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Research article

Trichothecenes B and ergosterol content evaluation in mature grains of durum wheat genotypes contaminated by *Fusarium culmorum*

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Abstract
The interest of this present work is to compare two selected durum wheat lines (G1 and G4) with their sensitive parents to fusarium head blight by determining the mycotoxin and ergosterol content in their mature grains. These lines are homozygous, fixed, obtained by the classical genealogical selection, and composed of seeds from diallel crosses between 4 parental varieties Saadi, Simeto, Ardente, and Waha. For this purpose, the grains of the studied genotypes (lines and parental varieties) were tested in the laboratory for their content of Trichothecenes B mycotoxins (TCTB) and ergosterol at full grain maturity using high-performance liquid chromatographic – diode array detection (HPLC-DAD). Fungal biomass was estimated by the content of ergosterol. However, the level of toxins was assessed by the levels of TCTB produced by the different studied isolates. The results obtained showed that the lines derived from crosses and, especially the G1 line, accumulated levels of TCTB and ergosterol significantly lower than their parents. Our findings open up a new avenue of investigation into fusarium head blight in Algeria, including the search for mycotoxins as potential causes of poorly understood human diseases and the factors that contribute to their accumulation in grains.

Keywords
Durum wheat, fusarium head blight, Algeria, Trichotecenes B, ergosterol

Introduction

Fusarium head blight (FHB) is one of the most destructive diseases of wheat crops (Spanic et al., 2010; Abedi-Tizaki and Sabbagh, 2012). The disease affects the quality of production and wheat yield causing significant losses and hindering the marketing of the harvest (Cuperlovic-Culf et al., 2017; Malbrán et al., 2020). The humid to sub-humid climate with very favorable humidity and temperature conditions in several wheat-producing regions of the world promotes the appearance of fusarium head blight during anthesis (Kikot et al., 2011). Fusarium head blight is the result of a complex of *Fusarium* species attack in small grain cereals such as wheat (Malihipour et al., 2012; Karlsson et al., 2021). Indeed, the infections caused by this disease are confirmed by laboratory analyses that showed

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a high proportion of grains infected by several species of the genus *Fusarium* (Abdallah-Nekache et al., 2019; Hadjout et al., 2022a). In recent years, several studies carried out in Algeria reported that *Fusarium culmorum* (Wm.G. Sm.) Sacc. is the most dominant and most dangerous species on wheat, thus causing the appearance of fusarium head blight (Yekkour et al., 2015; Touati-Hattab et al., 2016; Laraba et al., 2017; Abdallah-Nekache et al., 2019; Hadjout et al., 2022a).

In addition to yield losses, *F. culmorum* causes the contamination of cereal crops by the production of type B trichothecene mycotoxins as this group of mycotoxins are harmful to humans and animals (Oufensou et al., 2021; Palacios et al., 2021). Trichothecenes contaminate wheat kernels and act as pathogenic factors for fusarium head blight (Malbrán et al., 2020). The occurrence and level of *Fusarium* spp. contamination at harvest may be high depending on weather conditions (Savoie et al., 2019). Moreover, TCTB included deoxynivalenol (DON) and four other type B trichothecenes (TCTB), 3-acetyldeoxynivalenol (3-ADON), 15-acetyldeoxynivalenol (15-ADON), nivalenol (NIV) and fusarenon-X (FX) (Alassane-Kpembi et al., 2015; Huang et al., 2020; Stępień and Chełkowski, 2010). Isolates of *Fusarium culmorum* causing fusarium head blight of Algerian wheat produce 3-acetyl-deoxynivalenol (3-ADON) or nivalenol (NIV) (Laraba et al., 2017; Hadjout et al., 2017, Hadjout et al., 2022a). The DON is one of the most common TCTB in food and feed and is considered the most serious toxin worldwide produced primarily by *Fusarium* spp. (Pietsch et al., 2014; Mayer et al., 2017; Adami Ghamsari et al., 2021). Furthermore, it causes many undesirable effects in animals, such as anorexia and growth retardation (Jia et al., 2021). At the level of the plants, DON can have phytotoxic effects such as growth retardation, seedling inhibition, and regeneration of green plants (Rocha et al., 2005). Another formidable mycotoxin of the TCTB group is represented by NIV (Aupanun et al., 2019). Its appearance depends mainly on environmental conditions (Zingales et al., 2021). Recent studies have shown its cytotoxicity in vitro; it has been reported that NIV causes a marked decrease in cell proliferation (Nagashima et al., 2009; Zingales et al., 2021). In particular, NIV has resulted in the fragmentation of internucleosomal DNA, one of the characteristics of apoptosis (Nagashima et al., 2009).

