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# Pathogenicity and trichothecenes production of *Fusarium culmorum* strains causing head blight on wheat and evaluation of resistance of the varieties cultivated in Algeria

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**Abstract** Fusarium head blight (FHB) is a major disease of wheat that has been studied worldwide but never in Algeria where high quantities of both durum wheat and common soft wheat are grown and traditionally consumed as semolina and bread. Fusarium root rot has also been observed in this country. Here we show that *Fusarium culmorum* seems to be the major pathogen associated with these diseases in Algeria. The type of mycotoxins produced by four *F. culmorum* isolates and their capacity to confer the disease on spike and accumulate type B trichothecenes in the grain was evaluated. Two strains produced deoxynivalenol, 3-acetyldeoxynivalenol and zearalenone in vitro. The two other strains produced nivalenol and fusarenone X. The four strains were used for artificial spray inoculations on wheat spikes to determine their potential in generating FHB symptoms and accumulating mycotoxins in local field conditions. A panel constituted of four durum wheat and four soft wheat varieties generally cultivated in Algeria and of two newly created durum wheat lines were evaluated. The results show a correlation between the level of invasion of the grain and the quantity of accumulated toxins with a large diversity depending on the cultivars. Interestingly, two local durum wheat varieties and the two new durum lines showed a promising level of resistance to FHB with significantly lower trichothecene accumulation. The content in phenolic compounds of the different varieties was assessed and evaluated as possible factor of resistance to trichothecene accumulation. This is the first report evaluating the wheat varieties cultivated in Algeria for their susceptibility to Fusarium Head Blight caused by local strains of *F. culmorum* in semi-arid bioclimatic condition.

**Keywords** Wheat · Fusarium head blight · *F. culmorum* · Type B trichothecenes · Variety · Resistance

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## Introduction

Durum wheat is commonly used in Algeria to prepare the popular semolina used for couscous preparation. For this reason, it has been traditionally cultivated in all the regions where climatic conditions allow the growth of this cereal. Wheat is grown throughout the different agro-ecological areas of Algeria, but it is essentially concentrated in semi-arid and arid areas (Boulal et al. 2007). The areas reserved annually to cereal can reach 6

million hectares. Approximately one third of this surface is located in bioclimatic areas with an annual average rainfall greater than 450 mm (Feliachi et al. 2001). Durum wheat is cultivated approximately in 1.1 million hectares and its production was 1530 kg/ha in 2010 (Hamadache 2013).

Fusarium head blight (FHB) affecting the spike and Fusarium foot and root rot (FFR) (also known as the Fusarium crown root of wheat) are two types of devastating cereal diseases worldwide. These two diseases have also recently been observed on wheat in Algeria where they are caused mostly by the two *Fusarium* species: *F. culmorum* and *F. pseudograminearum* (Boureghda 2009).

FRR causes browning of wheat seedling, brown discoloration of sub-crown internodes and formation of bleached heads. Generally, the economic importance of the disease has been linked to environmental conditions. This disease has been reported as affecting wheat in South Africa (Klaassen et al. 1991; Lamprech et al. 2006), Iran (Saremi et al. 2007), many Mediterranean countries (Balmas et al. 2015), Australia (Akinsanmi et al. 2006; Murray and Brennan 2009), West Asia (Hogg et al. 2010) and USA (Smiley and Patterson 1996). It has been recently reported that FRR may be responsible for as much as 50 % of production losses (Hollaway et al. 2013). Mostly, four pathogenic species including *F pseudograminearum*, *F culmorum*, *F crookwellense* and *F graminearum* have been reported as responsible for root rot and crown rot.

Fusarium head blight (FHB) has been extensively studied worldwide (Parry et al. 1995; Xu and Berrie 2005). Economical losses caused by FHB due to poor yield and mycotoxins contamination was estimated to 6 billion $ in the US between 1993 and 2001 (Obanor and Chakraborty 2014). In addition to cause significant yield loss, FHB is of greater significance because it is often associated with kernel contamination by trichothecenes and zearalenone, two *Fusarium* mycotoxins produced in the field (Bottalico 1998). The most important pathogens responsible for FHB are *F. graminearum*, *F. culmorum*, *F. avenaceum* and *F. poae*, which can produce a range of different trichothecene molecules and also zearalenone (Bottalico and Perrone 2002) and *Microdochium nivale* (formerly *M. nivale* var. *nivale* and var. *majus*) (Glynn et al. 2005), which do not produce known mycotoxins. Among *Fusarium* mycotoxins, trichothecenes are frequently encountered on cereal crops in all parts of the world including North and South America, all Europe, Australia, South Africa, Japan and China (Bianchini et al. 2015).Trichothecenes are heat stable molecules and are not degraded during current food processing (Hazel and Patel 2004; Rocha et al. 2005). As these are potent inhibitors of eukaryotic protein synthesis (Rocha et al. 2005) and have a large range of undesirable effects, they constitute a toxin family of considerable concern to human and animal health (Bennett and Klich 2003). Type B trichothecenes (TCT B) are the major mycotoxins produced in cereals mainly by *F.culmorum* and *F. graminearum* as well as some other species of *Fusarium* (Desjardins 2006; Kimura et al. 2007). They include deoxynivalenol (DON) and its acetylated forms 3-acetyldeoxynivalenol and 15-acetyldeoxynivalenol (3- and 15-ADON), and nivalenol (NIV) and its acetylated form 4-acetylnivalenol or fusarenone X (FX) (Balzer et al. 2004; Bennett and Klich 2003; Sweeney and Dobson 1999). In Europe, maximum acceptable DON levels in food destined for human consumption were established (EC N°1881/2006). Accordingly, grains exceeding the established limits are not permitted for commercialization to human consumption. Currently in Algeria, there are no standards or regulatory limits fixing maximum levels of mycotoxins in food and feed.

The production of DON by *F. graminearum* has been shown to play a role in the progression of the disease from one spikelet to another through the rachis (Maier et al. 2006) and is a determinant of aggressiveness (Eudes et al. 2001; Proctor et al. 1995). Other reports suggest that the production of DON by *F. culmorum* also correlates with aggressiveness (Eudes et al. 2001; Hestbjerg et al. 2002; Miedaner et al. 2000), but the relationship between disease symptoms and DON content is not yet well understood. Today, the quantification of fungal ergosterol allows determining more precisely the quantity of pathogen development in the grain (Seitz et al. 1977; Schwadorf and Muller 1989). The development of quantitative PCR of DNA extracted from contaminated grains to assess the biomass of the fungus and of multi-toxin analyses using HPLC/MS technology for precise quantification of the toxin also contributed to improve the characterisation of the resistance level of wheat varieties and help understanding the factors that influence FHB. Such knowledge is determinant for the elaboration of strategies to reduce the risk of mycotoxin contamination of grains and foodstuffs.

The more promising way to prevent, or at least limit, trichothecenes occurrence in food and feedstuff is to

reduce their biosynthesis in the grain by the fungus before harvest. One of the best environmentally friendly and efficient strategy is breeding, selecting for varieties exhibiting natural mechanisms of plant resistance to both fungal spread and mycotoxin accumulation. Cereals can deploy various natural mechanisms to reduce mycotoxin accumulation. Among these natural mechanisms, the cereal plants can promote degradation or detoxification of mycotoxins (type V-1 resistance) or prevent their biosynthesis (type V-2 resistance) (Boutigny et al. 2008). The hypothesis was previously raised that resistance to *Fusarium* trichothecene accumulation in some durum wheat cultivars could be explained by a particular biochemical composition of the kernel, rich in specific endogenous compounds able to reduce trichothecene biosynthesis (Pinson-Gadais et al. 2007). Several reports suggest that resistance to *Fusarium* and toxin accumulation could be correlated with kernel phenolic content at maturity in maize (Assabgui et al. 1993; Bily et al. 2003; Reid et al. 1992) and wheat (McKeehen et al. 1999; Siranidou et al. 2002). More recent work showed that a phenolic fraction extracted from wheat bran exhibited a strong inhibitory effect on DON/ADON biosynthesis in vitro by *F. culmorum* (Boutigny et al. 2010) and strengthened the hypothesis of a possible role of phenolic compounds in resistance to trichothecene accumulation in durum wheat (Boutigny et al. 2009; Boutigny et al. 2008; Atanasova-Pénichon et al. 2012).

