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Morphological and cultural characterization of two *Fusarium* isolates causing wheat fusarium head blight in Algeria

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Abstract

In Algeria, fusarium head blight is a serious fungal disease of wheat caused by *Fusarium* genus. The study of the epidemiological cycle of the disease in the field is always hampered by a major problem which is the identification of the parasitic complex of *Fusarium* species. These species can only be identified after Petri dish culture and observation of a set of morphological criteria such as color, cultural aspect of colonies, growth rate of mycelium and the form of isolate macroconidia. To this end, the objective of this present study is to identify two *Fusarium* isolates based on a set of morphological and cultural criteria mentioned above. The results obtained for all the morphological characters studied show phenotypic variability between the two isolates. Based on these results, we were able to characterize these two isolates as *F. graminearum* and *F. culmorum*. Indeed, morphological and cultural characterization, although important, remains insufficient. It should therefore be further investigated by molecular characterization, in order to highlight any differences between the two isolates studied.

Keywords: Algeria; fusarium head blight; *Fusarium* isolates; morphological and cultural characterization

Introduction

Wheat is one of the most important cereal crops in the world, representing a major renewable resource for human, animal feed and industrial raw materials (Gómez *et al*., 2021). It provides 55% of carbohydrates and 20% of total food calories consumed (Shewry and Hey, 2015). Due to its economic importance, unfortunately, it is confronted by several cryptogamic diseases including fusarium head blight. This fungal disease affects practically all straw cereal crops, especially wheat, around the world, leading to lower yields and grain quality (Hadjout *et al*., 2017; Prat *et al.,* 2017). *Fusarium* species cause prejudicial damage with economic impact on wheat cultivation worldwide (Jaillais *et al*., 2015; Rebouh *et al*., 2019).

In infected plants, the disease causes the death of developing seeds (prematurely bleached ears) under humid conditions and mild temperatures during flowering (Figueroa *et al*., 2018). In many parts of the world, plant disease outbreaks are spreading and endangering the food security of vulnerable people (Ristaino *et al*., 2021). Severe epidemics have caused quantitative yield losses of up to 50-75% since their discovery in 1884

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(Parry *et al.,* 1995; McMullen *et al.,* 2012). Pests and diseases of food crops cause very significant global yield losses, which are in the order of 21.5% (10.1 to 28.1%) for wheat, 30.3% (24.6 to 40.9%) for rice, 22.6% (19.5 to 41.4%) for corn, 17.2% (8.1 to 21%) in potato and 21.4% (11 to 32.4%) in soybean (Savary *et al*., 2019). Globally, hundreds of billions of dollars in losses in food production have been recorded and losses in agricultural yields of several staple crops amounting to up to 30% (Rizzo *et al*., 2021). It also reduces grain quality because crops contaminated with high levels of mycotoxins are toxic to humans and animals (Maresca, 2013; Hadjout *et al.,* 2022). Mycotoxins have variable acute toxicity, with long-term effects such as cancer induction, DNA modifications or harmful effects on the fetus (Haque *et al*., 2020). Today, the great problem of food safety is linked mainly to cereal grains infected with mycotoxins due to their harmful effects on the quality and losses of production and cereal yields (Prat *et al.,* 2017).

When *Fusarium* pathogens infect seed, they cause the disease known as fusarium head blight (FHB). Grain infections can cause black spots, which are undesirable in milling as they can cause flour discoloration and change in flour quality (Stępień and Chełkowski, 2010; Polišenská *et al*., 2019). *Fusarium* species can develop after harvest if the wet grain is not dried effectively and quickly. The most common *Fusarium* species in cereals, especially wheat, are represented by *F. graminearum* and *F. culmorum* (Nelson *et al*., 1983; Wagacha and Muthomi, 2007; Houmairi *et al*., 2018; Hadjout *et al*., 2022; Gallé *et al*., 2022). *Fusarium* genus, are fungi belong to the hyalo-hyphomycetes and have a septate and colorless mycelium. In culture, colonies often show pink shades, yellow, red or purple (Booth, 1985; Alves-Santos *et al*., 1999; Ortoneda *et al*., 2004).