In addition to TCTB and particularly DON, the determination of ergosterol levels in cereals after *Fusarium* infection is also important to identify the susceptibility and resistance of cultivars to fusarium head blight (Hofstad et al., 2016). Ergosterol is a metabolite that reflects fungal biomass (Abramson et al., 1998). The ergosterol content was already used to evaluate the colonization of phytopathogenic fungi of the genera *Fusarium, Alternaria, Cladosporium, Mucor, Rhizopus, Aspergillus, Penicillium* and *Paecilomyces* in feedstuffs (Müller and Schwadorf, 1990). Ergosterol formation is influenced by a number of factors, including plant variety, related resistance, fungal species, and fungal growth circumstances (Dohnal et al., 2010). It has been demonstrated that this sterol is present mainly in cereals infected by *Fusarium* spp. (Perkowski et al., 2008; Ropelewska, 2018). High level of *Fusarium* colonization of wheat grains were found to have a strong correlation with ergosterol and deoxynivalenol concentrations (Wiwart et al., 2011). *Fusarium* isolates produce severe fungal infection on ears as they are highly pathogenic and can cause severe loss in seed number of around 80-90% and in particular in susceptible durum wheat variety (Mesterházy, 2002; Wiwart et al., 2011).

The objective of this study was to examine the concentrations of TCTB and ergosterol in the grains of a few durum wheat genotypes. The presence of TCTB was used as indicator of the grain infection of the tested *F. culmorum* strains, while ergosterol as indicator for the quantification of fungal biomass. Indeed, the grains of the studied genotypes (lines and parental varieties) were analyzed in the laboratory for their content of TCTB and ergosterol at full grain maturity. For this purpose, our study focused on the one hand on the comparison of the accumulation rates of TCTB type mycotoxins between the different genotypes inoculated by the different *F. culmorum* isolates at the flowering

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stage and, on the other hand, on the analysis of the accumulation rates of ergosterol in *F. culmorum* damaged grains, a parameter used to assess the existing fungal mass in the inoculated wheat grains.

Materials and methods

Plant material

The plant material used in our work includes a total of five durum wheat genotypes. Three parental varieties with the following codes: G10, Ardente; G11, Waha; G9, Simeto. Two genealogical lines carrying the symbols G1 and G4, composed of seeds resulting from diallel crosses between 5 parents: Ardente, Waha, Simeto, Vitron, and Saadi, obtained in June 2011 at the Laboratory of Crop Productions, ENSA, El-Harrach, Algiers.

Fungal material

The fungal material was composed of four isolates of *F. culmorum* called FC1, FC2, FC3, FC4. The choice of these isolates was made according to their pathogenic and toxigenic power because they cause significant damage to wheat ears by producing toxins on the grains and they deteriorate the quality and quantity of grain yield (Hadjout et al., 2017). The four isolates used were obtained from durum wheat spikelets of the "Vitron" variety, which showed typical symptoms of fusarium head blight (Hadjout et al., 2022b). The isolates were first identified using morphological criteria according to Leslie and Summerell (2006), which were then verified using molecular tests (PCR assays using species-specific primers) (Touati-Hattab et al., 2016; Hadjout et al., 2022a).

Inoculum preparation

A PDA medium was prepared in Petri dishes to cultivate the *Fusarium* isolates. The latter were then placed in conditions of darkness and an average temperature of 25 °C until sporulation of the fungus. After an incubation period of 20 days, a volume of sterile distilled water of approximately 10 to 20 mL was poured over the surface of the Petri dish containing the *Fusarium* mitospores (conidia). The concentration of the obtained conidial suspension was determined by counting of the number of conidia using the Malassez cell and adjusted to 5.10^4 spores mL^{-1} (Hadjout et al., 2017).

Source of samples analyzed

The samples analyzed in the laboratory were obtained after an experiment carried out in the field. The set-up of the tests comprises five trials: a control trial and four trials inoculated with the four isolates of *F. culmorum* (FC1, FC2, FC3, FC4). The experimental device chosen in our case was of the complete random block type, with three repetitions. To avoid any cross-contamination, rows of triticale were sown between the trials.

TCTB levels evaluation in mature durum wheat grains

In order to assess fusarium head blight, mycotoxin analyses were carried out on the healthy controls and the tests inoculated by the four isolates of *F. culmorum*.

TCTB extraction

The quantification of mycotoxins in the grains was carried out at the MycSA laboratory, INRAE of Bordeaux (France) according to the method described by Montibus et al. (2013) with some modifications. Ten grams of grains were ground with a centrifugal crusher (Tissuelyser, Retsh, Germany) (0.5 mm grid). In a 50 mL tube, 5 g of ground grains were added to 25 mL of an acetonitrile/water solution (84/16, v/v) under agitation at 50 rpm for 1 hour. After centrifugation, 5 mL of supernatant was filtered on a Trichothecene P® column (R-Biopharm, Darmstadt, Germany),

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from which 3 mL of this purified solution was transferred into a vial and evaporated at 50 ˚C under a nitrogen flow. The samples were then taken up in 300 µL of a methanol/water solution (1/1, v/v) and filtered on 0.22 µm porosity filters (Phenomenex, Torrance, USA) before analysis by UPLC-DAD.

TCTB determination by HPLC-DAD

TCTB quantification was performed with a Shimadzu Prominence UFLC device equipped with two LC-20 AD pumps, a DGU-20A3R degasser, a SIL-30 AC automatic passer, and an SPD-M20A diode array detector (Shimadzu Scientific Instruments, France). From the filtered extract, 0.5 µL were injected on a Kinetex column 2.6 µxB C18 - 100A (150 × 4.6 mm; 3.5 µm) (Phenomenex; France) maintained at 45 ˚C and a constant flow of 700 µL/min. The mobile phase consists of an ultrapure water solution (> 18 MΩ) with a pH-adjusted to 2.6 using a solution of ortho-phosphoric acid (solvent A) and acetonitrile (solvent B). The elution gradient was shown in Fig. 1.