The objectives of this study were to identify isolates of *F. culmorum* from Algerian climatic area and to characterise their capacity to produce mycotoxins in vitro. These Algerian strains were shown to be pathogenic toward wheat spikes of different varieties. Afterward, these strains were used during 2 years to assess in field conditions the behaviour of eight winter wheat varieties belonging to both soft wheat and durum wheat from indigenous or abroad origin. Not only resistance to FHB caused by *F. culmorum* but also the corresponding accumulation of trichothecenes and development of the fungus in the grain were evaluated. In addition, two new durum wheat lines were also assessed and shown to be potentially more resistant to *Fusarium* than all the varieties tested. Finally, phenolic acids were assessed in the set of varieties and lines to see if their content could be related to toxin accumulation. This is the first report evaluating the most frequently cultivated wheat varieties in Algeria for their susceptibility to Fusarium Head Blight caused by local strains of *F. culmorum* in such bioclimatic semi-arid condition.

## Materials and methods

### Fungal isolates

Four single-spore isolates (BT11, BD11, T5 06, T7 06) of *Fusarium species* were used for inoculation in the field (Table 1). These isolates were from durum wheat grains collected in Oued Smar and Rouiba located at the North of Algeria close to Algiers (S. Touati; unpublished results). They were identified as *F. culmorum* on the basis of conidial morphology according to Leslie and Summerell (Leslie and Summerell 2006).

### Plant materials

Four semi late and four early wheat genotypes with different levels of resistance to FHB were used. They belong to winter durum (*Triticum turgidum* L. subsp. *Durum*) and soft wheat (*Triticum aestivum*). Two wheat lines and two of their parent lines were also tested in the second year. Basic plots were established at the experimental farm of ENSA El Harrach. Four trials were two meters spaced and forty meters away from the test with control varieties. Each test is constituted of three blocks with 1 m spacing between the blocks, eight micro-plots of four lines with a 50 cm space between micro-plots. The seedling rate was ten seeds/line, 40 seeds/μ-plot. Inoculations were performed at anthesis (Zadoks et al. 1974) and the harvest was made at physiological maturity.

The inoculum was produced from four single-spore isolates of *F. culmorum* maintained on PDA plates at 4 °C for 10 days. Multiplication of the fungus to produce the required amount of inoculum for contamination of the plots was made with 60 plates/test. The mycelium was scraped and agar was ground in a blender with distilled water. After vortexing for 5 min, the solution was filtered through Nitex Sefar 100 μm in a bottle 1000 ml. Concentration of the spore suspension was assessed with a Malassez cell and was adjusted to the desired final concentration of $10^6$ spores/ml. Spray inoculations were made at full anthesis (stage 67–68), but they were not performed on the same day for all cultivars, as intervals of up to 15 days occurred between the early and late varieties. After inoculation, a sprinkler

**Table 1** characteristics of the Algerian isolates of *Fusarium culmorum* used in the field experiments

| Name | Species | Origin | Variety | Toxin produced (a) | | | | | DI%(b) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | DON µg/g | 3-ADON µg/g | NIV µg/g | Fx µg/g | ZEA µg/g | ARZ (SW) | VITRON (DW) |
| BD11 | *F. culmorum* | Rouiba | Vitron (DW) | 340.5 ± 5.9 | 284.2 ± 0.2 | nd | nd | 3179.3 ± 134.9 | 55.25 % | 70.00 % |
| BT11 | *F. culmorum* | Rouiba | Anza (SW) | 89.0 ± 9.9 | 52.6 ± 0.1 | nd | nd | 222.2 ± 26.5 | 52.50 % | 62.50 % |
| T5 06 | *F. culmorum* | Oued Smar | Vitron (DW) | nd | nd | 5.1 ± 1.0 | 7.9 ± 1.2 | nd | 43.50 % | 52.50 % |
| T7 06 | *F. culmorum* | Oued Smar | Vitron (DW) | nd | nd | 38.4 ± 4.7 | 15.2 ± 1.2 | nd | 48.75 % | 59.25 % |

a: main toxin produced on rice grains; b: primarily pathogenicity assay made in 2011, *DI* Disease Index, *SW* soft wheat, *DW* durum wheat, *DON* Deoxynivalenol, *3-ADON* 3-acetyldeoxynivalenol, *NIV* Nivalenol, *FX* Fusarenone X, *ZEA* Zearalenone

irrigation system was set up, with applications every 30 min for 20 s and 24 applications per day until maturity of the ears.

### Examination of FHB resistance traits

Head blight symptoms of wheat were evaluated 21 days after inoculation. The percentage of infected spikelets was evaluated from 100 spikes issued from each plot. The value obtained was defined as the disease index (DI). In total, up to 220 wheat samples issued from the field artificial inoculations were analysed during the two 2012 and 2013 years.

### *Fusarium culmorum* species-specific PCR

For DNA extraction, the isolates of *F. culmorum* were grown on potato dextrose agar medium (PDA) for 7 days. The mycelia were freeze-dried and ground to a fine powder. DNA was extracted by using a method adapted from Lamprecht et al. (1992). The DNA samples were amplified by PCR using the species-specific primers OPT18F/OPT18R (GATGCCAGACCAA GACGAAG/GATGCCAGACCAAGACGAAG) (Schilling et al. 1996). The amplification conditions were: 20 ng of fungal DNA, 1 mM of each dNTP, 1.5 mM MgCl2, 1 unit of GoTaq® DNA polymerase (Promega, USA), 1X PCR polymerase reaction buffer, and 0.25 µM of each forward and reverse primer. DNA amplification was performed in an iCycler™ (Bio-Rad) as described by Schilling et al. (1996). PCR assays with the primers Tox5 developed for the gene Tri5 were used to confirm the potential ability of the isolates to produce trichothecenes as described by the authors (Niessen and Vogel 1998). Primers specific for the sequence of Tri 12 gene of each chemotype were used to characterise NIV and DON producing chemotypes (L. Pinson Gadais, personal communication). The cycling protocol for identifying the NIV/FX chemotype was: Primer Tri12NivFx F and R (20 µm) with pre-incubation (1 cycle of 10 min at 95 °C) followed by amplification (45 cycles of 10 s at 95 °C, 40 s at 58 °C and 30s at 72 °C) followed by a final extension phase of 5mn at 72 °C. For identifying the 3-ADON chemotype, the primers Tri12DON3Adon F and R were used at 20 µm with one cycle at 94 °C for 10 min, then 45 cycles of 95 °C for 10s, 65 °C for 40s and 72 °C for 30s followed by a final extension step of 72 °C for 5 min.

### Culture on rice grains and toxins extraction

Toxin production by *Fusarium* strains was assessed on autoclaved rice grains. Rice used as the substrate was previously analysed for DON, NIV, 15-ADON, 3-ADON and FX content and found to no contain detectable levels of. Toxin. Before inoculation, rice was moistened with sterile distilled water to a water activity (aw) of 0.99. Rice grains (110 g) were placed in a 500 ml Erlenmeyer flask, and autoclaved twice for 25 min at 110 °C as described by Bakan et al. (2001). Each flask was inoculated with 0.5 ml of an aqueous suspension of $10^4$ conidia/ml harvested from 9 day-old cultures grown on PDA slant. Three flasks were inoculated for each strain. The flasks were incubated without shaking at 25 °C for 3 week in the dark. At the end of incubation, the culture material was dried in a forced-air oven at 50 °C for 72 h. The grains were homogenized and ground into a fine powder (particles size were about 100 μm). The controls were treated in the same way except that they were not inoculated. For analysis, a 5 g portion of each ground sample was extracted with 20 ml of acetonitrile:water (84:16, *v*/v). After agitation for 1 h followed by a centrifugation, 3 ml of the filtrate were purified using Trichothecene P columns (R-Biopharm, Darmstadt, Germany). The eluate was evaporated to dryness at 50 °C under a nitrogen stream. The samples were stored at −20 °C pending analysis.