At the taxonomic level, the *Fusarium* still constitute obstacles during their identifications (Divakara *et al*., 2014). The main identification tools for this fungi type are based on so-called "conventional" phenotypic methods based on the research for morphological and cultural characteristics of the strains (Quero, 2018). These methods are long, laborious, not very reproducible and require very extensive knowledge due to the great diversity of molds, making it difficult to differentiate very close species (Quero, 2018). The conventional methods of fungi identification used in routine are essentially based on the analysis of macroscopic morphological characters (vegetative apparatus aspect, relief, size, color) and microscopic characters (fruiting organs and spores). This identification requires a great knowledge of micromycetes field (Lecellier, 2013).

The objective of this research is to make a morphological identification of two *Fusarium* isolates responsible for fusarium head blight disease.

Materials and Methods

The morphological identification method used is mainly based on microscopic and macroscopic criteria. The strains were initially grown on a culture medium (PDA) and then examined under the microscope using the most well-known morphological identification keys.

Fungal material

The two *Fusarium* isolates, F.G.10.08 and F.C.T5, were obtained from soft wheat spikelets that showed typical disease symptoms. These symptoms manifested on the ear, a coloration ranging from pink to orange due to the presence of spore mass (Wegulo, 2021). The F.G.10.08 isolate comes from samples of soft wheat spikelets taken at Technical Institute of Field Crops of Algiers experimental station, whereas the F.C.T5 isolate is obtained from samples of soft wheat spikelets collected at the Higher National Agronomic School of Algiers experimental station, El-Harrach, Algiers. These two isolates were collected from the Laboratory of Phytopathology and Molecular Biology, Higher National Agronomic School, Algiers, Algeria.

Purification of both isolates by monospore culture

From each pure mycelial culture of any contamination containing spores of the two *Fusarium* isolates, a conidian suspension is prepared in glass tubes containing sterile distilled water. To prepare this, 10 mL of sterile distilled water was added into each sterilized glass tube. Subsequently, 5 mm of explant for each isolate taken from the Petri dish was introduced into the glass tube and mixed vigorously to allow the release of the spores into the sterile distilled water. Using a Pasteur pipette, a drop of each suspension containing small number of conidia, or even a single spore, and spread it uniformly in the center of a Petri dish containing Potato Dextrose Agar (PDA) medium. After incubation at 25 °C and in the dark for approximately seven days, the conidia germinated, thus giving a white-developed white airborne mycelium on the entire surface of the Petri dish and free from contamination.

Morphological identification criteria for the two isolates

The identification of the *Fusarium* species was carried out as described by Toussoun and Nelson (1976). Morpho-cultural characterization the two *Fusarium* isolates was carried out on a PDA culture medium. Some of the identification keys observed were: appearance and colour of mycelial colonies, size and shape of macroconidia, presence or absence of microconidia, and presence or absence of chlamydospores.

Conidia measurement

Conidia obtained from 12-day-old cultures on PDA medium were measured. For each isolate, using a Pasteur pipette. For each isolate, a small fragment containing the spores of the fungus is taken from the Petri dish using a Pasteur pipette. It is then placed between slide and coverslip of an optical microscope to observe the microscopic shape of the conidia. The device is previously calibrated and equipped with an eyepiece with a micrometer. Measurements of the length, diameter and number of septa for each isolate were taken from 50 spores randomly scattered between the slide and the coverslip.

Linear mycelial growth

The comparative study of the mycelial growth of isolates on the PDA culture medium, was done by measuring linear growth. This consists of measuring the mycelial growth (diameter of the colonies) as a function of time, from the transplanting of an initial explant of 5 mm of diameter, placed in the center of a Petri dish, containing a PDA medium, according to the formula of Rapilly (1968):

$L = (D-d)/2$

Where:

L: linear growth (mm)

D: colony diameter (mm)

d: initial explant diameter (mm)

Mycelial explants are taken using a sterile Pasteur pipette from cultures of the two isolates, approximately 7 days old, cultured on a PDA medium. Seeding is carried out in the center of the Petri dish containing the PDA culture medium. Four replicates (four boxes) are performed for each isolate. The Petri dishes are incubated at a temperature of 25 °C and in the dark. Colony growth is measured every 24 hours.