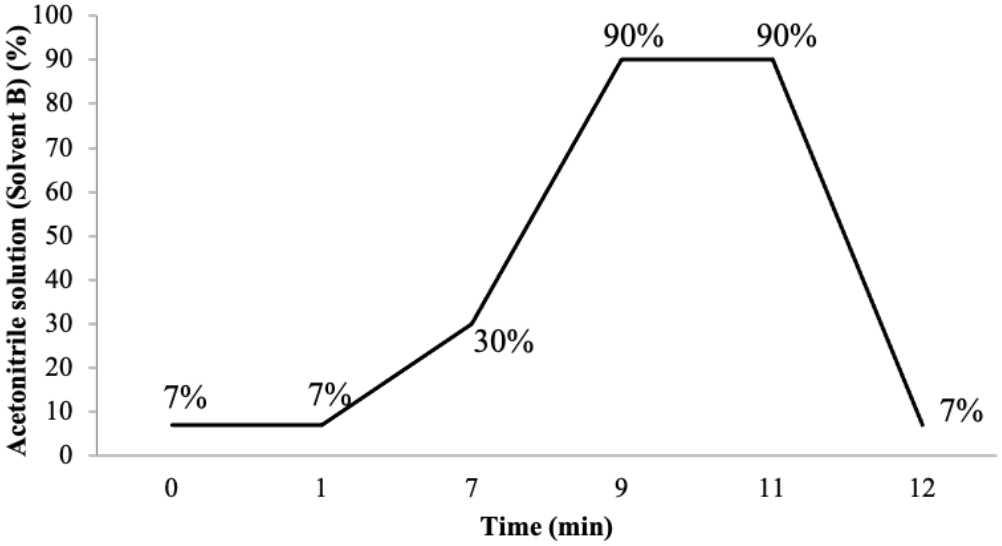

Fig. 1 - Representation of the water/acetonitrile gradient used for TCTB assay.

The visible UV spectra were measured from 190 to 400 nm, with the peak area at 230 nm. External calibration was used to perform the quantification, which was done with standard solutions from DON, 15-ADON, 3-ADON, NIV, and FX (Romer Labs, Austria).

Ergosterol content evaluation in mature durum wheat grains

Ergosterol extraction

The ergosterol extraction and assay methods were modified from those described by (Saraf et al., 1997; Marín et al., 2005). The following steps were taken: 30 mg of ground grains were mixed with a 2 mL solution of potassium hydroxide and methanol (01/10, w/v) in a 15 mL tube and agitated for 1 minute. After that, the alkaline hydrolysis was kept in the water bath for one hour at 80 ˚C, with regular tube degassing. After rapid sample cooling, 480 L of HCL 6 M and 500 L of MilliQ® water were added to stop the hydrolysis. Finally, two liquid-liquid extractions with 2 mL of hexane were performed in succession. The two organic phases were collected, grouped, and evaporated dry under nitrogen flux at room temperature after homogenization and centrifugation at 3000 rpm for 3 min. Before the HPLC-DAD assay, the dry samples were placed in 1 mL of methanol and filtered through a 0.22 m filter.

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Ergosterol assay by HPLC-DAD

Ergosterol elution was carried out on a Kinetex 2.6 xB C18 - 100A (150 × 4.6 mm) column (Phenomenex; France) kept at 40 ˚C. The injection volume was limited to 5 L for 15 min. Isocratic elution with 100% methanol was used at a fixed flow rate of 0.8 mL/min. The UV spectrum was measured from 190 to 400 nm, and ergosterol was quantified at 282 nm. The quantification was performed using an external calibration obtained from commercial ergosterol (Fulka, France). The detection limit for ergosterol was 6 µg g^{-1} DM.

Statistical analysis

For variance analysis, statistical analysis of the TCTB and ergosterol results was performed using the software statgraphics version 15.1.0. Then multiple comparisons of the means were carried out using the LSD test (Least Significant Difference) to determine the homogeneous groups at the 5% significance level.

Results

TCTB content evaluation in mature grains of durum wheat genotypes

Depending on the isolate, TCTB were represented under our conditions either by DON or NIV. Thus, two isolates (FC1 and FC2) produce NIV and two other isolates (FC3 and FC4) produce DON. Under these conditions, the detection limit for each toxin was 1 µg g^{-1} DM.

During the assays, FX was not detected with the NIV chemotype. The 3-ADON acetylated form was also not observed in samples inoculated with DON/3-ADON chemotype isolates. As for the developed symptoms, the quantity of mycotoxins in grains varies greatly depending on isolates and genotypes. For the controls, the contents were zero in all the samples.