### Quantification of type B trichothecenes (TCTB) and zearalenone (ZEA)

The dried samples were defrost at room temperature and directly suspended in 100 μl of methanol/water (1:1, *v*/v) (Methanol Fisher Scientific, Waltham, USA) and filtered through a 0.45 μm-pore-size Millipore filter. Quantification was performed as described previously (Montibus et al. 2013) on an Agilent Technologies 1100 series HPLC chain, equipped with an Agilent photodiode array detector (DAD) and the ChemStation chromatography manager software (Agilent, Waldbronn, Germany). Separation was achieved on a column kinetex 2.6 U XB-C18 (4.6 × 150 mm) maintained at 45 °C. The mobile phase consisted of water acidified with orthophosphoric acid to reach pH 2.6 (solvent A) and acetonitrile (solvent B). The flow was kept at 1 ml/min for a total run time of 27 min. The injection volume was set to 5 μl. TCTB were separated in gradient elution as follows: 93–7 % B, 70–30 % B in 10 min, 10–90 % B in 25 min, 93–7 % B in 12 min. UV-VIS spectra were measured from 220 to 550 nm and peak areas were measured at 230 nm. Quantification was performed using external calibration with standard solutions prepared from pure powders (Sigma Aldrich®, Saint-Louis, USA). The detection limit was 100 μg/kg for each of the toxins.

### Quantification of DON-Glu by LC-MS/MS analysis

Extraction was performed exactly as for trichothecenes. Before LC-MS/MS analysis the dry residue was suspended in 300 μl of methanol/water (1:1, *v*/v). Quantification of trichothecene glycoside was performed using a QTrap 2000 LC/MS/MS system (Applied Biosystems) equipped with a 1100 Series HPLC system (Agilent), a reverse phase Kinetex™ 2.6 μm XB-C18 column (150 × 4.6 mm, Phenomenex; France) maintained at 45 °C and a TurboIonSpray ESI source. Solvent A consisted of methanol/water (10/90, *v*/v) and solvent B consisted of methanol/water (90/10, *v*/v). The flow rate was kept at 700 μl/min and was split so that 300 μL/min went to the electrospray source. Gradient elution was performed with the following conditions: 4 min with a linear gradient from 80 % to 5 % A, 4 min held at 5 % A, 1 min linear gradient from 5 % to 80 % A and 80 % A for 8 min post run reconditioning. The injection volume was 10 μl. The electrospray interface was used in the negative ion mode at 400 °C with the following settings: curtain gas, 20 p.s.i.; nebulizer gas, 30 p.s.i.; auxiliary gas, 70 p.s.i.; ion spray voltage, −4200 V; declustering potential, −30 V; entrance potential, −10 V; collision energy, −30 eV; collision-activated dissociation gas, medium. Quantification was performed using external calibration with DON (Sigma-Aldrich, Lyon, France) and DON-3-O-glucose (Sigma-Aldrich, Lyon, France) standard solutions, ranging from 10 to 1000 ng/ml.

### Quantification of ergosterol

The extraction method and assay of ergosterol was adapted from Saraf (Saraf et al. 1997) and Marin (Marin et al. 2005). It was conducted as follow: In a tube, 30 mg of ground grain were added to 2 ml of potassium hydroxide/methanol solution (1:10, *w*/*v*). After 1 h at 80 °C, the tube was rapidly cooled on ice. An internal standard constituted of 40 μl of 7-dehydrocholesterol (Sigma, France) dissolved in

methanol at 500 mg/ml and mixed with 1 ml of ultrapure water was added to the sample. Extraction was carried out twice with 2 ml of hexane. After centrifugation at 3000 rpm for 3 min, the two organic phases were collected, grouped and evaporated to dryness under a nitrogen flow at room temperature. The dried extracts were then stored at −20 °C until analysis. The dried samples were suspended in 1 ml of methanol and filtered through a 0.45 mm filter before HPLC/DAD analysis. Ergosterol elution is conducted on a column Kinetex 2.6 μxB C18 - 100 A (150 mm × 4, 6) (Phenomenex; France) maintained at 40 °C. The injection volume was set at 5 μl. An isocratic elution with 100 % methanol was used. The flow rate was set at 0.8 ml/min for a total analysis time of 15 min. UV spectrum was measured from 190 to 400 nm and the identification or ergosterol was done using its specific maximum of absorbance at 282 nm. Quantification was done using external calibration obtained from the pure standard of ergosterol (Fulka, France).

### Extraction and analysis of phenolic acids from kernels

Phenolic acids were released from cell walls by alkaline hydrolysis. Four milliliters of sodium hydroxide (2 M) were added to finely grinded wheat kernels (100 mg). After agitation for 2 h, the filtrates were acidified to pH 2 with hydrochloric acid (12 M). Samples were extracted with 5 ml of ethyl acetate. After centrifugation (5000 rpm), 4 ml of the ethyl acetate fraction was collected**.** The extraction was repeated a second time and 8 ml of organic fraction evaporated to dryness at 35 °C under nitrogen stream. Finally, dried samples were dissolved in 100 μl of methanol/water (50:50, *v*/v) before analysis. HPLC/DAD separation was performed according to a previously described procedure (Atanasova-Pénichon et al. 2014) with some modifications. Separation of phenolic acids was achieved on a KINETEX 2.6 U XB-C18 column (4.6 × 150 mm) (Phenomenex, France) maintained at 45 °C. The mobile phase consisted of 2 % formic acid in water (*v*/v) (solvent A) and acetonitrile (solvent B). Phenolic acids were separated by a gradient elution as follows: from 5 to 15 % B in 30 min, from 15 to 50 % B in 10 min, from 50 to 90 % B in 5 min, 90 % B for 3 min, 90 to 5 % B in 2 min, and 5 % B for 10 min for post-run reconditioning. The injection volume was 5 μl and the flow rate was kept at 1 ml/min. The UV-VIS spectra were recorded from 200 to 550 nm and peak areas were measured at 260 nm, 280 nm and 320 nm and 360 according to the studied phenolic acid. Quantification was performed by using external calibrations with phenolic acid standard solutions prepared from commercial pure powders purchased from Sigma-Aldrich (France).

### Statistical analysis

Data were subjected to analysis of variance (ANOVA) with STATBOX Vegetal 7.2. Significance of mean differences were determined using the Tukey's test, and responses were judged significant at the 5 % level ($p = 0.05$) with a 95 % confidence interval, and the multiple comparison of means (CMM) for DI, EI and TCTB were determined using the Newman - Keuls test at 5 %. The correlation between DI, Ergo and mycotoxin accumulation in kernels were determined by Pearson correlation analysis and calculated using: XLSTAT software (PCA).

## Results

### Molecular and biochemical characterisation of the *Fusarium culmorum* strains

Four *Fusarium* strains where selected among a collection of isolates issued from wheat spikes of durum wheat collected in different area of the north of Algeria. The four strains where characterised as *F. culmorum* on the basis of morphological observation of mycelia on PDA plates and after observation of conidia under a microscope according to Leslie and Summerell (Leslie and Summerell 2006). Morphological identification of the isolates at the species level was confirmed by PCR using the specific primers described by Shilling et al. (1996). Analysis of the PCR product showed a fragment of 470 bp as expected for *F. culmorum* (result not shown). PCR assays with primer Tox5 specific of the gene *Tri5* were used to confirm the potential ability of the four isolates to produce trichothecenes B (Niessen and Vogel 1998). Specific PCR assays were used to determine the potential chemotype of each *F. culmorum* isolate by a genetic approach based on the use of primers specific of the Tri12 genes sequence (L. Pinson-Gadais; personal communication). Results showed the two isolates BT11 and BD11 yielded a 282 bp fragment with theTri12DON3Adon assay indicating a DON/3-ADON genotype. The two other isolates T5 06 and T7

06 yielded specific PCR products with the Tri12NivFX assay (465 bp) indicative of NIV producers.

The toxins produced when the four strains were grown in vitro on rice grains were analysed. The amounts of mycotoxins synthesised by *F. culmorum* BT11 and BD11 strains was deoxynivalenol (DON) and its acetylated form 3-ADON confirming the DON/3-ADON chemotype as determined by PCR. In addition, these two strains produced also large amounts of zearalenone (ZEA) on the rice substrate (Table 1). The strains T5 06 and T7 06 were confirmed as NIV/FX chemotype as they produced nivalenol (NIV) and its acetylated form fusarenone (FX). No ZEA was detected for these two strains. This definitely confirms the chemotype of the four isolates and characterise their toxigenic potential in vitro. The two DON/3-ADON isolates produce toxins levels well above those of the NIV/FX isolates and, in addition, the strain BD11 produces very high amounts of ZEA on rice substrate.

