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Isolation and characterization of Fusarium spp. responsible for crown rot and fusarium head blight in Algerian wheat; identification of associated species and assessment of their aggressiveness

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Full Title:	Isolation and characterization of Fusarium spp. responsible for crown rot and fusarium head blight in Algerian wheat; identification of associated species and assessment of their aggressiveness
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Constantine, 22/08/2021

Cover Letter

The Editor: Pr. Khaled A. El-Tarabily

Sub: Submission of Manuscript for publication

Dear Editor,

We wish to submit an original research article entitled "Isolation and characterization of *Fusarium* spp. responsible for crown rot and fusarium head blight in Algerian wheat; identification of associated species and assessment of their aggressiveness" for consideration by Journal of Plant Pathology. In this paper, we report the current diversity of *Fusarium* species associated with FCR and FHB, the main characteristics and life lines of the strains and the severity variables specific to each strain and their pathogenicity and aggressiveness on sensitive durum wheat.

We believe that this manuscript is appropriate for publication by journal of Plant Pathology because it is focused in the field of plant pathology. The best of our knowledge, we report herein for the first time the identification of *F. incarnatum*, *F. asiaticum*, *F. acuminatum* and *F. graminearum* from wheat in Algeria. The most relevant Highlights of this paper are:

- This study provides a simple in vitro test to predict the aggressiveness of *F. culmorum* on wheat FCR.
- The first report concerning the identification of *F. incarnatum*, *F. asiaticum*, *F. acuminatum* and *F. graminearum* from wheat in Algeria.
- No correlation between the two pathogenicity tests (in vitro and in vivo) with growth rate or sporulation rate, so these two life lines remain far from being parameters for measuring or predicting the pathogenicity of *Fusarium* strains.
- Some *Fusarium* species did not report symptoms of FCR but they did significantly reduce coleoptiles length.
- Correlation between growth rate and sporulation rate was observed ($r = 0.35$ $P = 0.012 < 0.05$).

We confirm that this work is original and has not been published elsewhere, nor is it currently under consideration for publication elsewhere. All authors have read and agree to the submission of the manuscript.

We can propose the following reviewers:

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We have no conflicts of interest to disclose. Thank you for your consideration of this manuscript.

Sincerely,

Dr. Ines Bellil

Signature

Isolation and characterization of *Fusarium* spp. responsible for crown rot and fusarium head blight in Algerian wheat; identification of associated species and assessment of their aggressiveness

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Declarations

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Competing interests

The authors declare that they have no competing interests

Availability of data and materials

All data generated or analysed during this study are included in this published article

Authors' contributions

HB conceived the work, designed and performed the experiments, analyzed the data, wrote the paper. **IB** was a major contributor in writing the manuscript, reviewing & editing the paper. **WH** contributed to the isolation of *Fusarium* strains. **DK** supervision, project administration. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

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Abstract

Fusarium crown rot (FCR) and fusarium head blight (FHB) are two destructive fungal diseases of wheat in the world. This research was conducted to study the most important species associated with these diseases in Algeria. One hundred and seventeen samples were collected. Sixty-three from the crown and fifty-four from wheat grains in several regions of northeastern Algeria. *Fusarium* spp. have been identified by sequencing regions of the internal transcribed spacer (ITS1-2) of the rDNA as well as their life lines (growth and sporulation rates). The FCR pathogenicity tests were performed by two methods in vitro and in vivo. A total of 34 isolates representing 10 *Fusarium* species were obtained. These results showed five species isolated from crown; *Fusarium culmorum* (16), *F. cerealis* (3), *F. acuminatum* (2), *F. graminearum* (1) and *Fusarium oxysporum* (1). And seven species from grains; *F. culmorum* (3), *F. incarnatum* (3), *F. graminearum* (1), *F. equiseti* (1), *F. asiaticum* (1), *F. fujikuroi* (1) and *F. brachygibbosum* (1). Strains of *F. culmorum* were dominant and more aggressive. However, the rest of the species were more saprophytic than aggressive. A strong correlation was recorded between the tube and the pot tests ($r = 0.62$, $P = 0.007 < 0.01$). This result provides a simple in vitro test to predict the aggressiveness of *F. culmorum* on wheat FCR. In addition, this is the first report concerning the identification of *F. incarnatum*, *F. asiaticum*, *F. acuminatum* and *F. graminearum* from wheat in Algeria.

Keywords: Algeria, fungus, *Fusarium*, pathogenicity, *Triticum*.

1. Introduction

Wheat (*Triticum* spp.) being a staple food for about 40% of the world's population plays a major role in global food security (Bockus et al. 2010). Unfortunately, it can be infected by many diseases caused by microorganisms that reduce the quality and quantity of the harvest. Among these diseases, fusarium crown rot (FCR) and fusarium head blight (FHB). These are two of the most destructive and damaging fungal diseases of wheat, transmitted by soils and residues in many countries of the world (Tunali et al. 2008; Shikur Gebremariam et al. 2017).

This *Fusarium* species complex, varies according to time, geography and environmental factors (Xu and Nicholson 2009), including, *F. pseudograminearum, F. culmorum* and *F. graminearum* known for their high pathogenicity and cause more severe symptoms and yield losses, and they are the most common species in many countries (Smiley et al. 2005; Dyer et al. 2009).

Fusarium include several genera and species that have different characteristics and life lines that can potentially influence their development or production of toxins during an epidemic (Picot et al. 2011). Quantification of the life lines of *Fusarium* spp. and the associated FCR severity variables is an essential step in order to define the links between strain characteristics and their ability to induce FCR.

The objectives of this study were; (i) To assess the current diversity of *Fusarium* species associated with FCR and FHB in Algeria. (ii) To identify and measure in vitro the main characteristics and life lines of the strains and the severity variables specific to each strain. (iii) To evaluate their pathogenicity and aggressiveness on a sensitive durum wheat. (iv) And to try to highlight links between these life lines, easily and rapidly measurable in vitro, and these in vivo severity measures.

2. Material and methods

2.1. Isolation, purification and conservation of *Fusarium* spp.

Sampling was carried out in six of the most wheat-producing provinces (Constantine, Mila, Guelma, Oum elbouaghi), located in north-eastern Algeria. Plants symptomatic of FCR, or those suspected of being symptomatic of FHB, were collected from different fields in several communes of the regions surveyed (**Table 1**). The infected crowns of the plant were cut into fragments of about 10mm, while seeds were peeled from their glumes. The samples were disinfected in 2% sodium hypochlorite (NaClO) for 5 minutes, and then rinsed in 3 successive baths of sterile distilled water. Dried between two sheets of sterile paper towels, then deposited on potato sucrose agar (PSA) medium in sterile Petri dishes (5 fragments per dish), and incubated at 28°C for 7 days. The fungal colonies suspected to be of the genus *Fusarium* were transplanted on the same medium. All isolates were purified by the single-spore technique and stored in Eppendorf tubes in 20% glycerol water at -80°C.