Nivalenol (NIV) levels evaluation

The mean NIV levels produced by fungal isolates were calculated for each genotype and presented in Fig. 2. In terms of NIV accumulation, the variance analysis for this parameter in each of the two tests (test with FC1 isolate and test with FC2 isolate) showed a significant difference between the different genotypes studied (Fig. 2). In addition, individual statistical analysis results showed that in the FC1 test, both lines G1 and G4 presented the lowest NIV levels of the series respectively 4.63 µg g^{-1} DM for G1 and 6.37 µg g^{-1} DM for G4. The NIV content in the G1 line is below the detection limit (6 µg g^{-1} DM), and therefore this line practically did not accumulate NIV in its grains. In contrast, the two varieties G9, and G11, showed higher levels of this toxin, respectively 31.15 µg g^{-1} DM for G9 and 39.34 µg g^{-1} DM for G11, while the G10 variety accumulated an average NIV content in its kernels of 19.57 µg g^{-1} DM. For the FC2 assay, the NIV levels in all genotypes were lower than those produced by the FC1 isolate. Therefore, the FC1 isolate is more aggressive than FC2 regarding NIV production. Genotypes G1, G4, and G10 had low NIV levels of 1 µg g^{-1} DM, 2.95 µg g^{-1} DM, and 2.89 µg g^{-1} DM, respectively. All of these values were below the detection limit (< 6 µg g^{-1} DM), in which case the NIV quantities in the grains of these three genotypes cannot be detected by the method used. As for the two varieties G9 and G11, the NIV levels were higher, i.e 10.09 µg g^{-1} DM and 7.78 µg g^{-1} DM, respectively. The overall analysis of variance for the two inoculated tests with FC1 and FC2 revealed a significant difference between the two trials and another for the genotype * assay interaction (Fig. 2). However, the genotype effect is not significant ($P > 0.05$).

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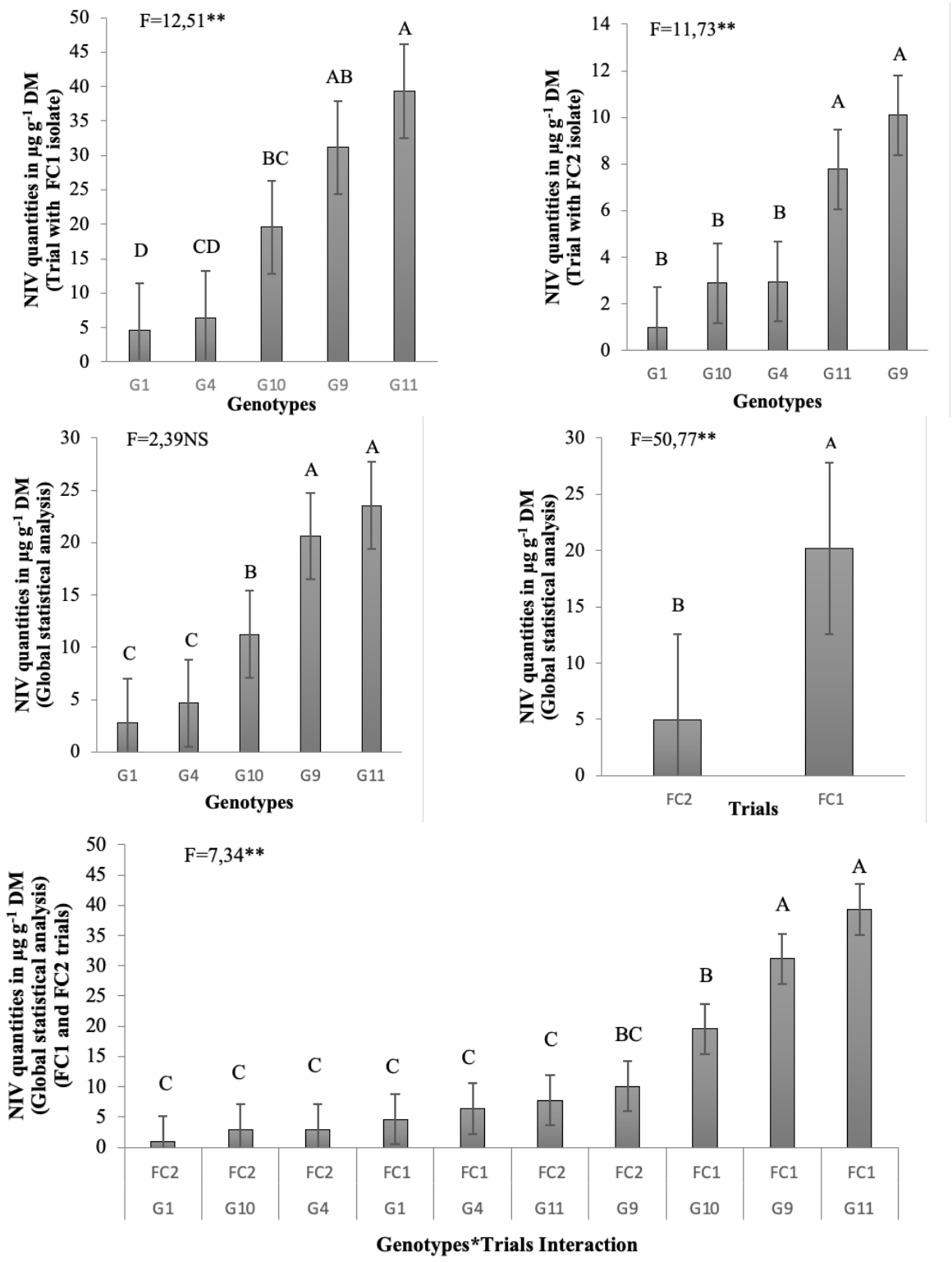