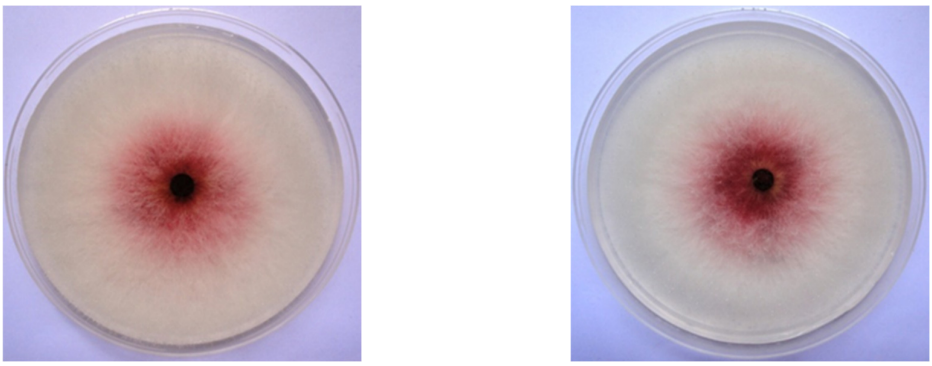
Statistical analysis

Statistical analysis of the results is performed using SASTM software, version 9.0. A multiple comparison of the means was conducted using the LSD test (Least Significant Difference) to determine homogeneous groups at the 5% significance level.

Results

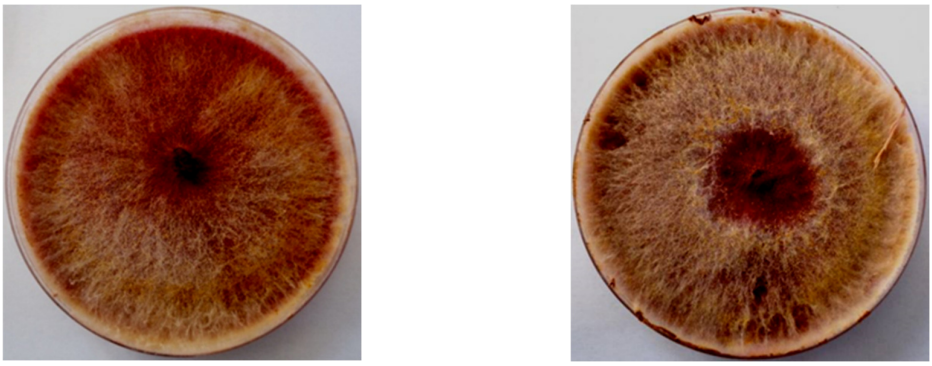
Color and cultural appearance of colonies

The two isolates studied presented slightly different cultural and morphological characteristics. Based on these criteria, morphological identification results revealed that F.C.T5 and F.G.10.08 isolates belong to *F. culmorum* and *F. graminearum*, respectively. On PDA medium, *F. culmorum* grew rapidly with an orange pale mycelium, but becomes dark brown with age with more or less yellow reflections in its aerial part and sporulates very abundantly. These isolate forms a red pigment on PDA medium (Figures 1 and 2). *F. graminearum* also grows rapidly on PDA medium with a dense mycelium which varies from white to pale orange to yellow with more or less yellow reflections in its aerial part and sporulates very abundantly. This isolate also produces a variable red pigment with pH (Figures 1 and 2).



A B

Figure 1. Cultural aspect of colonies on PDA medium of both isolates after 4 days of incubation at 25 °C and in the dark (young cultures)
A: F.C.T5 isolate, B: F.G.10.08 isolate



A B

Figure 2. Cultural aspect of colonies on PDA medium of both isolates after 20 days of incubation at 25 °C and in the dark (Aged cultures)
A: F.C.T5 isolate, B: F.G.10.08 isolate

Growth speed

Analysis of the variance for linear mycelial growth trait revealed very highly significant differences between the two isolates ($P<0.001$; $F=21.66$). Indeed, the mycelial growth rate of F.C.T5 isolate is slightly faster than that of F.G.10.08isolate. The almost total covering of the Petri dish by the mycelium is done after 4 days of incubation reaching a diameter of 39.75 mm for F.C.T5 isolate; i.e., an average speed of 9.94 mm/day. On the other hand, F.G.10.08 isolate shows a slower speed since on the fourth day of incubation, the mycelium

occupation of the Petri dish is not yet reached, the diameter of the colony is 37.88 mm, i.e., an average growth rate of 9.47 mm/day (Figure 3).