2.2. Molecular identification and Phylogenetic analysis

The identification was first carried out on the basis of macroscopic data and microscopic characteristics on three culture media; Carnation Leaf-Piece Agar (CLA), Spezieller Nährstoffarmer Agar (SNA) and PSA. These media were prepared according to Leslie and Summerell (2006). To confirm the identification of the *Fusarium* spp. obtained. Molecular identification was performed 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 of lysis buffer (50mM Tris-HCl pH 7.5, 50mM EDTA, 3% SDS and 1% 2-mercaptoethanol) were added and the nucleic acids were isolated using the microwave mini-preparation procedure described by (Goodwin and Lee 1993). 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. Internal transcribed spacer (ITS) regions of rDNA were amplified using a fungal-specific primer ITS-1 (5' CTT GGT CAT TTA GAG GAA GTA A '3) (Gardes and Bruns 1993). All amplification reactions were carried out in a reaction volume of 25µl containing the Sample Volume: 14.1 µl of ultra pure water, 5 µl buffer of Taq Promega, 1.5 µl $MgCl_2$, 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).

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.

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.

Sequencing of PCR products, The isolated and purified PCR products were sequenced using Sanger technique (Sanger et al. 1977) using the Applied Biosystems BigDye v3.1 kit and PCR primers used for the amplification

of the fragments of interest. The obtained sequences are analyzed and cleaned by the use of software SnapGene® Viewer 5.2. The final sequences are 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 of *Fusarium* spp. 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 (1987). The phylogenetic distance was based on the Kimura 2-parameter (K2P) model (Kimura 1980).

2.3. Characterization of strains and measurement of their life lines

2.3.1. Growth rate

From the mother strains in conservation, transplantation was carried out on PSA medium on Petri dishes (9 cm diameter). After 6 days of growth, a second transplantation of 6 mm diameter of the culture was performed on the same medium. The resulting Petri dishes were incubated at 28 °C in the dark for 5 days. Then the radial growth diameters were measured to determine the growth rate of each strain in mm per day. Three replicates were made for each isolate.

2.3.2. Sporulation rate

The conserved *Fusarium* strains were previously transplanted on a Petri dish containing PSA medium and incubated for 30 days at 28°C. After microscopic confirmation that all isolates have been sporulated, an 18mm plug of mycelium from the center of each dish was removed and transferred to a 50 ml Erlenmeyer flask containing 10 ml of sterile distilled water. After 20 minutes of rotary agitation at a speed of 200 rpm, the suspensions obtained were filtered separately using fine cheesecloth. Then the absorbencies were measured by a spectrophotometer at 530nm, and the sporal concentration was determined by the following linear formula (Concentration = 20090760 × Abs - 100439) (Caligiore-Gei and Valdez 2015). Spore concentrations were also confirmed by counting at the Malassez cell. Three counts were made for the same sporal suspension as well as 2 repetitions for each strain.

2.4. FCR pathogenicity tests

The study of FCR pathogenicity and aggressiveness of *Fusarium* isolates were evaluated by two methods in vitro and in vivo. The cultivar of Italian origin Simeto, known for its sensitivity to wheat fusarium, was chosen as the plant material for both tests (Purahong et al. 2014).

2.4.1. In vitro test in growth chamber

This test was performed according to Bouanaka et al. (2021a). The bottoms of 105 essay tubes (14 cm × 3 cm) were filled with 4cm of cotton. Twenty milliliters of distilled water were added to each tube and then covered with aluminum foil. Afterwards the tubes were sterilized by autoclaving at 180°C for 2 hours twice with an interval of 24 hours. The wheat (Simeto) seeds were surface disinfected with 2% NaClO for five minutes and rinsed three times in succession with sterile distilled water. After drying, the seeds were carefully placed on moist cotton in each essay tube (5 seeds/ tube).

An 8 mm disc of 7-days old fungal isolate was placed adjacent to the seeds (3 discs in 3 tubes for each isolate). The tubes were closed with transparent caps and incubated at 28ºC for 4 days, then transferred to the growth chamber (25/19ºC day/night temperature, 16/8 h light/dark cycle). The tubes were arranged according to a complete randomization design. After two weeks, the disease severity (DSt) and the percentage of coleoptiles length (%CL) were measured. FCR disease severity classes based on symptoms that included brown and necrotic discoloration of crown tissue were assigned to the laboratory on a scale of 0 to 4 where: Class 0 = healthy crown; 1 = light browning of the crown; 2 = browning of half of the crown; 3 = complete browning of the crown; and 4 = death of the seedlings.

The DSt of each treatment was calculated using McKinney's (1923) index, which expresses the percentage of maximum disease severity (i.e., 100) according to the formulas: $DSt = \{\sum(c \times f)/n \times N\} \times 100$.

Where c = disease class, f = frequency, n = number of observations, and N = the highest value of the empirical scale adopted (class 4).

$\%CL = (CLt \times 100)/CLc$. Where %CL = the percentage of coleoptiles length, CLt = the average coleoptiles length, CLc = the average coleoptiles length of the control.

2.4.2. In vivo test in greenhouse

This test was performed according to Bouanaka et al. (2021b). Only some isolates among the isolated *Fusarium* spp. that showed significant pathogenicity in the first in vitro test were studied for this in vivo test. Five seeds of durum wheat Simeto were surface-sterilized with 2% NaClO for 8 min and then rinsed six times with sterile

distilled water. Then they were sown in a pot of (8cm x 12 cm height) containing a mixture of soil and compost (1/2). This mixture was previously sterilized at 180° for 2h (2 repetitions with 24h interval). For each isolate, two replicates and two non-inoculated controls (5 pots/ isolate) were used. The pots were placed in the growth chamber (25/19ºC day/night temperature, 16/8 h light/dark cycle). After 3 weeks, when the seedlings have reached the two-leaf stage (Zadoks' GS 12: Zadoks et al. 1974), the soil around the stems was removed and a PSA disc (13 mm diameter) of a 7-days-old culture of the tested isolate was placed around the stems, 2 cm below the soil to allow the mycelium to reach the base of the stems. The soil was then put back in place around the seedlings. The pots were organized according to a completely random plan. They were watered every 3 days throughout the test. Three weeks after inoculation (Zadoks' GS 20), each plant was carefully removed from the soil and washed with tap water.

Severity classes were assigned to the laboratory on a scale of 0 to 4. As well as the severity of disease (DSp) of each treatment were calculated using the McKinney (1923) index as described above (in vitro test). The DSp value ranged from 0% (non-pathogenic, absence of infected crown) to 100% (highly pathogenic, all seedlings dead). Koch's postulates were satisfied by re-isolation of *Fusarium* sp. from small or large brown spots on the crowns for both tests.

2.5. Statistical analysis

Data analyses, graphs, tables, and descriptive statistics (mean standard deviation and variance), were performed using SPSS software (IBM SPSS Statistics version 25). Correlations between the different parameters were determined by Pearson correlation, the coefficients of the correlation were considered significant at the 5% level ($P \leq 0.05$) and highly significant at the 1% level ($P \leq 0.01$). The means for the different treatments were compared using Tukey's honestly significant difference test.