Fig. 2- Average Nivalenol (NIV) quantities contained in the samples analyzed (µg g^{-1} DM). FC1, FC2 - *Fusarium culmorum* isolates; G1, G4, G9, G10 et G11 – Durum wheat genotypes; the *p* value of independent test is presented with its threshold of significance; **p* < 0.05; ***p* < 0.01; NS – Not Significant *p* > 0.05); values with the same letters in a column were not statistically different at the 5% significance level according to Fisher's Least Significance Difference (LSD).

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Comparing the two tests, it appears that the two lines G1, and G4, have very low mean NIV levels of 2.82 µg g^{-1} DM for G1 and 4.66 µg g^{-1} DM for G4. In contrast, the G9 and G11 varieties accumulate very high quantities, averaging 20.62 µg g^{-1} for G9 and 23.56 µg g^{-1} for G11; the G10 variety had an average intermediate content of only 11.23 µg g^{-1} DM.

Deoxynivalenol (DON) levels evaluation

For the accumulation of DON in the two assays, the variance analysis for this parameter revealed a significant difference between the genotypes (Fig. 3). In the FC3 isolate test, both genotypes G1 and G10 had low DON levels of 2.47 µg g^{-1} DM for G1 and 3.49 µg g^{-1} DM for G10. These quantities were below the detection limit (< 6 µg g^{-1} DM), which explains the absence of DON in the grains of these two genotypes. In contrast, the highest quantity of DON is found in the G9 variety at 56.52 µg g^{-1} DM. Finally, the G4 genotype accumulated 23.54 µg g^{-1} DM of DON, while the G11 genotype accumulated 32.64 µg g^{-1} DM of DON.

In the FC4 assay, The G1 line accumulated less DON, only 10.85 µg g^{-1} DM, followed by G10 with 19.25 µg g^{-1} DM, while G11 had a very high DON content of 40.00 µg g^{-1} DM. The two genotypes G4, and G9, showed mean DON levels with 36.32 µg g^{-1} DM and 28.07 µg g^{-1} DM, respectively. Variance analysis for both assays (FC3 isolate and FC4 isolate assays) showed a significant difference for the DON parameter only for the interaction (Fig. 3), but the effect of genotypes and assays is not significant.

The quantities of DON produced by the other two isolates (FC3 and FC4) were slightly higher than the NIV. Indeed, the G1 line accumulated less DON, i.e., 6.66 µg g^{-1} DM, and G10 with 11.37 µg g^{-1} DM. In contrast, The G4, G9, and G11 genotypes showed a high accumulation of DON, averaging 29.93 µg g^{-1} DM, 42.30 µg g^{-1} DM, and 36.32 µg g^{-1} DM, respectively.

Based on the results of the quantification of TCTB (NIV and DON) in grains of all genotypes, the G1 line was the most resistant to TCTB accumulation, thus accumulating fewer toxins in their grains. However, the G9 and G11 varieties resulted with lower quantities of mycotoxins. Furthermore, the DON content of the G4 line grains was higher than that of the G10 variety.

Ergosterol content evaluation in mature grains of durum wheat genotypes

Figure 4 depicts the accumulated ergosterol levels in mature wheat grains of durum wheat genotypes, contaminated at flowering with *F. culmorum* isolates. The individual statistical analysis for ergosterol showed a significant difference between genotypes at the level of the assays with FC1, FC2, FC3 isolates and a non-significant genotypic difference in the assay with isolate FC4. Similarly, the analysis of variance for all four assays revealed a difference between genotypes, while the effect of the four essays and the genotype * assay interaction is non-significant.

The comparison of the genotypes (parental varieties and lines) for the ergosterol content shows that at the level of the test with the FC1 isolate, the G1 line has the lowest content, the G4 line comes in the second position, or 8.74 µg g^{-1} DM and 15.80 µg g^{-1} DM, respectively. In contrast, the two G9 and G11 varieties had higher levels of 73.82 µg g^{-1} DM and 82.73 µg g^{-1} DM, respectively; the G10 variety had an average ergosterol level of 37.20 µg g^{-1} DM. In the FC2 isolate trial, the lowest concentration was recorded in the G1 line with only 6.03 µg g^{-1} DM, whereas the highest concentration was recorded in the G9 variety with 51.79 µg g^{-1} DM. The genotypes G4, G10, and G11 have ergosterol levels of 15.49 µg g^{-1} DM, 10.37 µg g^{-1} DM, and 34.42 µg g^{-1} DM, respectively.