## Pathogenicity and aggressiveness of the four *F. culmorum* isolates in field assay on wheat

The two most important commercial wheat types are the durum wheat (DW) *Triticum durum L.* subsp. *Durum* and the soft wheat (SW) *Triticum aestivum L*. In Algeria, 12 varieties are currently cultivated for production of durum wheat. Since 1980, five of these varieties have been introduced as improved material. Today, the two introduced varieties VITRON and WAHA occupy up to 65 % of the total cultivated areas. Furthermore, growth of the more recently introduced variety "CHEN's" has increased in the recent years to up to a level of 7 % of the durum wheat seed sole occupancy (Boufnar-Zaghouane and Zaghouane 2006). In our assay, we evaluated the resistance to *F. culmorum* infection for the two types of durum wheat cultivars including both the autochthon cultivars and the introduced varieties. This information is a prerequisite to the development of improved genetic material with effective resistance to FHB. For the comparison, we also used a few common soft wheat varieties grown in Algeria as summarised in Table 2. In a primarily assay performed in 2011, the four *F. culmorum* strains were evaluated for their capacity to confer FHB in field experiments. They were spray inoculated on spikes using VITRON, a variety of durum wheat and ARZ, a variety of soft wheat (Table 2). The four T5 06, T7 06, BT11 and BD 11 strains where pathogenic giving slightly different Disease Index (DI) increasing from 43.5 % to 55.25 for the soft wheat variety ARZ and from 52.5 to up to 70 % for the durum wheat variety VITRON (Table 1). The four isolates were then used as reference strains for the evaluation of resistance of a set of eight wheat varieties. Spray inoculation onto spikes was used for the determination of the pathogenicity and evaluation of respective aggressiveness of the four *F. culmorum* strains. Four were semi late varieties and four were early genotypes and they were chosen as representative of the endogenous and introduced varieties generally used in Algeria (Table 2). The assays were performed for the 2 years 2012 and 2013, and a control assay was performed for each variety. Disease index (DI), ergosterol content reflecting the development of the fungus in the grain (hereby called the Ergosterol Index (EI)) and mycotoxins contents were also evaluated for the 2 years. The results are reported in Tables 3 and 4.

### *Correlations between variables*

It is noteworthy that the present results from field assays are based on 2-year studies and variables are calculated on the results of the two cumulative years. Overall, the disease index DI was found highly correlated to EI, the fungal ergosterol content, and to the toxin content in the four assays ($r = 0.9$, $r = 0.88$; $r = 0.85$, $r = 0.9$; $r = 0.77$, $r = 0.72$; $r = 0.70$, $r = 0.69$ respectively for T5 06, T7 06, BT11 and BD11). As well, the toxin content was found highly correlated to the EI for T5 06 and T7 06 assays $r = 0.87$ and $r = 0.88$ respectively and it was correlated only with an $r = 0.37$ for BT11 and an $r = 0.41$ for BD11. These relationships were further explored with a variance analysis ANOVA. For the three variables DI, EI and TCTB, the analysis of variance gives highly significant results for the factor 1 (variety), factor 2 (isolate) and the F1 * F2 interaction ($p < 0.0001$). The variability between blocks is non-significant, suggesting that the blocks are homogeneous. Both strains and varieties had a significant effect on DI, EI and on toxin content. The disease index DI appears to be a good indicator of the aggressiveness of the strain and, of the impact on its development in grains (fungal biomass represented by the ergosterol index EI) and its production of toxins in grain. Regarding the control experiments, our results showed only a very low natural contamination for VITRON in 2012 and the fungal levels of ergosterol were below the detection limit of our method.

**Table 2** Different varieties and lines used in the field assays

| Name of genotype | Short Name used | Specie | Origin | Vegetative cycle |
|---|---|---|---|---|
| ARZ | ARZ | SW | Local | Semi-early |
| HD1220 | HD1220 | SW | Local | Early |
| Florence Aurore | FLORENCE | SW | FRANCE | Late |
| Mahon Demias | MAHON | SW | SPAIN | Late |
| Mohammed Ben Bachir | MBB | DW | Local | Late |
| BIDI17 | BIDI17 | DW | Local | Late |
| Chen's | CHEN's | DW | ICARDA | Early |
| Vitron | VITRON | DW | SPAIN | Early |
| G1-F14 line 1 | G1 | Line from P3-P4 | ENSA Algiers | Early |
| G4-F14 line 4 | G4 | Line from P1-P2 | ENSA Algiers | Early |
| G9-P1 line G4 | G9 P1-G4 | DW | FRANCE | Early at very early |
| G10-P2 line G4 | G10 P1-G4 | DW | FRANCE | Semi-early |

*SW* Soft wheat, *DW* Durum wheat

However, in 2013 the fungal ergosterol analysis indicated a slight contamination for VITRON, and HD1220.

*Comparison of symptom severity and ergosterol content in grain*

The four strains of *F. culmorum* were pathogenic towards all the varieties but the aggressiveness varied greatly among the isolates in the severity of the visual disease symptoms induced (See Tables 3 and 4, Disease Index (DI)). A test of Tukey performed (on the whole results) (HSD) showed that the strains differed significantly ($p < 0.0001$) in their ability to cause head blight. The multiple comparison of means (CMM) for (DI) using the Newman - Keuls test at 5 % shows that BD11 and BT11 strains producing DON/3-ADON are the most aggressive, while the T5 06 and T7 06 isolates producing NIV/FX are the less aggressive.

For the four strains, the DI vary from 27.67 for ARZ; (T5 06, 2012) to 87 % for VITRON (BD11, 2013) (See Tables 3 and 4), but they present similar general pattern for the DI based classification of varieties. The maximum index of severity was measured for susceptible DW varieties VITRON and CHEN's in the 2 years but the 2013 DI was superior to the 2012 DI. The test of Newman-Keuls showed that the VITRON and CHEN's were the most susceptible and ARZ was the most resistant and durum wheat seems less resistant than soft wheat.

Visual evaluation of symptoms is prone to errors and is not always fully representative of the fungal invasion. Actually, for equivalent values of symptoms annotation, there were large differences observed among scabby grains once harvested. Measuring the ergosterol in harvested grains allows a more relevant evaluation of fungal development inside the grain (Seitz et al. 1977). We have performed such an analysis on all the samples and the result is reported as Ergosterol Index (EI) in Tables 3and 4. Result of Newman-Keuls for EI shows that the homogeneous group with the highest EI represents BD11 strain, followed by a group represented by BT11 and then a which represent both T5 06 and T7 06 and shows the lower DI. For all varieties, the ergosterol level turned being higher in 2013 compared to 2012. This probably reflects differences in climatic condition between the 2 years. For classification of varieties, the Newman-Keuls test showed that the variety CHEN's is the one presenting the highest EI, followed by VITRON, while ARZ, BIDI 17 and FLORENCE exhibited the lower EI. Higher concentration of ergosterol were found in the introduced durum wheat varieties VITRON and CHEN's, which were also the two that had the highest susceptibility to FHB as judged with the DI. In our experiment, they behave as the most sensitive varieties when assayed in Algerian conditions. The results also clearly show that in general, soft wheat is more resistant than durum wheat in terms of mycelium development. The autochthon SW ARZ variety is the one exhibiting the best tolerance to fungal spreading among the four strains used. The two autochthon late durum wheat varieties