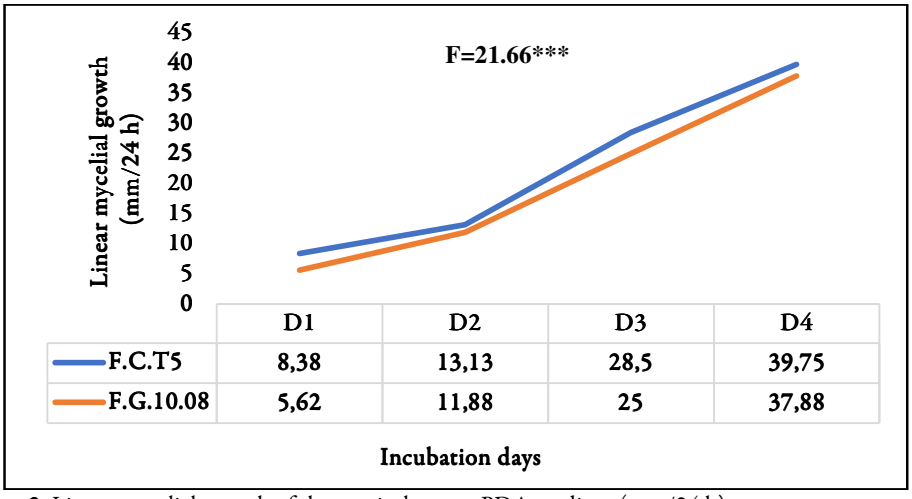
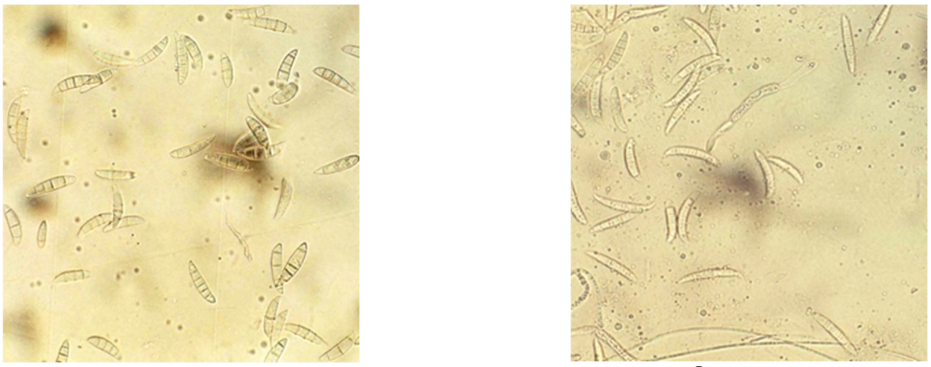


Figure 3. Linear mycelial growth of the two isolates on PDA medium (mm/24 h)

Macroconidia measurement

The macroconidia of both isolates are hyaline. For the F.C.T5 isolate, they are thick, curved, fusiform and septate (3 to 5 septa) with a short pointed apical cell. In F.G.10.08 isolate, they are straight or slightly arched and septate (3 to 5 septa). The apical cell is slightly elongated and strongly curved at the end. For both isolates, microconidia and chlamydospores are absent in this case (Figure 4).



Gr. 25 × 3,2 **Gr. 25 × 3,2**

A B

Figure 4. Microscopic appearance of macroconidia from both isolates (22 days old)
A: F.C.T5 isolate, B: F.G.10.08 isolate

Macroconidia length

The analysis of variance for this parameter revealed a very highly significant difference between the two isolates (Figure 5) ($P<0.001$; $F=25.03$). The classification of the means by LSD (Least Significant Difference) test highlights two heterogeneous groups. The average length recorded in F.C.T5 isolate is 35 µm while F.G.10.08 isolate has the highest average length (39.32 µm) (Figure 5).