3. Results

3.1. *Fusarium* spp. isolated

Out of 117 samples collected (crown + grain), sixty-three samples from wheat plants showing symptoms of FCR and fifty-four samples from wheat grains suspected of being affected by FHB. A total of 34 isolates of *Fusarium* spp. were the subject of our study. Eighteen isolates of *Fusarium* spp. were obtained in this study and sixteen others were provided by the national institute of agronomic research of Algeria (INRAA), Constantine unit.

Among the 34 isolates obtained, a high diversity of *Fusarium* spp. was observed, 23 isolates from plant crown and 11 from wheat grain (**Table 1**). The majority of isolates were obtained during the 2019 crop year. But also from the previous seasons 2016 and 2017 for some isolates exclusively from wheat grain (**Table 1**).

3.2. Molecular identification and Phylogenetic analysis

Identification was performed on the basis of macroscopic data and microscopic characteristics (Leslie and Summerell 2006; Hoshino et al. 2009) and confirmed by molecular identification. In the total of 34 isolates, ten *Fusarium* species were identified (**Table 2**). *F. culmorum* is the predominant species for the FCR and a remarkable variety of species for the FHB. The sequence of each isolate was compared to the reference sequence collected from GenBank (**Table 2**). Phylogenetic analysis showed that our isolates are form several groups (**Fig. 1**).

3.3. Growth and sporulation rates

A huge variety between isolates for both lines is noted. Very high diversity in growth rates, with *F. culmorum* species leading the way with its strains (FC20, FC5, FC11 and FC10) recording the fastest growth rates. While isolates FC21, FE10, FE2, FC7, FE9 and FE6 ranked in ascending order according to their growth rate recorded rates of less than 10mm/d (**Table 3**). What is remarkable is that all these last isolates belong to the single species *F. acuminatum, F. brachygibbosum, F. equiseti, F. oxysporum, F. fujikuroi, F. asiaticum*, respectively.

3.4. FCR pathogenicity tests in vitro and in vivo

The in vitro test showed that all isolates were pathogenic and caused symptoms of FCR. None of the strains had a zero DSt, their DSt values varied between 88.33% for FC20 and 5% for FE7 (**Table 3**). It was also clearly noticed that the DSt value is in a very significant relation with the species. *F. culmorum* with its 19 strains caused FCR with a wide range of DSt but also the highest among all other species **(Table 3).**

The 17 *Fusarium* spp. tested for their FCR aggressiveness in pots in greenhouse, were only those that showed the most severe pathogenicity in the tube test with a DSt > 60%. It was *F. culmorum* with 16 strains and one strain of *F. graminearum* (**Table 3**). The results of the aggressiveness in the pot test revealed significant differences among *F. culmorum* (**Table 3**).

4. Discussion

This study was conducted to isolate and identify *Fusarium* spp. causing FCR and FHB from wheat in the north-eastern region of Algeria and to characterize their life lines and their aggressiveness (in vitro and in vivo) towards FCR.

From 34 isolates of *Fusarium* spp. obtained, the results after identification (**Table 2**) showed a diversity of species isolated and the presence of new species never reported in Algeria. Also the results allowed to distinguish five *Fusarium* species from the crown; *F. culmorum* (16), *F. oxysporum* (1), *F. graminearum* (1), *F. cerealis* (3) and *F. acuminatum* (2) and seven species from grains distributed as follows; *F. culmorum* (3), *F. incarnatum* (3), *F. graminearum* (1), *F. asiaticum* (1), *F. fujikuroi* (1), *F. brachygibbosum* (1), and *F. equiseti* (1) (**Table 2**). This high diversity of species isolated from wheat grains appears to be quantitatively similar to that obtained by Nielsen et al. (2011), where they found up to seven different species of *Fusarium* in the grain samples, confirming the existence of a complex of diverse *Fusarium* species at the origin of FHB epidemics. Thus Audenaert et al. (2009) recorded up to nine *Fusarium* spp. in a sample of cereals and found clear associations and direct synergy between some species, although FHB species frequently compete in the same host.

Although the pathogenicity of *F.culmorum* strains to FCR is high overall, there is as yet little potential for variation between strains due to differentiation in aggressivity. This illustrates the variability between isolates of the same species, so it is necessary to continue to monitor trends in this pathogenic species in the future. This complex variation between pathogenic isolates associated with FCR has been previously studied using various evaluation standards and inoculation techniques by Yang et al. (2010), and Poole et al. (2012). Most of these studies showed that *F. pseudograminearum* and *F. culmorum* were the most pathogenic species, and *F. avenaceum*, *F. equiseti* and *F. poae* were the least pathogenic species. While *F. pseudograminearum* and *F. graminearum* cause greater crown rot, and *F. culmorum* causes the greatest seedling blight.

Pathogenicity results in the tube assay revealed significant differences in aggressiveness among *Fusarium* isolates (**Table 3**). The single species of *F. oxysporum, F. equiseti, F. asiaticum, F. fujikuroi* and *F. brachygibbosum* recorded low to negligible pathogenicity values (**Table 3**). These species have not been competitive against strains of *F. culmorum*. Our results seem to be compatible with the works of Eslahi (2012), Shikur Gebremariam et al. (2017) and Dehghanpour et al. (2019), where they found that *F. proliferatum, F. acuminatum, F. oxysporum* and *F. flocciferum* species had medium to low levels of pathogenicity on wheat, they are rather saprophytic.

According to the study of Smiley and Patterson (1996), *F. acuminatum* includes a few isolates capable of causing brown discoloration of the wheat crown and some strains capable of even killing wheat plants in greenhouse. In contrast to our results, the two strains of *F. acuminatum* were without influence on coleoptiles growth and with very low DSt (**Table 3**).

In this study, *F. oxysporum* and *F. brachygibbosum* were among the non-pathogenic species. However, Demirci and Dane (2003) also reported that *F. oxysporum* is weakly aggressive. Also Shikur Gebremariam et al. (2017) in their work reported that *F. oxysporum, F. equiseti, F. incarnatum, F. brachygibbosum* and *F. acuminatum* were non-pathogenic on winter wheat.

Pathogenicity was also manifested by a large reduction in %CL (**Table 3**). On the other hand, other isolates did not influence the length of the coleoptiles at all, with values even higher than those of the control. This was the case for FE11 and FC21, which represent two strains of *F. incarnatum* and *F. acuminatum,* respectively. Three other isolates recorded almost negligible reduction in coleoptiles length, FE2, FC19 and FE10 represent the species (*F. equiseti, F. acuminatum* and *F. brachygibbosum*), respectively (**Table 3**). In contrast to our single strain of *F. equiseti*, Bencheikh et al. (2020) found that *F. equiseti* had little negative effect on certain growth parameters such as length and fresh weight of the root and vegetative system.