**Table 3** Resistance of eight wheat varieties to *F. culmorum* DON/ADON isolates causing Fusarium head blight

| Fusarium strain | | | | BT11 | | | BD11 | | |
|---|---|---|---|---|---|---|---|---|---|
| Name | Year | Species | Index | DI % ± SD | EI μg/g ± SD | DON μg/g ± SD | DI % ± SD | EI μg/g ± SD | DON μg/g ± SD |
| ARZ | 2012 | SW | R | 27.94 (±1.87) | 41.19 (±1.13) | 22.57 (±1.81) | 28.96 (±1.98) | 43.65 (±5.40) | 28.65 (±3.16) |
| BIDI 17 | 2012 | DW | R/S | 40.67 (±1.15) | 28.18 (±2.55) | 10.75 (±0.29) | 57.17 (±2.57) | 64.25 (±4.35) | 30.47 (±4.07) |
| MBB | 2012 | DW | R/S | 50.07 (±0.90) | 31.73 (±2.89) | 26.66 (±1.41) | 57.00 (±2.65) | 68.75 (±6.54) | 31.95 (±1.88) |
| FLORENCE | 2012 | SW | R/S | 50.22 (±2.01) | 37.48 (±2.88) | 21.38 (±2.27) | 57.00 (±3.00) | 69.64 (±2.10) | 41.26 (±2.95) |
| HD1220 | 2012 | SW | S | 61.87 (±2.27) | 53.72 (±3.05) | 29.22 (±4.51) | 60.81 (±2.55) | 86.83 (±2.75) | 62.43 (±5.46) |
| MAHON | 2012 | SW | S | 72.40 (±5.79) | 84.74 (±8.89) | 71.34 (±4.21) | 69.67 (±4.51) | 79.56 (±11.06) | 83.06 (±1.36) |
| VITRON | 2012 | DW | S | 77.80 (±2.55) | 80.49 (±5.27) | 87.05 (±7.29) | 74.78 (±4.67) | 144.98 (±5.04) | 142.99 (±14.53) |
| CHEN's | 2012 | DW | S | 69.10 (±0.85) | 102.48 (±3.52) | 33.51 (±2.76) | 72.77 (±2.54) | 152.37 (±6.13) | 52.33 (±2.65) |
| ARZ | 2013 | SW | R | 40.27 (±2.91) | 62.01 (±8.84) | 20.50 (±0.86) | 43.95 (±3.35) | 63.50 (±8.84) | 23.00 (±2.52) |
| MBB | 2013 | DW | R/S | 45.29 (±4.67) | 69.35 (±3.07) | 25.08 (±2.80) | 55.13 (±4.85) | 123.47 (±3.07) | 40.36 (±3.26) |
| BIDI 17 | 2013 | DW | R/S | 47.87 (±2.32) | 52.37 (±1.96) | 30.20 (±3.72) | 50.87 (±3.87) | 93.81 (±1.96) | 35.60 (±1.06) |
| FLORENCE | 2013 | SW | R/S | 48.10 (±2.52) | 59.63 (±7.85) | 31.21 (±4.69) | 46.77 (±3.40) | 83.38 (±7.85) | 36.80 (±2.91) |
| MAHON | 2013 | SW | S | 60.27 (±4.91) | 84.71 (±12.91) | 37.74 (±2.28) | 66.61 (±3.23) | 112.26 (±12.91) | 56.75 (±5.76) |
| HD1220 | 2013 | SW | S | 71.17 (±3.40) | 89.95 (±8.99) | 50.11 (±6.79) | 70.73 (±3.99) | 150.74 (±8.99) | 78.12 (±15.21) |
| VITRON | 2013 | DW | S | 79.77 (±0.87) | 87.70 (±6.51) | 72.00 (±1.50) | 86.97 (±7.51) | 153.25 (±6.51) | 84.95 (±8.13) |
| CHEN's | 2013 | DW | S | 83.34 (±6.13) | 161.92 (±24.52) | 29.64 (±3.46) | 70.32 (±4.75) | 233.90 (±24.52) | 44.95 (±3.08) |

Varieties are arbitrarily ordered following the disease index observed with BT11 except for CHEN 's in 2012 which has been manually classed at the last position on the basis of EI

*SW* soft wheat, *DW* durum wheat, Index is the indexation in Algerian catalogue: *R* resistant, R/S = moderately resistant *S* sensible, *DI* Disease Index, *EI* Ergosterol Index (μg/g), *DON* deoxynivalenol +3 acetyldeoxynivlalenol

**Table 4** Resistance of eight wheat varieties to *F. culmorum* NIV/Fx isolates causing Fusarium head blight

| Fusarium strain | | | | T5 06 | | | T7 06 | | |
|---|---|---|---|---|---|---|---|---|---|
| Name | Year | Species | Index | DI % ± SD | EI μg/g ± SD | NIV μg/g | DI % ± SD | EI μg/g ± SD | NIV μg/g ± SD |
| ARZ | 2012 | SW | R | 27.67 (±2.52) | 23.68 (±1.32) | 6.90 (±1.41) | 30.99 (±1.16) | 28.00 (±2.84) | 8.32 (±0.77) |
| BIDI 17 | 2012 | DW | R/S | 37.09 (±1.72) | 24.72 (±1.25) | 5.09 (±0.26) | 36.57 (±1.36) | 21.96 (±1.96) | 8.34 (±1.88) |
| MBB | 2012 | DW | R/S | 40.43 (±0.59) | 31.92 (±1.64) | 7.41 (±1.71) | 42.00 (±2.65) | 47.75 (±2.33) | 5.80 (±0.72) |
| FLORENCE | 2012 | SW | R/S | 36.43 (±2.23) | 28.17 (±1.95) | 5.21 (±0.53) | 40.51 (±2.48) | 28.86 (±1.17) | 7.52 (±0.94) |
| HD1220 | 2012 | SW | S | 42.77 (±2.04) | 27.97 (±2.08) | 7.12 (±1.33) | 55.53 (±0.85) | 41.33 (±3.71) | 13.04 (±1.21) |
| MAHON | 2012 | SW | S | 45.43 (±0.75) | 30.53 (±0.65) | 9.28 (±0.83) | 64.67 (±1.53) | 59.83 (±1.58) | 16.48 (±2.66) |
| VITRON | 2012 | DW | S | 61.70 (±2.86) | 75.60 (±4.50) | 15.88 (±1.91) | 71.33 (±3.21) | 85.37 (±5.06) | 29.70 (±4.03) |
| CHEN's | 2012 | DW | S | 62.44 (±3.60) | 77.60 (±2.45) | 10.58 (±2.57) | 78.74 (±1.56) | 111.97 (±2.14) | 29.02 (±2.16) |
| ARZ | 2013 | SW | R | 34.50 (±1.90) | 33.62 (±2.40) | 8.85 (±0.07) | 36.48 (±3.08) | 38.35 (±3.02) | 10.94 (±0.87) |
| MBB | 2013 | DW | R/S | 45.03 (±4.95) | 39.00 (±2.14) | 10.32 (±0.41) | 49.20 (±0.92) | 72.60 (±6.17) | 12.96 (±0.91) |
| BIDI 17 | 2013 | DW | R/S | 41.22 (±3.39) | 34.18 (±1.12) | 9.10 (±1.43) | 46.20 (±1.99) | 32.96 (±5.45) | 8.99 (±0.36) |
| FLORENCE | 2013 | SW | R/S | 43.71 (±1.64) | 41.51 (±1.79) | 9.22 (±0.26) | 46.17 (±4.40) | 38.24 (±5.81) | 10.53 (±0.56) |
| MAHON | 2013 | SW | S | 49.66 (±0.78) | 35.42 (±0.74) | 14.68 (±0.25) | 60.17 (±5.20) | 72.06 (±6.83) | 17.70 (±0.33) |
| HD1220 | 2013 | SW | S | 59.85 (±2.29) | 48.67 (±2.90) | 13.92 (±0.41) | 58.03 (±2.30) | 54.94 (±6.00) | 17.43 (±1.49) |
| VITRON | 2013 | DW | S | 76.25 (±3.81) | 113.62 (±4.55) | 43.10 (±3.07) | 75.15 (±4.83) | 121.55 (±12.13) | 42.11 (±3.96) |
| CHEN's | 2013 | DW | S | 71.42 (±3.60) | 114.80 (±5.27) | 24.97 (±2.57) | 76.67 (±2.89) | 178.23 (±16.41) | 34.05 (±2.52) |

Varieties are arbitrarily ordered following the disease index observed with BT11 except for CHEN 's in 2013 which has been manually classed at the last position on the basis of EI

*SW* soft wheat, *DW* durum wheat, Index is the indexation in Algerian catalogue: *R* resistant, R/S = moderately resistant *S* sensible, *DI* Disease Index, *EI* Ergosterol Index (μg/g), *NIV* nivalenol + Fx

BIDI17 and MBB also appeared less susceptible compared to the other DW varieties and even when compared to most SW varieties.