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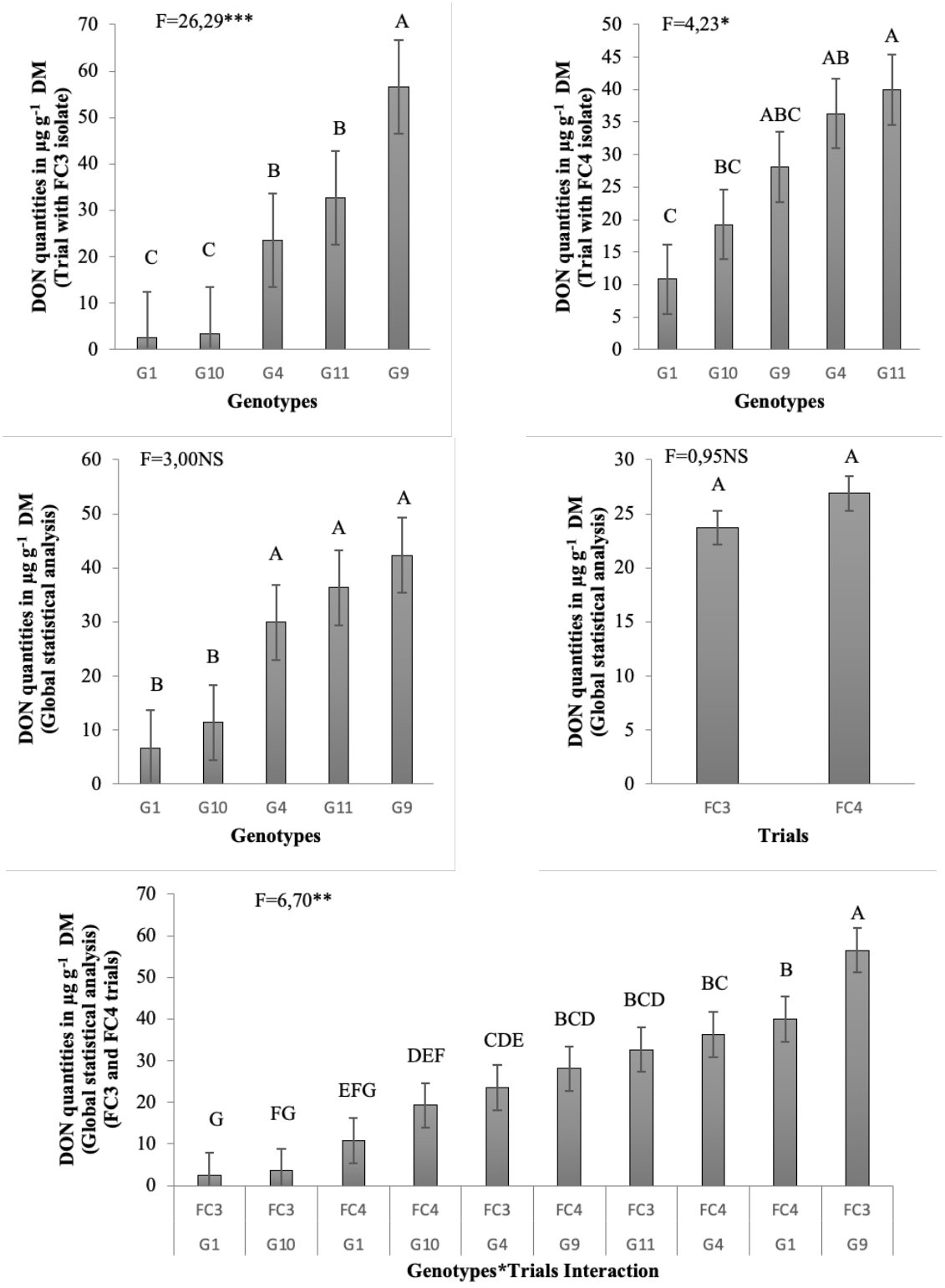

Fig. 3 - Average deoxynivalenol (DON) quantities contained in the samples analyzed (µg g^{-1}DM). FC3, FC4 - *Fusarium culmorum* isolates; G1, G4, G9, G10 et G11 – Durum wheat genotypes; the *p* value of independent test is presented with its threshold of significance; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; NS – Not Significant $p > 0.05$); values with the same letters in a column were not statistically different at the 5% significance level according to Fisher's Least Significance Difference (LSD).