Overall, isolates of *F. cerealis* were more aggressive than those of *F. incarnatum*, resulting in greater DSt and a greater reduction in coleoptiles length, particularly FC23. On the other hand, *F. acuminatum* isolates were less aggressive than *F. incarnatum* isolates, and so was the reduction in %CL, where it is negligible for *F. acuminatum* (**Table 3**). Inoculations in the tube test by the single strains *F. equiseti F. brachygibbosum F. asiaticum F. fujikuroi* had no effect on the length of the coleoptiles. While *F. oxysporum* significantly reduced the %CL, And had a very small effect on FCR with a DSt = 6.66% (**Table 3**).

The results for the pot test showed that FC20, FC1and FC2, recorded the highest aggressiveness, while FE1, FC4 and FC15 recorded the lowest (**Table 3**). The only strain of *F. graminearum* showed no significant difference from those of *F. culmorum.*

F. culmorum was the most common species among the main pathogenic *Fusarium* species identified in this study. This result is consistent with those obtained in other countries such as Turkey (Tunali et al. 2008), Italy (Scherm et al. 2012), and Iran (Eslahi 2012) where *F. culmorum* was the dominant species as the causative agent of FCR. However, our results appear to be slightly different from a survey conducted in five provinces of China, where *F. asiaticum* was considered the dominant species (Zhang et al. 2015).

A strong correlation was found between the tube test and the pot test (r =0.62, P=0.007< 0.01) (**Fig. 2.a**). This result will allow us to predict the aggressiveness of *F. culmorum* strains towards wheat FCR by a simple in vitro tube test. Also another correlation between growth rate and sporulation rate was observed (r =0.35 P=0.012<0.05) (**Fig. 2.b**) (**Table 4**).

Total lack of correlation between DS in both tests with the reduction in coleoptiles length was noticed (r = -0.03, p = 0.90 for DSt, and r = 0.17, p = 0. 50 for DSp). This can be explained by the fact that some species did not report symptoms of FCR but they did significantly reduce coleoptiles length, the case of *F. oxysporum* and some strains of *F. incarnatum* (FE7) and *F. cerealis* (FC14) (**Table 4**). No correlation between the two tests in vitro and in vivo with growth rate or sporulation rate was observed, so these two life lines remain far from being parameters for measuring or predicting the pathogenicity of *Fusarium* strains (**Table 4**). Such pathogenicity, phylogeny and genetic diversity will be useful for monitoring trends in pathogen distribution in different regions and for the adoption of wheat FCR control strategies in Algeria.

5. Conclusion

This study reveals a wide range of *Fusarium* species associated with wheat FCR in Algeria. The main finding was that the different *Fusarium* spp. do not all have the same FCR aggressiveness even between strains of *F. culmorum*. This confirms the need for a vigilant surveillance strategy for infected material. The predominant species isolated was *F. culmorum*, followed by *F. cerealis* and *F. incarnatum*. Here we report the appearance of *F. acuminatum, F. asiaticum, F. incarnatum* and *F. graminearum* for the first time from wheat in Algeria. These strains collected in this research constitute a characterized fungal source for many studies in the future.

References

Audenaert K, Van Broeck R, Bekaert B, De Witte F, Heremans B et al. (2009) Fusarium head blight (FHB) in Flanders: population diversity, inter-species associations and DON contamination in commercial winter wheat varieties. Eur J Plant Pathol 125: 445–458. https:// doi. org/10.1007/s10658-009-9494-3

Bencheikh A, Rouag N, Mamache W, Belabed I (2020) First report of *Fusarium equiseti* causing crown rot and damping-off on durum wheat in Algeria. Arch Phytopathol Plant Protection 1-17. https:// doi. org/10.1080/03235408.2020.1804303.

Bockus WW, Bowden RL, Hunger RM, Morrill WL, Murray TD et al. (Eds) (2010) Compendium of wheat diseases and pests (3rd ed.). St Paul: The American Phytopathological Society Press

Bouanaka H, Bellil I, Harrat W, Boussaha S, Benbelkacem A et al. (2021a) On the biocontrol by *Trichoderma afroharzianum* against *Fusarium culmorum* responsible of fusarium head blight and crown rot of wheat in Algeria, Egypt J Biol Pest Control 31, 68. https:// doi. org/10.1186/s41938-021-00416-3

Bouanaka H, Bellil I, Khelifi D (2021b) Multiple methods for varietal resistance assessment of durum wheat cultivars against *Fusarium culmorum* the causal agent of Fusarium Head Blight and Crown Rot in Algeria, Physiol Mol Plant Pathol 115, 101683. https:// doi. org/10.1016/j.pmpp.2021.101683

Caligiore-Gei PF, Valdez JG (2015) Adjustment of a rapid method for quantification of *Fusarium* spp. spore suspensions in plant pathology. Revista Argentina de Microbiología 47(2), 152–154. https:// doi. org/10.1016/j.ram.2015.03.002

Dehghanpour FS, Taheri P, Falahati RM (2019) Identification and pathogenicity of *Fusarium* spp., the causal agent of wheat crown and root rot in Iran. J Plant Pathol https:// doi. org/10.1007/s42161-019-00400-9

Demirci E, Dane E (2003) Identification and pathogenicity of *Fusarium* spp. from stem bases of winter wheat in Erzurum, Turkey. Phytoparasitica 31, 170–173

Dyer AT, Johnston RH, Hogg AC, Johnston JA (2009) Comparison of pathogenicity of the Fusarium crown rot (FCR) complex (*F. culmorum, F. pseudograminearum* and *F. graminearum*) on hard red spring and durum wheat. Eur J Plant Pathol 125(3), 387–395. https:// doi. org/10.1007/s10658-009-9488-1

Eslahi MR (2012) Fungi associated with root and crown rot of wheat in Khuzestan province. Iran Journal of Crop Protection 1, 107–113

Gardes M, Bruns TD (1993) ITS primers with enhanced specificity for basidiomycetes - application to the identification of mycorrhizae and rusts. Mol Ecol 2(2), 113–118. https:// doi. org/10.1111/j.1365-294x.1993.tb00005.x

Goodwin DC, Lee SB (1993) Microwave mini prep of total genomic DNA from fungi, plants, protists and animals for PCR. Biotechniques 15:438–444

Hoshino T, Xiao N, Xiao N, Tkachenko OB (2009) Cold adaptation in the phytopathogenic fungi causing snow molds. Mycoscience 50(1), 26–38. https:// doi. org/10.1007/s10267-008-0452-2

Kimura M (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J Mol Evol 16(2), 111–120. https:// doi. org/10.1007/bf01731581

Leslie JF, Summerell AB (2006) The Fusarium laboratory manual p. 388. Ames: Blackwell Publishing

McKinney HH (1923). Influence of soil temperature and moisture on infection of wheat seedlings by *Helminthosporium sativum.* J Agric Res 26**,** 195–217

Nielsen LK, Jensen JD, Nielsen GC, Jensen JE, Spliid NH et al. (2011) Fusarium head blight of cereals in Denmark: species complex and related mycotoxins. Phytopathology 101:960–969. https:// doi. org/10.1094/PHYTO-07-10-0188