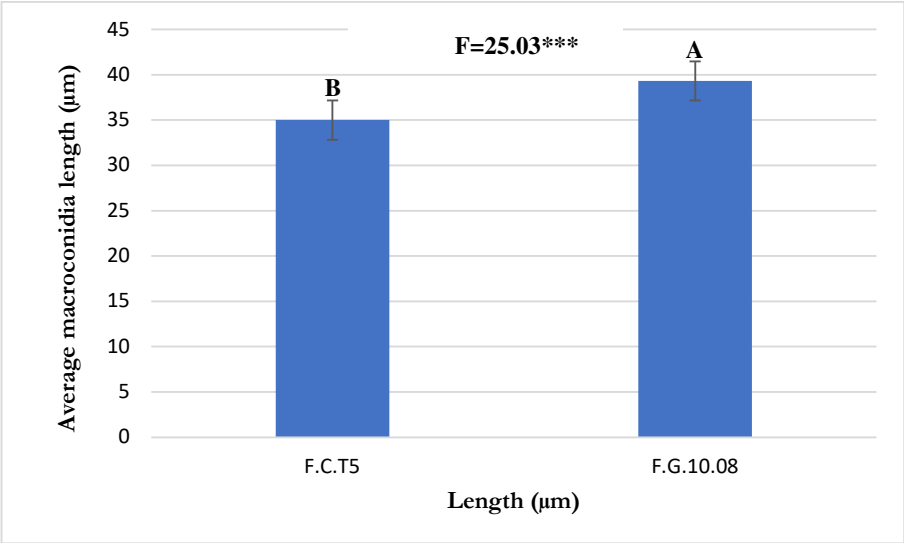


Figure 5. Average macroconidia length of the two isolates (µm)

The highest length of macroconidia is obtained in F.G.10.08 isolate, which is between 28.27-46.26 µm. On the other hand, F.C.T5 isolate has a shorter length, which varies from 23.13 to 43.69 µm (Figure 6).

The lengths of macroconidia observed were classified into three groups: the group which has a length less than 30 µm, the group which has a length between 30 and 40 µm and the group which has a length greater than 40 µm. Indeed, the percentages of macroconidia which are less than 30 µm in length are 16% in F.C.T5 isolate and only 2% F.G.10.08in isolate. On the other hand, the highest percentages of macroconidia with a length between 30 and 40 µm are recorded in F.C.T5 isolate with 66% and the lowest in F.G.10.08 isolate with 46%. Finally, macroconidia having a length of 40 µm present the highest percentages and are obtained by F.G.10.08 isolate with 52% while F.C.T5 isolate records the lowest percentage with only 18% (Figure 6).

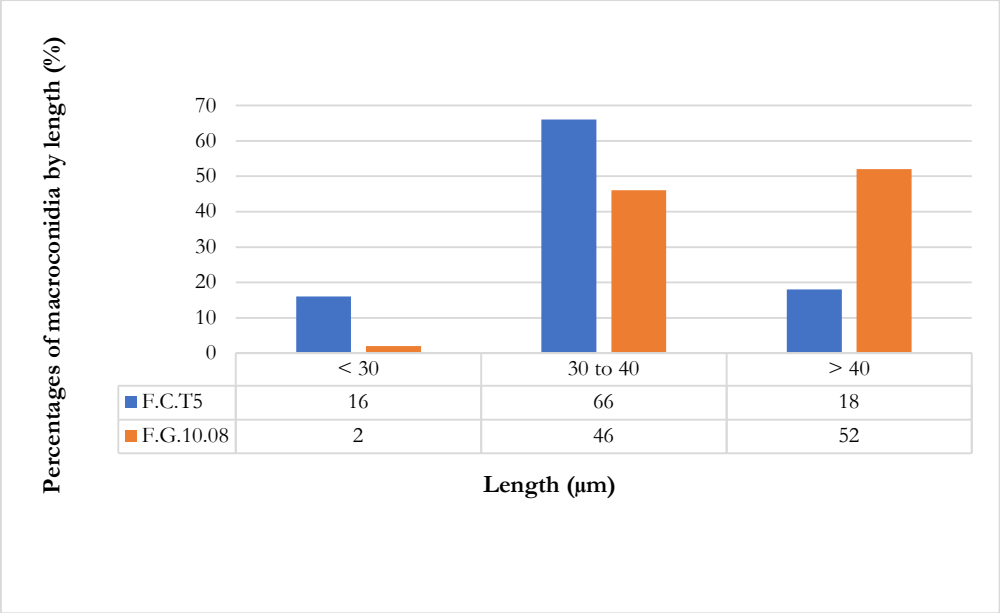


Figure 6. Percentage of macroconidia from each isolate by length (%)