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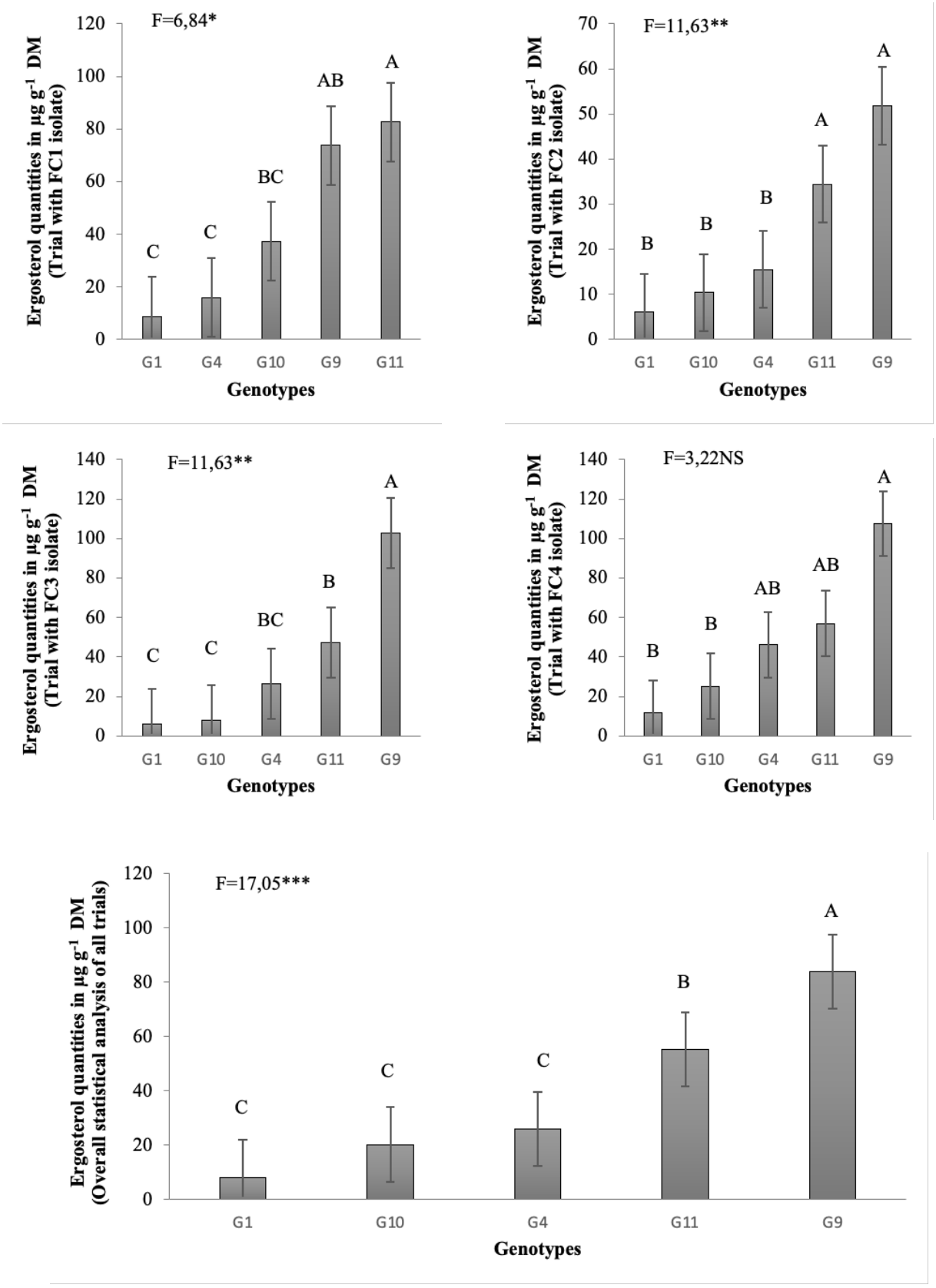

Fig.4-Ergosterol levels accumulated in mature grains of durum wheat genotypes contaminated at flowering with *Fusarium culmorum* isolates.

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In the FC3 isolate assay, the G1 and G10 genotypes had similar but low ergosterol levels with 6.00 µg g^{-1} DM and 8.07 µg g^{-1} DM, respectively. The highest level was recorded in the G9 variety with 102.62 µg g^{-1} DM. At the same test level, the G4 and G11 genotypes showed levels of 26.27 µg g^{-1} DM and 47.19 µg g^{-1} DM, respectively. Finally, in the FC4 isolate test, the G1 line recorded the lowest ergosterol levels in its grains, with 11.80 µg g^{-1} DM, followed by the G10 variety with 25.16 µg g^{-1} DM, and the G9 variety with 107.37 µg g^{-1} DM. The G4 and G11 genotypes had average ergosterol levels of 46.08 µg g^{-1} DM and 56.96 µg g^{-1} DM, respectively.

The comparison of essays indicated that the G1 lines showed lower ergosterol levels than the other genotypes, with only 8.14 µg g^{-1} DM. In contrast, the G11 and G9 genotypes showed higher levels, with 55.33 µg g^{-1} DM for G11 and 83.90 µg g^{-1} DM for G9, respectively. The G10 genotype has averaged 20.20 µg g^{-1} DM and 25.91 µg g^{-1} DM for the G4 genotype.

Discussion

Durum wheat is one of the most important crops in Mediterranean countries (Alkadri et al., 2013) and worldwide (Bouanaka et al., 2021). In Algeria, durum wheat dominates the diet of the population. Production cannot satisfy the needs of a population which currently exceeds 40 million inhabitants and which is potentially and traditionally a consumer of this product, hence the recourse to imports. This decrease in production is due to several factors, of which the attacks of *Fusarium* spp. were confirmed by laboratory analysis, especially showing a high proportion of grains infected by several species of *Fusarium* (Hadjout et al., 2017).