Picot A, Hourcade-Marcolla D, Barreau C, Pinson-Gadais L, Caron D et al. (2011) Interactions between *Fusarium verticillioides* and *Fusarium graminearum* in maize ears and consequences for fungal development and mycotoxin accumulation. Plant Pathol 61(1), 140–151. https:// doi. org/10.1111/j.1365-3059.2011.02503.x

Poole GJ, Smiley RW, Paulitz TC, Walker CA, Carter AH et al. (2012) Identification of quantitative trait loci (QTL) for resistance to Fusarium crown rot (*Fusarium pseudograminearum*) in multiple assay environments in the Pacific Northwestern US. Theor Appl Genet 125(1), 91–107. https:// doi. org/10.1007/s00122-012-1818-6

Purahong W, Nipoti P, Pisi A, Lemmens M, Prodi A (2014) Aggressiveness of different *Fusarium graminearum* chemotypes within a population from Northern-Central Italy. Mycoscience 55(1), 63–69. https:// doi. org/10.1016/j.myc.2013.05.007

Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol. https:// doi. org/10.1093/oxfordjournals.molbev.a040454

Sanger F, Nicklen S, Coulson AR (1977) DNA sequencing with chain-terminating inhibitors. Proceedings of the National Academy of Sciences 74(12), 5463–5467. https:// doi. org/10.1073/pnas.74.12.5463

Scherm B, Balmas V, Spanu F, Pani G, Delogu G et al. (2012) Fusarium culmorum: causal agent of foot and root rot and head blight on wheat. Mol Plant Pathol 14(4), 323–341. https:// doi. org/10.1111/mpp.12011

Shikur Gebremariam E, Sharma-Poudyal D, Paulitz TC, Erginbas-Orakci G, Karakaya A et al. (2017) Identity and pathogenicity of *Fusarium* species associated with crown rot on wheat (*Triticum* spp.) in Turkey. Eur J Plant Pathol 150(2), 387–399. https:// doi. org/10.1007/s10658-017-1285-7

Smiley RW, Gourlie JA, Easley SA, Patterson LM (2005) Pathogenicity of Fungi Associated with the Wheat Crown Rot Complex in Oregon and Washington. Plant Dis 89(9), 949–957. https:// doi. org/10.1094/pd-89-0949

Smiley RW, Patterson LM (1996) Pathogenic fungi associated with Fusarium foot rot of winter wheat in the semiarid Pacific Northwest. Plant Dis 80, 944–949

Tunali B, Nicol JM, Hodson D, Uçkun Z, Büyük O et al. (2008) Root and Crown Rot Fungi Associated with Spring, Facultative, and Winter Wheat in Turkey. Plant Dis 92(9), 1299–1306. https:// doi. org/10.1094/pdis-92-9-1299

Xu XM, Nicholson P (2009) Community ecology of fungal pathogens causing wheat head blight. Ann Rev Phytopathol 47:83–103. https:// doi. org/10.1146/annurev-phyto-080508-081737

Yang X, Ma J, Li H, Ma H, Yao J et al. (2010) Different genes can be responsible for crown rot resistance at different developmental stages of wheat and barley. Eur J Plant Pathol 128(4), 495–502. https:// doi. org/10.1007/s10658-010-9680-3

Zadoks JC, Chang TT, Konzak CF (1974) A Decimal Code For The Growth Stages Of Cereals. Weed Res 14(6), 415–421. https:// doi. org/10.1111/J.1365-3180.1974.Tb01084.X

Zhang X, Sun H, Shen C, Li W, Yu H et al. (2015) Survey of *Fusarium* spp. Causing Wheat Crown Rot in Major Winter Wheat Growing Regions of China. Plant Dis 99(11), 1610–1615. https:// doi. org/10.1094/pdis-04-14-0422-re

Table captions

Table 1. Summary table of varieties, locations, and sampling period.

Table 2. Comparison of the DNA sequence of *Fusarium* spp. isolates (obtained with ITS1) with reference sequences collected from the Genbank (NCBI)

Table 3. Molecular identification of *Fusarium* isolates with their life lines (sporulation rate and growth rate) and pathogenicity *in vitro* and *in vivo* on FCR

Table 4. The different correlations between the different parameters measured

Figure captions

Figure 1. Phylogenetic relationships of the 34 *Fusarium* isolates inferred from the analysis of ITS sequences by MEGA software.

Figure 2. Simple Scatter with fit line of Correlations between; (**a**) DSt by DSp test ($r = 0.627$, $P = 0.007 < 0.01$), (**b**) Sporulation rate (mm/d) by Growth Rate (sp/ml) $r = 0.355$, $P = 0.012 < 0.05$ determinate by Pearson correlation coefficient.

Table 1 Summary table of varieties, locations, and sampling period

Isolate code	Region	Province	Wheat	Location Coordinates latitude and longitude	Year
FC1	Carrefour Djbel aougueb Oued Athmania	Mila	Durum	36°14′56″N 6°21′08″E	2019
FC2	Messaoud Boudjriou	Constantine	Durum	36°26′03″N 6°29′12″E	2019
FC3	Oued Zenati	Guelma	Durum	36°19′43″N 7°09′31″E	2019
FC4	Bekkouche Ahmed	Guelma	Durum	36°16′52″N 7°07′00″E	2019
FC5	Ain Mlila	Oum El Bouaghi	Durum	36°07′28″N 6°36′07″E	2019
FC6	Guettar El Aich	Constantine	Bread	36°13′35″N 6°36′22″E	2019
FC7	Ain Fakroun	Oum El Bouaghi	Bread	35°49′28″N 7°01′36″E	2019
FC8	Sigus	Oum El Bouaghi	Durum	36°03′14″N 6°49′06″E	2019
FC9	El khroub	Constantine	Bread	36°11′37″N 6°41′55″E	2019
FC10	Oued Arama	Mila	Durum	36°13′59″N 6°26′12″E	2019
FC11	Bordj Lagar	Mila	Durum	36°12′37″N 6°24′15″E	2019
FC12	Oued Seguin	Mila	Durum	36°14′21″N 6°25′05″E	2019
FC13	Oued Seguen	Mila	Durum	36°10′10″N 6°24′53″E	2019
FC14	Zegrour Elarbi Hamma Bouziane	Constantine	Durum	36°14′23″N 6°19′14″E	2019
FC15	Oued Athmania	Mila	Durum	36°21′30″N 6°41′38″E	2019
FC16	El Mridj	Constantine	Durum	36°26′23″N 6°33′17″E	2019
FC17	Constantine	Constantine	Bread	Nl	2019
FC18	Constantine	Constantine	Bread	Nl	2019
FC19	Constantine	Constantine	Durum	Nl	2019
FC20	Constantine	Constantine	Durum	Nl	2019
FC21	Constantine	Constantine	Durum	Nl	2016
FC22	Constantine	Constantine	Durum	Nl	2017
FC23	Constantine	Constantine	Durum	Nl	2017
FE1	Constantine/Nord	Constantine	Durum	Nl	2016
FE2	Constantine/Sud	Constantine	Bread	Nl	2016
FE3	Constantine/Sud	Constantine	Bread	Nl	2016
FE4	Constantine/Nord	Constantine	Durum	Nl	2016
FE5	Constantine/Sud	Constantine	Durum	Nl	2017
FE6	Constantine/ Nord	Constantine	Bread	Nl	2017
FE7	Constantine	Constantine	Durum	Nl	2017
FE8	Constantine	Constantine	Durum	Nl	2017
FE9	Constantine	Constantine	Bread	Nl	2017
FE10	El Mridj	Constantine	Durum	36°21′10″N 6°45′44″E	2019
FE11	Ain Smara	Constantine	Bread	36°15′31″N 6°28′21″E	2019