Even if the DI and levels of ergosterol are sensibly different between the 2 years 2012 and 2013, the ranking remains roughly identical for these two criteria. Only CHEN's behaved quite differently for the 2 years, showing the highest EI each year, but with a DI sensibly lowest in 2012 with BT11 strain. Otherwise, it is also remarkable that the order remains more or less the same whatever the *F. culmorum* strain used. It appears also that globally, the introduced varieties MAHON DEMIA, VITRON and CHEN's are more susceptible to FHB than the endogenous varieties ARZ, MBB and BIDI17.

*Trichothecenes accumulation in grains*

In order to have a complete view of the FHB incidence, we also analysed the toxin accumulated inside the grains. Type B trichothecenes, either DON or NIV depending of the strain, were observed in all samples. However, quite different levels were observed, depending of the strain used, the variety considered and the year. No FX was detected for assays with NIV chemotypes. The acetylated form of DON, 3-ADON, was not observed in most of the samples inoculated with the DON/3-ADON strains and when present, it was detected at very low level. In Table 3, DON values represent the sum of DON +3-ADON. The two *F. culmorum* BT11 and BD11 strains produced high amounts of zearalenone in the in vitro assay but the presence of ZEA was erratic and limited to only a few 2013 wheat samples. In addition, the level observed was rather low in these field assays. For this reason, the result of ZEA quantification has not been considered in this field study.

Overall, in terms of accumulation of trichothecenes in grains, the 2 years of experimentation can be considered as two very favourable years. Even if the 2 years showed differences for DI or EI contents, the differences for toxin accumulation in the varieties were quite similar for the 2 years and the accumulation of toxin generally reflected the DI or EI. However, quite strong differences

were found for the mean performance of isolates with different toxin-producing capacities. The two DON/3-ADON isolates produce significantly higher levels of toxin (from about 21 μg/g to about 115 μg/g) than the two NIV/FX isolates (from about 2 μg/g to 42 μg/g of toxins). The Newman-Keuls test classify VITRON as the variety accumulating more TCTB, followed by the HD1220, CHEN's and MAHON DEMIAS varieties, and lastly, ARZ that accumulated the lowest concentration in TCTB.

Classification of varieties based on the toxin content is conserved compared to the classification with DI and EI except for the durum wheat CHEN's. Strikingly, CHEN is the more susceptible variety in term of *Fusarium* contamination as measured by ergosterol level, but it accumulates significantly (p 0.004) much less toxin than the DW VITRON. CHEN's shows the highest EI (233.9 μg/g) with BT11 in 2013 and the DON content is only about 40 μg/g, i.e. more than 2 times less than the other susceptible variety VITRON, which shows a DON and EI value of about 80 μg/g and 153.35 μg/g respectively. This result is confirmed regarding the results obtained with the three other *F. culmorum* strains and also for the 2 years. Regarding the ratios for DON/EI or NIV/EI, the susceptible genotype CHEN's has always significantly lower values than the resistant varieties suggesting that although CHEN's is favourable to *F. culmorum* spreading in grains, something restricts trichothecene accumulation in this variety. It has been shown that in some resistant varieties, the DON is efficiently transformed into the glucosylated form DON-Glucoside (DON-Glu) (Berthiller et al. 2005, Poppenberger et al. 2003). In order to investigate if the lower content in DON observed for the CHEN's variety could be explained by efficient transformation of DON into DON-Glu, we analysed the content in DON-Glu for ARZ, MBB, CHEN's and VITRON inoculated with the strain BD11 in the trial 2013. No higher accumulation of DON-Glu was observed for CHEN's (0.58 μg/g) compared to VITRON (0.67 μg/g) or MBB (0.5 μg/g) which could explain the lower accumulation of DON in this variety. Only ARZ (1.0 μg/g) contained about two time more DON-Glucoside than the others varieties.

Although the 2 years conducted to slightly different DI, they give very similar results for the classification of varieties for resistance to FHB. A PCA was realized on the eight varieties for the 2 years and three variables DI, EI and TCTB content. Figure 1 shows the contribution of the variables in the two axes F1 and F2, representing 78.63 % and 15.90 % of the total variance. This represents the better distribution of varieties for the 2 years. Zone 1 regroups all susceptible varieties and zone 2 groups the resistant ones. The coordinates on F1 show that the behaviour of varieties is similar for 2012 and 2013 except for HD1220 which grouped in zone 2 in 2012 and, because it had a higher DI and accumulated higher level of toxin, grouped in zone 1 in 2013. CHEN's is apart from the other varieties in F2 axis. This can be explained by the significantly lower level of toxin accumulated for this variety even if the DI is high as mentioned previously. Again in general, durum wheat is more susceptible than soft wheat for toxin accumulation as it was observed for DI and EI. However, BIDI 17 is as resistant to fungal development and toxin accumulation as the less susceptible soft wheat variety ARZ (Fig. 1 and Tables 3 and 4). Figure 1 also shows that the endogenous varieties ARZ for SW and BIDI 17 and MBB for DW seem less susceptible to *F. culmorum* than the introduced varieties MAHON DEMIAS for SW and VITRON and CHEN's for DW.

### *Evaluation of resistance of new genetic material*

During the year 2013, in parallel to the eight varieties, new genetic material from ENSA El Harrach, Algiers (Mekliche, unpublished results) was assessed with the strain T5 06. Two durum wheat lines hereby called G1-F14-Line 1and G4-F14-line 4 corresponded to $F_{14}$ seeds were chosen in function of the result of preliminary resistance field tests performed in 2012 (result not shown). The field assay also included the G4 parental varieties G9 (Parent 1-G4) and G10 (Parent 2 G4). For technical reason, the two others parental varieties could not be assessed in our 2013 assay. On the basis of this sole experiment, the results presented in Table 5 show that the two parental durum wheat varieties can also be considered as material susceptible to *Fusarium* infection and toxin accumulation. It can be noticed than the parent G10 behaves as CHEN's, showing a high EI and DI but a relative tolerance to toxin contamination compared to VITRON. Interestingly, the two lines G1 and G4 behaved as resistant material in term of DI and toxin production (Table 5). Particularly, G4 presented much better characteristics than ARZ, the best soft wheat tested showing the highest resistance level. In this assay, the DI (25.33) and the EI (30.21) were the lowest and

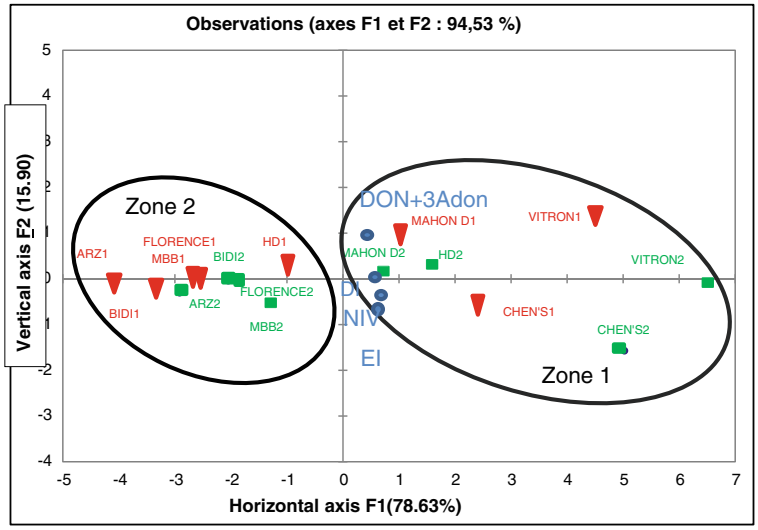


**Fig. 1** Correlation of variables () and samples in the axis defined by the two components F1 and F2. (*red down-pointing triangle*) V arieties year1 (*green square*) varieties year2

the toxin content was as low as 2.04 μg/g. Actually, G4 has a NIV/EI ratio two fold lower than G1 and three to eight times lower than any of the other varieties tested in the 2013 assay.

