimia, where the contamination by *F. brachygibbosum* did not influence at all on the coleoptiles length or weight, with even negative values recorded (-2.95% and -6.19%), respectively, although it recorded the highest DS (36.53%) (Table 3).

Table 3. The disease severity (DS), and the percentage reduction in coleoptile length and weight (%RCL and %RCW) obtained by the tube test.

Parameters	Disease Severity (DS%)	Coleoptile Length (mean± Std. Dev)(mm)		Coleoptile Weight (mean± Std. Dev)(mg)		% Reduction in coleoptiles Length and Weight	
Grain / Cultivar	DS *F. brachygibbosum*	Infected CL (mm) *F. brachygibbosum*	Control CL (mm)	Infected CW (mg) *F. brachygibbosum*	Control CW (mg)	*F. brachygibbosum*	
						RCL	RCW
Medracen	14.58±1.86^{a}	132.40±3.60^{ab}	166.15±10.97^{a}	85.30±3.54^{c}	109.97±14.23^{a}	20.31	22.43
El Hachimia	36.53±2.63^{b}	177.27±13.37^{d}	172.18±8.68^{a}	118.54±2.68^{e}	111.63±5.36^{a}	-2.95	-6.19
Boumerzoug	14.28±2.70^{a}	166.55±6.52^{cd}	174.84±6.17^{a}	99.83±5.52^{d}	112.10±2.70^{a}	4.74	10.94
Akhamokh	10.71±0.92^{a}	145.02±3.38^{bc}	169.97±11.37^{a}	84.46±3.89^{bc}	110.73±9.58^{a}	14.67	23.72
Nif Encer	15.38±1.89^{a}	114.55±5.93^{a}	162.67±14.89^{a}	52.77±5.21^{a}	103.66±9.11^{a}	29.58	49.09
Arz	14.28±1.98^{a}	112.09±13.86^{a}	171.11±8.81^{a}	52.00±2.88^{a}	100.84±4.12^{a}	34.49	48.43
Ain Abid	13.46±3.77^{a}	128.31±4.21^{ab}	181.79±17.08^{a}	72.26±2.48^{b}	110.48±3.87^{a}	29.41	34.59
Bordj Mehis	16.66±1.68^{a}	130.42±3.58^{ab}	165.65±18.78^{a}	89.38±6.08^{cd}	118.09±14.09^{a}	21.26	24.31
Hidhab	30.76±3.95^{b}	148.36±3.62^{bc}	184.26±3.85^{a}	99.60±6.00^{d}	124.79±2.65^{a}	19.48	20.18

For each column, the values (mean± Std. Deviation) with different letters denote the statistical significance determined by ANOVA followed by Tukey test ($p < 0.05$).

DISCUSSION

The genus *Fusarium* causes the most serious diseases affecting the roots, stem and spike of wheat and at all stages of growth. In Algeria, some studies have been carried out on the main agent responsible for FCR and FHB of wheat which is *F. culmorum*, focusing on its occurrence, pathogenicity and diversity [31, 32]. However, other *Fusarium* pathogenic species have not yet been well studied. Little research has been conducted on the pathogenicity of *F. brachygibbosum* on wheat worldwide, including quantitative studies of the effect on coleoptile length and weight, as well as initial infection by FCR. This study was conducted to isolate and identify the FE10 isolate causing FCR of wheat, but also to characterize their aggressiveness (*in vitro* and *in vivo*). Our encoded *F. brachygibbosum* strain (FE10) was submitted to the Genbank database (NCBI) and accession number MW450596 was assigned.

FE10 was isolated from infected wheat grains collected from the region of El Mridj in the province of Constantine (36°21′10″N 6°45′44″E) Algeria. Isolate FE10 was morphologically suspected to belong to the species *F. brachygibbosum*, but to avoid confusion with other species, molecular identification was paramount and was done with sequencing of the ITS1-2 region of the rDNA internal transcribed spacer (contains the sequences of internal transcribed spacer 1, ribosomal RNA 5.8S and internal transcribed spacer 2). Comparison of the ITS1-2 rDNA sequence of isolate FE10 with the referenced sequences collected in the Genbank database (NCBI), showed us a 100% similarity rate with the *F. brachygibbosum* strain (isolate LWU_40), whose host is Medicinal plant from India with the accession number MK299139 (Table 1). This confirms the result of the morphological identification.

All our tests were performed under controllable conditions in the laboratory, or in the growth chamber, in order to reduce the variability associated with environmental factors, and that can distort our results, and as proven by Wallwork et *al.* [33], field trials are often unreliable due to uneven natural infection of contamination by other pathogens or adverse environmental conditions that affect the results.

The pathogenicity test of FE10 on *in vitro* germination was performed on 9 bread wheat varieties to determine its impact on seedlings, germination but also initial infection of germinated coleoptiles by the AUDPC parameter (Table 2). The results showed that there was a statistically significant difference ($p<0.001$) between the artificially infected plants and the uninfected control plants. This result confirms that our isolates were the cause of the wheat seed germination disease. Despite the difference in resistance/susceptibility levels of our tested cultivars. *F. brachygibbosum* negatively affected germination capacity and germinated seed growth of all cultivars except cv. Ain Abid (Table 2).

The *in vitro* pathogenicity test on FCR showed that none of the cultivars had a null DS and that FE10 caused FCR symptoms on all tested varieties, with more or less low DS, varying from 10.71% for cv. Akhamokh to 36.53% for cv. El Hachimia which was at the top of the list for its FCR sensitivity (Table 3). No statistically significant differences between the lengths and weights of the control coleoptiles of the different varieties tested (Table 3).

F. brachygibbosum (FE10) pathogenicity seems to be far from comparable with the results obtained by Hudec [34], who reported in his work that *F. culmorum* and *F. graminearum* were the most severe pathogens on the wheat coleoptile length development, with a reduction of 91.32% for *F. culmorum* and 78.32% for *F. graminearum*.

The value of RCL is statistically significantly related to RCW with a correlation coefficient r=0.859, $p< 0.001$, which is very logical. The cv. El Hachimia created the exception by negative RCL and RCW (-2.95 and -6.19%), respectively. While all other values were positive (Table 3).

The infection risk is associated with climate change with increasing rainfall and temperature [35]. This makes the crops exposed to FCR invasions, and forces us to do more and more experimentation on the different cultivars sensitivity for more control and promotion of *Fusarium* disease control.

Our study shows the importance of searching for new *Fusarium* species in FCR wheat samples. As well as the need for further research on these species pathogenicity which could be a potential threat to wheat cultivation.

CONCLUSION

This study identified *F. brachygibbosum* on Algerian wheat. Its pathogenicity was manifested by FCR reduction of seed germination, as well as negative effect on length and fresh weight of the vegetative system. This information will have an impact on future scientific research. Especially regarding the prevalence of this pathogen, and its effects on crop productivity.

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