Nl: Not located, FC: isolated from wheat crown, FE: isolated from wheat grain, INRAA: National Institute of Agronomic Research of Algeria

Table 2 Comparison of the DNA sequence of *Fusarium* spp. isolates (obtained with ITS1) with reference sequences collected from the Genbank (NCBI)

Isolate code	*Fusarium* species	Query length (bp)	Similar strain description (small subunit ribosomal RNA gene,partial sequence ITS1, 5.8S ribosomal RNA gene)	Max score	Query cover	Per. ident	Accession number of the nearby strain
FC1	*F. culmorum*	468	*Fusarium* sp. isolate RL682	865	100%	100%	MT557469
FC2	*F. culmorum*	482	*F. culmorum* isolate M62A	883	100%	99.79%	MT640271
FC3	*F. culmorum*	471	*F. culmorum* isolate Montana-ex4	870	100%	100%	MK729631
FC4	*F. culmorum*	489	*F. culmorum* isolate S68	904	100%	100%	MH681156
FC5	*F. culmorum*	475	*F. culmorum* isolate ITS75_IQ-BMSP-CS- ITS1,	878	100%	100%	MK281302
FC6	*F. culmorum*	474	*F. culmorum* isolate Montana-ex4	876	100%	100%	MK729631
FC7	*F. oxysporum*	483	*F. oxysporum* f. sp. cumini isolate Anna2	863	100%	98.96%	MN497239
FC8	*F. graminearum*	472	*F. graminearum* isolate N7_ITS1,	872	100%	100%	MT465509
FC9	*F. culmorum*	471	*F. culmorum* isolate Montana-ex4	870	100%	100%	MK729631
FC10	*F. culmorum*	482	*F. culmorum* isolate W_ARSO2_6_19	889	99%	100%	MK594970
FC11	*F. culmorum*	473	*F. culmorum* isolate FC50	874	100%	100%	MT077212
FC12	*F. culmorum*	471	*F. culmorum* isolate Montana-ex4	870	100%	100%	MK729631
FC13	*F. culmorum*	485	*F. culmorum* isolate Montana-ex4	896	100%	100%	MK729631
FC14	*F. cerealis*	472	*F. cerealis* strain TH11-2	876	100%	100%	MT558863
FC15	*F. culmorum*	479	*Fusarium* sp. isolate RL20	885	100%	100%	MT557083
FC16	*F. culmorum*	481	*F. culmorum* isolate S68	889	100%	100%	MH681156
FC17	*F. cerealis*	484	*F. cerealis* isolate 9703	856	100%	98.55%	MG274303
FC18	*F. culmorum*	475	*F. culmorum* isolate Montana-ex4	878	100%	100%	MK729631
FC19	*F. acuminatum*	501	*F. acuminatum* strain DZ-4-2-1	920	100%	99.80%	KT192260
FC20	*F. culmorum*	460	*F. culmorum* isolate FC50	850	100%	100%	MT077212
FC21	*F. acuminatum*	490	*F. acuminatum* strain DZ-4-2-1	905	100%	100%	KT192260
FC22	*F. culmorum*	471	*F. culmorum* isolate Montana-ex4	870	100%	100%	MK729631
FC23	*F. cerealis*	491	*Fusarium* sp. PSU-ES115	889	98%	99.79%	JN116651
FE1	*F. culmorum*	472	*F. culmorum* isolate Montana-ex4	872	100%	100%	MK729631
FE2	*F. equiseti*	477	*F. equiseti* strain UMAS MS25	651	80%	90.84%	MK228625
FE3	*F. culmorum*	470	*F. culmorum* isolate Montana-ex4	869	100%	100%	MK729631

FE4	*F. culmorum*	483	*Fusarium* sp. isolate RL682	893	100%	100%	MT557469
FE5	*F. graminearum*	449	*F. graminearum* isolate ITS71_IQ-BTI3-CS-	527	90%	89.98%	MK271251
FE6	*F. asiaticum*	484	*F. asiaticum* isolate 97b	889	100%	99.79%	KY466889
FE7	*F. incarnatum*	503	*Fusarium* sp. OTU930	904	99%	99.20%	GU934527
FE8	*F. incarnatum*	492	*F. incarnatum* isolate 15	909	100%	100%	MN534779
FE9	*F. fujikuroi*	475	*F. fujikuroi* isolate DE-2016-15	872	100%	99.79%	MT603307
FE10	*F. brachygibbosum*	484	*F. brachygibbosum* isolate LWU_40	894	100%	100%	MK299139
FE11	*F. incarnatum*	500	*Fusarium* sp. isolate BW20PH	924	100%	100%	KU612374

Table 3 Molecular identification of *Fusarium* isolates with their life lines (sporulation rate and growth rate) and pathogenicity *in vitro* and *in vivo* on FCR