*Phenolic acid content of the grains from the varieties and lines*

Monomeric forms of phenolic acids have been quantified in the mature grains of the different samples using HPLC-DAD. Results are presented in Table 6. Cinnamic derived phenolic acids were clearly the most abundant phenolics. Ferulic acid is representing up to 90 % of total monomeric phenolic content and was from far the most abundant. The second was sinapic acid followed by *p*-coumaric acid. The benzoic acid derived vanilic acid and *P*-hydroxybenzoic acid and vanillin were also detected but in lower amounts. The content in phenolic acids varies dramatically from one variety to another (from about 52 μg/g in HD1220 to up to 1 636 μg/g in CHEN's for ferulic acid). There is a trend toward the fact that the varieties and lines such as CHEN's, FLORENCE, G1 and G4, having the higher content in ferulic acid are the one that have the lower NIV/EI ratio; however, this is not true for all the varieties so that no clear conclusion can be drawn.

**Table 5** Evaluation of the G1 and G4 durum wheat lines compared to the varieties used in Algeria

| Genotype | Specie | DI% (+SD) | EI μg/g (+SD) | NIV μg/g (+SD) | NIV/EI ratio |
|---|---|---|---|---|---|
| G4 F14 line 4 | DW | 25.33 (0.31) | 30.21 (2.03) | 2.04 (0.30) | 0.07 |
| ARZ | SW | 34.50 (1.90) | 33.62 (2.40) | 8.85 (0.07) | 0.26 |
| G1 F14 line 1 | DW | 36.67 (2.89) | 63.99 (1.76) | 8.78 (1.06) | 0.14 |
| BIDI 17 | DW | 41.22 (3.39) | 34.18 (1.12) | 9.10 (0.43) | 0.27 |
| FLORENCE | SW | 43.71 (1.64) | 41.51 (1.79) | 9.22 (0.26) | 0.22 |
| MBB | DW | 45.03 (4.95) | 39.00 (2.14) | 10.32 (0.41) | 0.26 |
| MAHON | SW | 49.66 (0.78) | 35.42 (0.74) | 14.68 (0.25) | 0.41 |
| HD1220 | SW | 59.85 (2.29) | 48.67 (2.90) | 13.92 (0.41) | 0.29 |
| CHEN's | DW | 71.42 (3.60) | 114.80 (5.27) | 24.97 (2.57) | 0.22 |
| G10 (Parent 1-G4) | DW | 73.67 (1.53) | 128.72 (1.53) | 24.16 (1.90) | 0.19 |
| VITRON | DW | 76. 25 (3.81) | 113.62 (4.55) | 43.10 (3.07) | 0.38 |
| G9 (Parent 2-G4) | DW | 78.33 (2.89) | 144.89 (5.24) | 34.11 (4.36) | 0.24 |

Assays were performed in 2013 with the *F. culmorum* isolate T5 06. Classification of varieties is based on the DI

**Table 6** Content in cinnamic derived phenolic acids measured in grains of the different varieties

| | | Phenolic acid content in mature grains in 2013 (μg/g) | | | | | |
|---|---|---|---|---|---|---|---|
| Variety or line | Specie | Ferulic ±SD | Sinapic ±SD | *p*-coumaric ±SD | Vanillic ±SD | Vanillin ±SD | 4-hydroxy- benzoic ±SD |
| CHEN's | DW | 1636 (±26.5) | 216 (± 6.4) | 28(± 2.9) | 13 (± 1.5) | 16 (± 2.9) | 3 (± 0.1) |
| FLORENCE | SW | 1438 (± 5.7) | 151 (±28.8) | 57 (± 4.2) | nd | 1(± 0.1) | nd |
| G1 | DW | 1126 (± 7.1) | 150 (± 5.6) | 42 (± 1.6) | nd | nd | nd |
| BIDI17 | DW | 1035 (±28.4) | 93 (± 1.5) | 32 (± 1.5) | 18 (± 1.5) | 28 (± 4.3) | 6 (± 0.1) |
| G4 | DW | 988 (±11.3) | 133 (± 8.4) | 54(± 2.8) | nd | nd | nd |
| MAHON | SW | 780 (± 1.1) | 110 (± 1.4) | 11 (± 0.3) | nd0 | 32 (± 2.8) | 12 (± 1.1) |
| MBB | DW | 763 (±17.1) | 158 (±17.0) | 32 (± 1.4) | 12 (± 0.14) | 8 (± 0.1) | 19 (± 1.6) |
| G10 Parent1 G4 | DW | 730 (±12.7) | 76 (± 3.0) | 22 (± 2.8) | nd | nd | nd |
| ARZ | SW | 730 (±15.5) | 67 (± 4.2) | 50 (± 1.4) | nd | nd | nd |
| G9 Parent2 G4 | DW | 555 (±14.3) | 107 (± 6.9) | 46 (± 4.2) | nd | nd | nd |
| VITRON | DW | 483 (±14.1) | 69 (± 2.8) | 3 (± 0.6) | 20 (± 1.4) | 16 (± 1.5) | 13 (± 1.4) |
| HD1220 | SW | 52 (± 0.1) | 46 (± 1.5) | 2 (± 0.1) | 11 (± 0.1) | 16 (± 4.3) | 12 (± 1.5) |

*nd* not detected

## Discussion

Because of its rapidly increasing population, Algerian consumption of wheat (both durum wheat and common wheat) is largely superior to its actual capacity of production. Due to the semi-arid climate, areas of culture cannot be sufficiently extended to increase the production. Consequently, the internal market has been depending on huge level of importation in the recent years. In addition, yields are quite low for the wheat grown locally and should be improved. Various reasons are responsible of this situation including historical ones, but the semi-arid and arid climatic area where it is cultivated lead generally to the use of locally adapted indigenous durum wheat varieties with poor yield. Search for new varieties adapted to the climatic areas and showing acceptable yields is a necessity for this country. However, adaptation of abroad varieties and breeding for new material must also take into account the potential risk of *Fusarium* and its mycotoxins as Fusarium Root Rot (FFR) and Fusarium Head Blight (FHB) were reported in Algeria (Boureghda 2009). Such diseases can result in important losses in yields and quality of cereals (Hollaway et al. 2013). Then *Fusarium* epidemic is a threat as it could greatly impair the progress provided by improvement of varieties by breeders for local adaptation to climatic condition if no care to select also for varieties resistant to *Fusarium* is taken. Up to now, very little is known in Algeria about the endemic *Fusarium* species, their capacity to cause Fusarium head blight and mycotoxin contamination and the tolerance of the varieties used to these local species in arid and semi-arid condition.

In this work we characterized four isolates of *Fusarium* for their capacity to produce high level of mycotoxins and to infect wheat spikes. These strains were isolated from wheat spikes of durum wheat collected in Oued Smar and Rouiba located close to Algiers. The four *Fusarium* isolates were visually identified as *F. culmorum* and the species confirmed by molecular analysis. The result of a survey in Algeria in the years 2008–2010 showed that *F. culmorum* is the predominant species observed in Algeria on wheat (unpublished results), although *F pseudograminearum* was also quite frequently observed and a few *F. graminearum* isolates were detected too. Presence of the two species *F. culmorum* and *F. pseudograminearum* are typically predominantly observed in the southern hemisphere (Obanor and Chakraborty 2014) and also reported as dominant species in many Mediterranean countries such as Tunisia, (Kammoun et al. 2010, 2009), Syria and Egypt (Alkadri et al. 2013; Balmas et al. 2015), as well as in Italy (Rossi et al. 1995; Scherm et al. 2013; Balmas et al. 2015) including the island of Sardinia (Tyrrhenian Islands) (Balmas et al. 2015). In these countries, both FHB and FRR have been described suggesting that typical climatic conditions with arid or semi-arid area may be the driver for these species. Another remark is that in

these countries, both durum wheat and soft wheat are extensively grown, an agronomic situation that may favour these two *Fusarium* species.

The four *F. culmorum* Algerian isolates where characterized for their chemotype by molecular analyses. Two strains were typed as having the DON/3-ADON chemotype, the two other strains being of the NIV/FX chemotype. These two chemotypes are the one more generally described for *F. culmorum* worldwide with the proportion between DON/3-ADON and NIV/FX isolate varying between the years and the countries (For review see: (Pasquali and Migheli 2014)). These predicted chemotype were confirmed by analysis of the toxin produced in vitro when grown on rice grains. As generally found, the DON/3-ADON production in vitro is one order of magnitude higher than the production of NIV/FX. This difference was further confirmed in vivo with the results of field assays. In addition to the production of trichothecenes, the two DON/3-ADON isolates produced high amount of zearalenone in vitro. Production of this estrogenic toxin has been frequently reported for *F. culmorum* on different cereals (Bottalico 1998; Bakan et al. 2001). Strikingly, these strains produced no or trace amount of zearalenone when inoculated on wheat spike in field experiment. This suggests that either the wheat varieties used or the environmental conditions during the field experiments were not conductive for the biosynthesis of zearalenone.