Macroconidia diameter

The diameter of the macroconidia measured in F.C.T5 isolate varies from 3.85 to 6.42 µm, while that of F.G.10.08 isolate is between 3.25 and 4.8 µm. The analysis of variance for this parameter also revealed a very highly significant difference between the two isolates (Figure 7) ($P<0.001$; $F=35.84$). The classification of the means by LSD test distinguished 2 heterogeneous groups. Indeed, the highest average diameter was found in the F.C.T5 isolate (4.78 µm), on the other hand the isolate F.G.10.08 recorded a lower diameter (3.9 µm) (Figure 7).

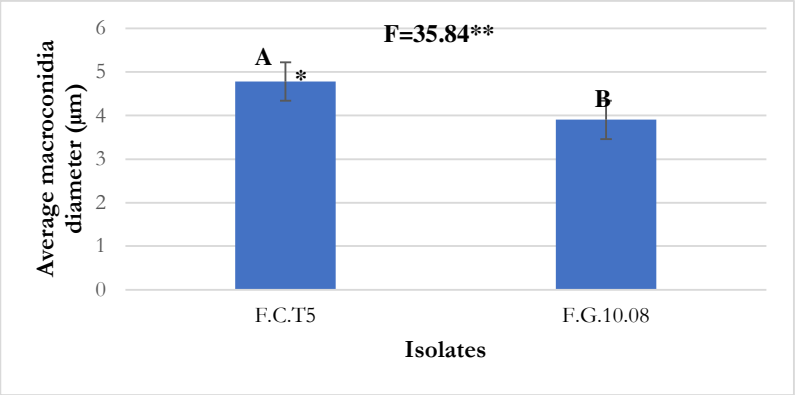


Figure 7. Average macroconidia diameter of the two isolates (µm)

Depending on the diameter of the macroconidia, the latter have been classified into three distinct categories: the first has a diameter of less than 4 µm, the second having a diameter between 4 and 5 µm and the third category has a diameter greater than 5 µm. For this purpose, in F.G.10.08 isolate, the percentage of macroconidia with a diameter of less than 4 µm is 58% and is greater than that of macroconidia with a diameter between 4 and 5 µm (42%). No conidia have a diameter greater than 5 µm (0%). On the other hand, in F.C.T5 isolate, the percentage of macroconidia with a diameter greater than 5 µm is 68% and is greater than that of macroconidia with a diameter less than 4 µm (32%). No conidia presented a diameter between 4 and 5 µm (0%) (Figure 8).

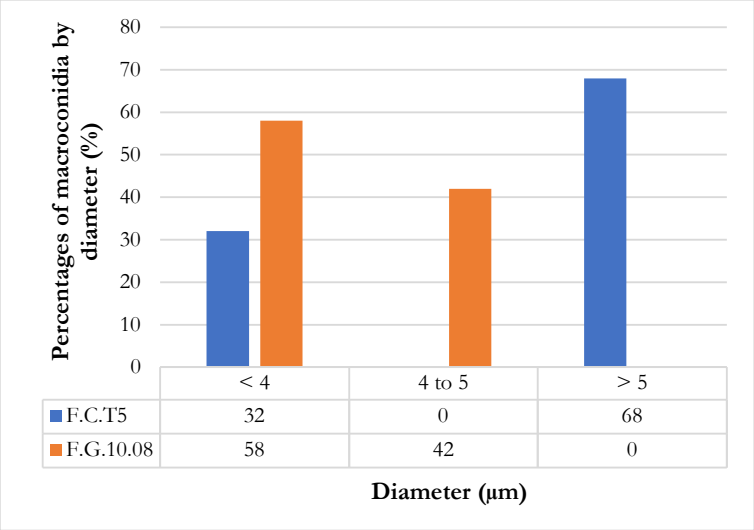


Figure 8. Percentages of macroconidia of each isolate according to diameter (%)

Macroconidia septa

The analysis of variance for this parameter revealed a very highly significant difference between the two isolates (Figure 9) (P<0.001; F=14.73). The classification of the means by LSD test distinguished tow heterogeneous groups. The macroconidia generally have three to five septa in both isolates. In fact, F.G.10.08 isolate has the highest average number of septa (4.48), the lowest average is found in F.C.T5 isolate (3.92) (Figure 9).