Isolate code	*Fusarium* species	GenBank accession number	Sporulation Rate (sp/ml)		Growth Rate (mm/d)		Disease Severity Tube test (DSt)	the average coleoptiles length (CLt) mm ± Std. Deviation	the coleoptile length (%CL)	Disease Severity Pot test (DSp)
			Mean	Std. Deviation	Mean	Std. Deviation				
FC1	*F. culmorum*	MW366637	776420,33^{ab}	449168,89	13,40^{fghij}	0,87	75,00	66,73±34,80^{abcdefg}	59,58	68,33
FC2	*F. culmorum*	MW165423	555532,00^{a}	407759,70	13,46^{fghij}	0,46	83,33	46,53±40,84^{abcde}	41,54	68,33
FC3	*F. culmorum*	MW363060	2469897,67^{abcdef}	371900,84	14,60^{hij}	1,03	76,66	58,73±50,02^{abcdefg}	52,44	56,66
FC4	*F. culmorum*	MW165434	977228,00^{abc}	771826,13	14,66^{hij}	1,10	81,66	43,60±40,80^{abc}	38,92	48,33
FC5	*F. culmorum*	MW349658	3721598,67^{bcdef}	1628193,45	15,73^{ij}	1,33	81,66	60,20±48,40^{abcdefg}	53,75	60,00
FC6	*F. culmorum*	MW358285	475209,33^{a}	220279,00	14,00^{ghij}	1,74	36,66	41,33±59,39^{ab}	36,90	NA
FC7	*F. oxysporum*	MW353146	4558296,67^{defg}	387303,58	8,93^{cd}	0,41	6,66	66,60±51,84^{abcdefg}	59,46	NA
FC8	*F. graminearum*	MW349656	4404344,00^{defg}	573035,18	13,80^{ghij}	0,20	78,33	37,13±28,47^{ab}	33,15	58,33
FC9	*F. culmorum*	MW353156	2771109,33^{abcdef}	362011,21	13,60^{fghij}	0,52	60,00	43,67±45,79^{abc}	38,98	55,00
FC10	*F. culmorum*	MW353149	4765798,00^{efg}	801806,47	15,00^{hij}	0,87	76,66	44,20±37,42^{abc}	39,46	56,66
FC11	*F. culmorum*	MW151664	3761760,00^{cdef}	1164742,20	15,33^{ij}	0,70	85,00	30,80±32,27^{a}	27,50	61,66
FC12	*F. culmorum*	MW349657	916985,67^{abc}	258982,17	14,80^{hij}	0,20	70,00	26,53±37,70^{a}	23,69	56,66
FC13	*F. culmorum*	MW353157	676016,67^{a}	548588,23	14,66^{hij}	0,11	56,66	39,93±46,07^{ab}	35,65	NA
FC14	*F. cerealis*	MW358286	4437812,33^{defg}	654707,01	13,40^{fghij}	0,72	25,00	65,86±55,76^{abcdefg}	58,80	NA
FC15	*F. culmorum*	MW447383	883518,00^{abc}	456147,35	14,80^{hij}	0,52	63,33	52,33±46,56^{abcdef}	46,72	50,00
FC16	*F. culmorum*	MW450585	896905,00^{abc}	206092,87	14,73^{hij}	1,50	66,66	59,27±54,47^{abcdefg}	52,91	51,66
FC17	*F. cerealis*	MW447299	214159,00^{a}	101070,62	12,26^{efgh}	0,70	56,66	69,20±42,51^{abcdefg}	61,78	NA
FC18	*F. culmorum*	MW353140	3768453,33^{cdef}	707496,50	14,00^{ghij}	1,21	76,66	50,40±47,68^{abcdef}	45,00	60,00
FC19	*F. acuminatum*	MW353145	200772,00^{a}	60242,00	10,66^{def}	0,57	8,33	110,13±44,76^{defg}	98,33	NA
FC20	*F. culmorum*	MW165435	6767180,33^{g}	4052086,57	15,80^{j}	0,20	88,33	50,00±36,60^{abcdef}	44,64	71,66
FC21	*F. acuminatum*	MW447502	2443123,33^{abcdef}	655732,68	3,20^{a}	0,52	13,33	112,67±32,14^{fg}	100,59	NA
FC22	*F. culmorum*	MW353148	2777803,00^{abcdef}	815519,40	13,66^{ghij}	0,83	75,00	45,40±38,30^{abcd}	40,53	65,00
FC23	*F. cerealis*	MW447774	649242,33^{a}	239570,21	12,80^{fghi}	1,74	60,00	40,93±41,37^{ab}	36,54	NA

FE1	*F. culmorum*	MW353147	1144567,33^{abc}	278970,96	13,00^{fghij}	0,34	65,00	41,27±49,07^{ab}	36,84	40,00
FE2	*F. equiseti*	MW448184	1104406,33^{abc}	313671,47	6,93^{bc}	0,50	10,00	110,93±39,10^{efg}	99,04	NA
FE3	*F. culmorum*	MW353158	5247736,00^{fg}	425661,64	12,13^{efgh}	1,13	61,66	52,80±55,01^{abcdef}	47,14	NA
FE4	*F. culmorum*	MW450591	4866201,67^{efg}	645505,90	14,40^{hij}	2,11	73,33	54,20±44,38^{abcdefg}	48,39	56,66
FE5	*F. graminearum*	MW358916	3801921,67^{cdef}	162310,81	14,13^{hij}	0,11	65,00	53,20±56,38^{abcdef}	47,50	NA
FE6	*F. asiaticum*	MW366557	736259,00^{a}	209007,59	9,60^{cde}	0,34	11,66	98,07±54,48^{bcdefg}	87,55	NA
FE7	*F. incarnatum*	MW450593	388192,33^{a}	258202,32	12,93^{fghij}	0,30	5,00	82,00±67,51^{abcdefg}	73,21	NA
FE8	*F. incarnatum*	MW448396	1713522,33^{abcd}	960173,70	14,33^{hij}	0,98	41,66	86,00±61,36^{abcdefg}	76,78	NA
FE9	*F. fujikuroi*	MW450594	1258358,33^{abc}	421855,34	9,06^{cd}	1,00	10,00	96,40±41,80^{bcdefg}	86,07	NA
FE10	*F. brachygibbosum*	MW450596	950453,67^{abc}	543418,61	4,93^{ab}	0,30	20,00	106,67±51,36^{cdefg}	95,23	NA
FE11	*F. incarnatum*	MW450597	2068282,67^{abcde}	348388,17	11,06^{defg}	1,00	30,00	118,27±35,92^{g}	105,59	NA
Control								112,00±33,47^{fg}	100	

For each column, the values (mean±SD) with different letters denote the statistical significance determined by ANOVA followed by Tukey test ($P < 0.05$). NA : not available

Table 4 The different correlations between the different parameters measured

different parameters compared	Pearson Correlation (r)	P-value	P > F
DSt X DSp	0,627**	0,007	<0.01
DSt X Sporulation Rate	0,315	0,218	>0.05
DSt X Growth Rate	0,314	0,219	>0.05
DSt X Coleoptile length	-0,030	0,908	>0.05
DSp X Sporulation Rate	0,236	0,362	>0.05
DSp X Growth Rate	0,043	0,871	>0.05
DSp X Coleoptile length	0,173	0,508	>0.05
Sporulation Rate X Growth Rate	0,355*	0,012	<0.05
Sporulation Rate X Coleoptile length	0,078	0,766	>0.05
Growth Rate X Coleoptile length	0,276	0,284	>0.05