The purpose of this study is the analysis of TCTB, ergosterol in durum wheat seeds, and the characterization of the toxinogenic potential of *F. culmorum* isolates by HPLC. High-performance liquid chromatography with fluorescence detection coupled to a diode array detector (HPLC-FLD/DAD) was used as an effective analytical method for ergosterol (Álvarez et al., 2021). The use of HPLC/MS technology makes it possible to evaluate the quantity of ergosterol which may be linked to the fungal biomass quantification of *F. culmorum* and this technique also allows to estimate the quantify the TCTB type toxin (Touati-Hattab et al., 2016). The obtained results showed that resistance levels to fusarium head blight and mycotoxin accumulation in the G1 line are very high compared to the two susceptible parent varieties (G9 and G11). The latter two have higher levels of ergosterol in the grains than G1, thus indicating a greater fungal infection on the ears of wheat at the time of flowering. The results of Zhao et al. (2018) revealed that the "ND2710" wheat line is resistant to fusarium head blight compared to other parents (Grandin and Wheaton), which are very susceptible to FHB. The same authors report that the sources of resistance of this line (ND2710) are probably derived from the wheat cultivar Sumai 3 because the other parents (Grandin and Wheaton) are very sensitive to FHB. Furthermore, some triticale lines showed a weak infection of the ear by *Fusarium* spp. at the flowering stage but showed significant damage at the grain level, with a higher rate of accumulation of the TCTB type toxin (Góral et al., 2021). In particular, the levels of TCTB accumulated in grains are correlated with fungal contamination. A strong correlation between the level of *Fusarium* spp. infection in the grain and the quantity of stored toxin was observed (Touati-Hattab et al., 2016; Hadjout et al., 2017). Thus, the G1 line accumulates few TCTB (NIV: 4.63 µg g^{-1} DM, DON: 2.47 µg g^{-1} DM), while G9 (NIV: 31.15 µg g^{-1} DM; DON: 56.52 µg g^{-1} DM) and G11 (NIV: 39.34 µg g^{-1} DM; DON: 32.64 µg g^{-1} DM) store a greater quantity. The results showed that both lines were less susceptible to fusarium head blight than commercial varieties, especially the G1 line. TCTB and ergosterol values associated with the G1 line were 5 to 10 times lower than those assessed for susceptible commercial varieties [Ardente (G10), Waha (G11), Simeto (G9)]. For this purpose, ergosterol analysis of seeds of wheat genotypes infected with *F. culmorum* showed different resistance to fusarium head blight

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(Snijders and Krechting, 1992). As a result, these data strongly support a strong correlation between levels of trichothecene production and observed symptoms. The amount of toxin and the growth of the fungus in the grain, as assessed by ergosterol, have a strong correlation (Touati-Hattab et al., 2016). The results of TCTB and ergosterol were also consistent with those of Atlin et al. (1983), who found a high and significant correlation between DON concentration and fusarium head blight intensity in maize. However, Miller et al. (1985) observed cases of high concentrations of ergosterol, representing a quantitative index of the presence of *Fusarium* in wheat tissues. In addition, there is still a strong correlation between the quantity of toxin and the development of *F. culmorum* in the grain as measured by the ergosterol index. This correlation was subsequently reported by many authors (Mirocha et al., 1994; Perkowski et al., 1996; Miedaner et al., 2000).

Furthermore, it should be noted that *F. culmorum* is known to produce two types of trichothecenes: NIV and DON. The NIV is the most neglected toxin because it is found in the lower parts in wheat. This toxin is responsible for the inhibition of DNA synthesis, which explains the course of several toxic phenomena leading to cell death (Pasquali et al., 2010). By against, DON plays a role in pathogenicity, the development of the disease and the destruction of chlorophyll, which explains the early drying of *Fusarium* spikes. It is produced during infection, when moisture conditions are important. Thus, the harvest delay caused by rainy weather increases the production of DON (Wagacha and Muthomi, 2007; Lori et al., 2009).

Miller and Arnison (1986) demonstrated DON accumulation resistance in some wheat cultivars. Furthermore, the results of our trials showed that the difference in DON accumulation resistance between genotypes would be easily discernible in the presence of significant infection, i.e. disease severity at the time of flowering. On the other hand, mycotoxin concentrations are lower in low sensitivity lines compared to their more sensitive counterparts from the same cross (Atanassov et al., 1993).

Snijders and Krechting (1992) also demonstrated that DON could be translocated to the young ear before being colonized by the pathogen, indicating that a line resistant to *Fusarium* hyphal invasion could prevent DON translocation. According to Snijders and Krechting (1992), resistance to DON could increase resistance of wheat and prevent the accumulation of trichothecenes. Some wheat cytoplasm is trichothecenes tolerant cytologically, which can be attributed to improved membrane stability and the presence of modified peptidyl transferase (Wang and Miller, 1988; Miller, 1989).

Conclusion

In Algeria, the attacks of durum wheat by fusarium head blight are confirmed by laboratory analyzes, which showed a high proportion of grains contaminated by *F. culmorum* species in sensitive genotypes. This work focused on biochemical characterization in the laboratory of trichothecenes type B (TCTB) and ergosterol. The obtained results revealed that the isolates from crosses showed higher resistance than their parents, which showed a significantly lower accumulation of TCTB and ergosterol. Under our crop conditions, no genotype has shown complete disease resistance. However, the G1 line has a high resistance level under our conditions. The toxin and ergosterol values associated with the G1 line were 5 to 10 times lower than those assessed for sensitive commercial varieties. In general, the results presented here showed the possibility of selecting new durum wheat genotypes adapted to Algerian climatic constraints and displaying a better tolerance to fusarium head blight.

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