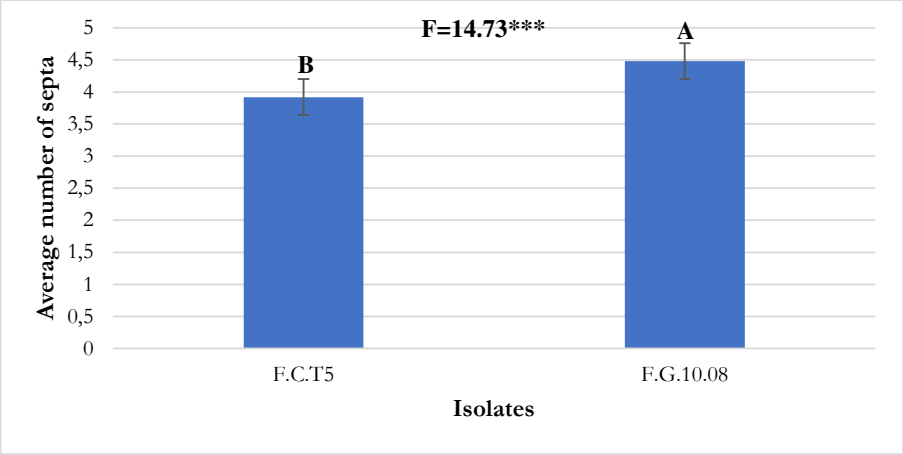


Figure 9. Mean number of septa of the two isolates

Considering the number of septa of the two isolates, the observed macroconidia were classified into three different classes: the class presenting macroconidia having 3 septa, the class of macroconidia with 4 septa and the third class which includes macroconidia having 5 septa. Indeed, it was found that in F.G.10.08 isolate, macroconidia with 5 septa recorded the highest percentage (58%) while the lowest percentage was represented by macroconidia with 3 septa (10%). On the other hand, macroconidia with a number of septa of 4 (intermediate class) represent a percentage of 32%. On the other hand, in F.C.T5 isolate, the macroconidia with 4 septa represent the highest percentage (40%) then come the macroconidia with 3 septa with a percentage of 34% and finally the macroconidia which have 5 septa with only 26% (Figure 10).

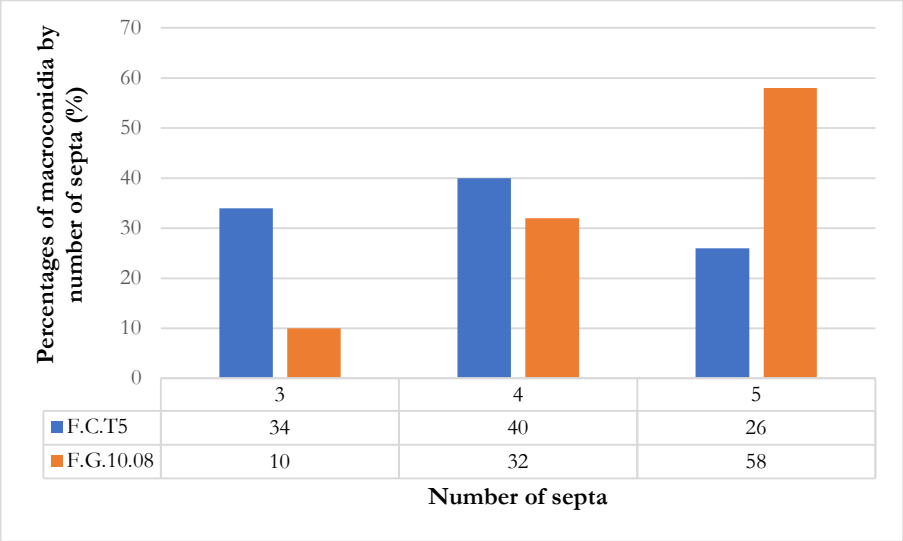


Figure 10. Percentages of macroconidia of each isolate according to the number of septa (%)