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

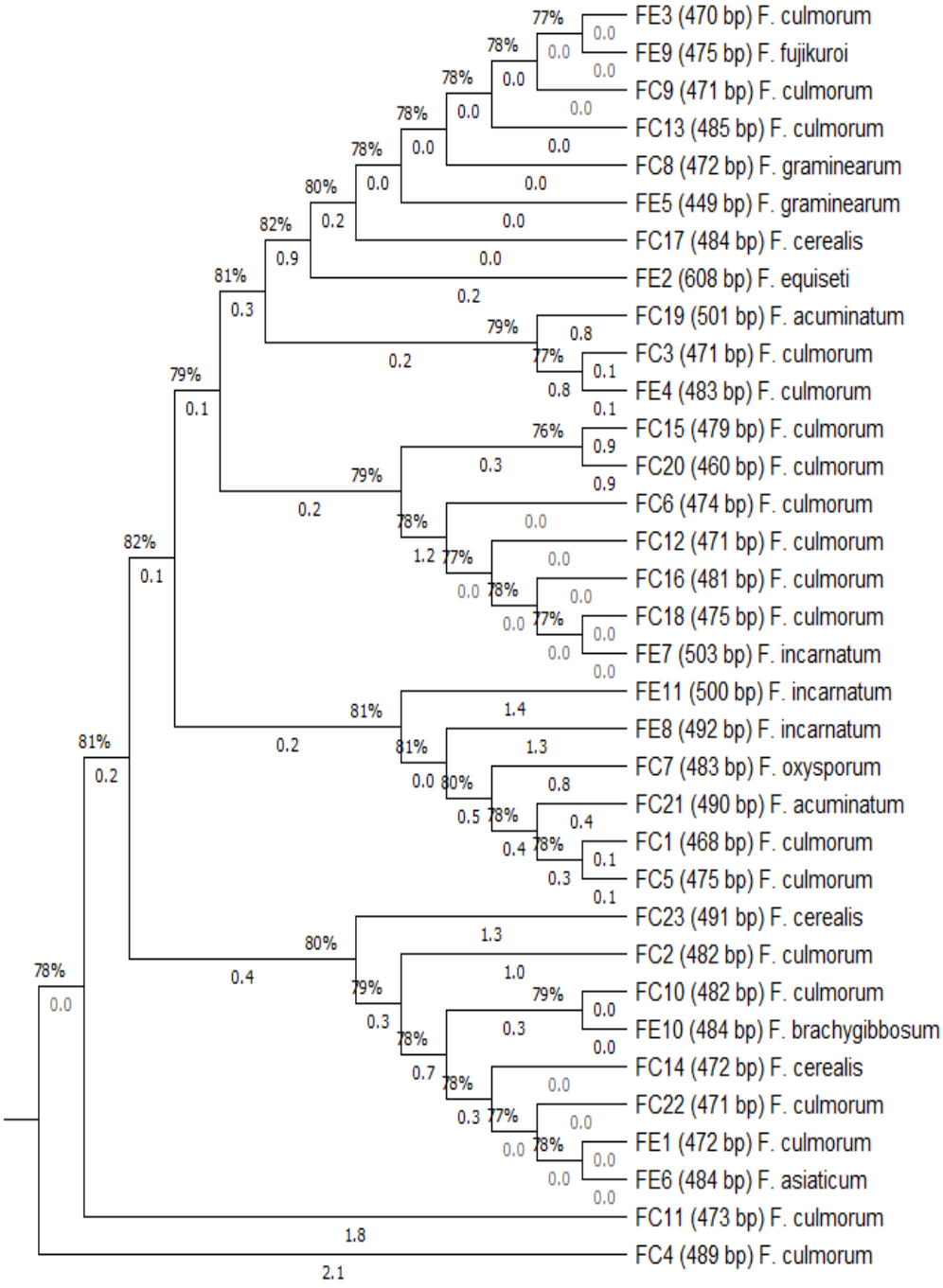

Fig. 1 Phylogenetic relationships of the 34 *Fusarium* isolates inferred from the analysis of ITS sequences by MEGA software.

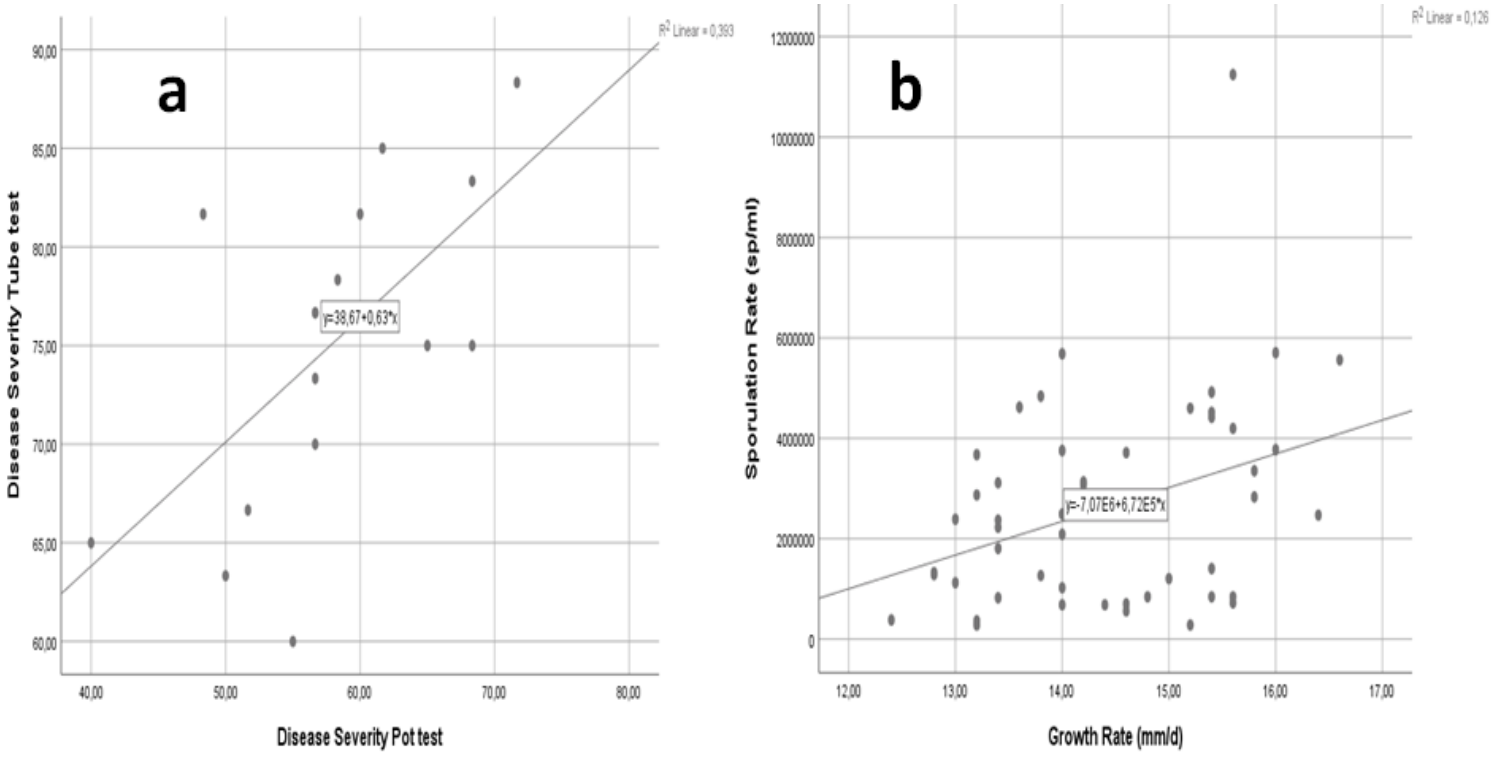

Fig. 2 Simple Scatter with fit line of Correlations between; (**a**) DSt by DSp test (r =0.627, P=0.007 < 0.01), (**b**) Sporulation rate (mm/d) by Growth Rate (sp/ml) r =0.355, P=0.012 < 0.05 determinate by Pearson correlation coefficient.