Discussion

There are a number of *Fusarium* species that can cause fusarium head blight, the distribution and predominance of these species and their associated mycotoxins is very significant from one region to another (Cerón-Bustamante *et al*., 2018). It is accepted that *F. graminearum* and *F. culmorum* are the two most frequently identified and dominant *Fusarium* species associated with fusarium head blight (Pancaldi *et al*., 2010; Kebede *et al*., 2020). In Europe, *F. graminearum*, *F. culmorum* and *F. poae* are the main species responsible for FHB (Senatore *et al*., 2021). In Algeria, following the results of morphological and molecular identifications of *Fusarium* isolated from wheat ears, *F. culmorum* and *F. pseudograminearum* represent the two most dominant species (Abdallah-Nekache *et al*., 2019; Hadjout *et al.,* 2022).

The three most common morphological identification characters currently used in *Fusarium* species are mycelium color, growth rate and pigmentation. For the color of the mycelium, it varied from light pink, pink, dark pink to brownish pink. Regarding pigmentation, all *Fusarium* isolates produce pink pigmentation on PDA medium. As far as mycelial growth is concerned, it is generally measured for at least 24 hours, which is more than enough for the isolates to cover the entire Petri dish. However, the growth of the mycelium generally marks a significant difference between *Fusarium* isolates (Akshay Kumar et *al*., 2021). After 7 days of incubation of *Fusarium* colonies at 25 °C on PDA medium, white and fluffy aerial mycelia were well developed, with a diffuse pink pigment on the back (Leyva-Mir *et al*., 2022; Beacorn and Thiessen, 2021). Indeed, macroconidia exhibited five to six septa thus measuring 23.47 ± 7.74 µm long and 3.47 ± 0.66 µm wide with foot-shaped basal cells (Beacorn and Thiessen, 2021).

Moreover, wheat heads infected with *Fusarium* showed bleaching symptoms with dense colonies of whitish mycelium typical for *Fusarium* species responsible for fusarium head blight (Ghimire *et al*., 2020). Inoculations of *Fusarium* species on bread wheat produced ear bleaching symptoms with a fusarium head blight severity of 12.46%, with significant differences between infected varieties ($P > 0.05$) (Mohammed -Ameen *et al*., 2021).

In addition, the microscopic and macroscopic characteristics used according to the colonies of *Fusarium* species make it possible to better identify them (Kebede *et al*., 2020). Other studies have shown that the use of morphological characteristics made it possible to identify a set of isolates whose set belonged only to *Fusarium graminearum* and *F. culmorum* with a dominance of the first species (Davari *et al*., 2014). To distinguish *Fusarium pseudograminearum* from *F. graminearum*, several morphological criteria have been established such as the difference in the growth rates of the *Fusarium* colonies, the width of the growth of the conidia, the reaction to black-light blue light near ultraviolet for the conidia with 3 and 5 septa and finally the absence of homothallic production of peritheces (Aoki and O'Donnell, 1999).

The optimum temperature for growth for *F. culmorum* and *F. graminearum* is 25 °C, exhibiting an average growth of 8.2 and 6.8 mm/day respectively where *F. culmorum* exhibits a faster growth rate than *F. graminearum* (Brennan *et al*., 2003). *F. graminearum* isolates from the United States had an optimal *in vitro* growth temperature of 25 °C (Campbell and Lipps, 1998).

Conclusions

In conclusion, the cultural aspect of the colonies and the morphological characterization of the macroconidia of each of the two isolates identified in this present study, isolated from the ears of wheat and which are responsible for the symptoms of fusarium head blight observed on the ears of wheat, corresponds exactly to the descriptions and identification keys of the morphological characters currently used in the morphological identification method of *Fusarium* isolates. Although necessary, this characterization remains

insufficient. Given the similarity of the two isolates, it is important to complete it with a molecular study in order to observe any differences between the two isolates studied and avoid any confusion.

Authors' Contributions

HS designed and performed the experiments and also wrote the manuscript. ZM performed the statistical analysis. Both authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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