The first question we addressed in this work concerned the capacity of Algerian *F. culmorum* strains to confer FHB on spike and to produce toxins in the grain in our local conditions. The second question was to evaluate the tolerance to FHB of the varieties used in Algeria for the production of wheat. A preliminary assay to assess the pathogenicity of our isolates of *F. culmorum* strains when inoculated on spike of different varieties showed that the strains were all pathogenic as they conferred FHB symptoms. Then, the four isolates were used as reference strains in a 2 year field experiment for inoculation on eight varieties, including local and foreign ones, of both soft and durum wheat. The toxigenic potential and the aggressiveness of the four strains of *F. culmorum*, of both DON/3-ADON and NIV/FX chemotypes, used for the artificial inoculation has been one component of the success of our experiments. These resulted in 2012 and 2013 in consequent fungal growth and symptoms associated with a significant production of toxins. The results obtained allow us to draw several clear elements of conclusion. First, based on DI and EI, higher levels of severity were found for the two DON/3-ADON strains. The other two NIV/FX strains proved slightly less virulent in term of symptoms and of mycelium development and, depending of the variety, accumulated 5–10 times less trichothecene than the DON/3-ADON strains. This chemotype effect with a slightly slower spread of the NIV/FX strains in the ears has also been abundantly described before for *F. culmorum* (Miedaner et al. 2001; Wegulo 2012) and it was also observed for the species *F. graminearum* (Mirocha et al. 1994) and *Fusarium asiaticum* (Zhang et al. 2012; Shen et al. 2012). However, in term of symptoms, the difference of aggressiveness between the two chemotypes remains moderate in our experiments when compared to the reports in the literature, suggesting our two Algerian NIV strains are quite aggressive.

The data for the 2 years 2012–2013 strongly support a tight correlation between the level of trichothecene production and the symptoms observed when each of the two chemotype is considered apart. This correlation is even stronger between the quantity of toxin and the development of the fungus in the grain as measured by the ergosterol index. This was described first by Miller et al. (Miller et al. 1985) a long time ago and has been since reported by various authors (Mirocha et al. 1994; Perkowski et al. 1996; Miedaner et al. 2000). Higher the DI is, more important the fungal development is, and higher is the quantity of toxin accumulated in the grains (For review see: (Foroud and Eudes 2009)). This seems to be the rule in our samples and the variability in development and toxin production is clearly due to a variety effect. However, this rule does not apply for the CHEN's variety. This durum wheat variety exhibits a high susceptibility with a DI comparable to VITRON and a development of the fungus similar or even greater than VITRON or some of the other susceptible varieties but strikingly, with the four fungal strains, the accumulation of trichothecenes is significantly lower for this variety. A resistance to the accumulation of trichothecene has already been and discussed before (Boutigny et al. 2008). Two main reasons can explain such a result: i) the toxin is produced but modified by a detoxification process; and ii) the accumulation of the toxin remains limited due to a grain composition unfavourable for the biosynthesis (Boutigny et al. 2008). It has been shown that in some resistant varieties, the DON is efficiently transformed by the plant into the glucosylated form DON-Glucoside (DON-Glu) (Berthiller et al. 2005;

Poppenberger et al. 2003). However, in our case, the lower content in DON observed for the CHEN's variety in 2013 could not be explained by an efficient transformation of DON into DON-Glu, as the content of DON-Glu measured for CHEN's is similar to the one of VITRON or MBB. Plant biochemical inhibitory compounds present at the moment the fungus invade the forming grain could explain a lower biosynthesis of DON (Boutigny et al. 2008; Miller and Arnison 1986). It has been shown that, for example, phenolic acids can strongly modulate trichothecenes biosynthesis by *F. graminearum* (Ponts et al. 2011). As phenolic acid profiles vary between wheat varieties (Lempereur et al. 1997; Moore et al. 2006; Albermann et al. 2013), they could be good candidate compounds for the reduced trichothecene biosynthesis in kernels (Boutigny et al. 2009, 2008). Several reports suggested that resistance to *Fusarium* is correlated with kernel phenolic content in maize at maturity (Reid et al. 1992; Assabgui et al. 1993; Bily et al. 2003) and wheat (McKeehen et al. 1999; Siranidou et al. 2002). A more recent work (Boutigny et al. 2009) showed that a phenolic fraction extracted from wheat bran exhibited a strong inhibitory effect on in vitro DON/3-ADON biosynthesis by *F. culmorum*. Ferulic acid is generally abundantly present in the grain of durum wheat and this suggested a possible role in resistance to trichothecene accumulation *in planta* (Boutigny et al. 2009, 2008). Our results of phenolic acids analyses in 2013 show that ferulic acid is by far the most abundant phenolic acid (90 % of total monomeric phenolic content), in accordance with previously published studies (Onyeneho and Hettiarachchy 1992; Kim et al. 2006). It is noteworthy that the CHEN's variety has the highest content in cinnamic derived phenolic acids, especially in ferulic acid and sinapic acids. This seems coherent with the known inhibitory effect of cinnamic derived phenolic acids (Ponts et al. 2011) and the limited accumulation of trichothecenes in this variety. This observation is true also for the lines G1 and G4, but for the two other varieties Florence and BIDI 17 which also present a high content in ferulic acid, no such clear effect of lower toxin accumulation was observed. However, it is noteworthy that the content in phenolic acid has been determined in the mature grain. Accumulation of phenolic compounds varies greatly during the filling of the grain and the level is generally the highest in the early stage of grain filling as shown in maize (Atanasova-Pénichon et al. 2012). If a significant effect on modulation of trichothecenes accumulation is expected, it would mainly depend of the content in phenolic acids at the moment the fungus invades the grain during the days just following the inoculation. It should be suitable to investigate the timing of accumulation of the phenolic compounds in the varieties that accumulate less trichothecene.

Our results provide a first evaluation of the wheat varieties used in Algeria for the tolerance to FHB caused by local *Fusarium* strains of *F. culmorum*. The following classification from the less susceptible to the most susceptible can be drawn from all our results for Algerian climatic conditions:

$$ARZ_{(SW)} + BIDI_{(DW)} + MBB_{(DW)} >> FLORENCE_{(SW)} > HD1220_{(SW)} > MAHON\ DEMIAS_{(SW)} > VITRON_{(DW)} > CHEN's_{(SW)}$$

First, these results show that globally, durum wheat is more susceptible to *F. culmorum* than soft wheat. This is in agreement with the generally admitted tendency that durum wheat is more susceptible than common wheat to FHB caused by species such as *F. graminearum* or *F. culmorum* (Buerstmayr et al. 2009; Lionetti et al. 2015). The classification established after these field assays showed that the two DW BIDI17 and MBB behaved as the less susceptible material meanwhile the introduced varieties VITRON and CHEN's were very susceptible. A general conclusion that can be drawn from this study is that before a variety of wheat is going to be introduced and grown to large extend in a specific climatic area, it should be judicious to correctly assess its resistance to FHB in this climatic condition. Another remark is related to the fact that the DW variety BIDI17 and MBB are autochthonous. This may suggests that they are best adapted either to the semi-arid climatic conditions or to the local fungal strains we used. However, it must be taken into account that generally, no significant resistance has ever been observed for durum wheat (Buerstmayr et al. 2009). Then, the lower susceptibility of some varieties we observed in our assay may be uncertain. This need also to be modulated by the fact the soft wheat varieties used in our assay are the one grown in Algeria. These varieties are not known as

being the best resistant varieties to FHB available worldwide to date. It should be worthwhile to introduce reference resistant material in such field assays to have a good reference for a better classification of resistance.

One of the different reasons for initiating this work was to characterise genetic material adapted to the Algerian geographic conditions and showing acceptable tolerance to FHB. Among the traditionally cultivated local varieties, ARZ for soft wheat and BIDI 17 or MBB for durum wheat seem the best ones. However, the two new DW lines developed recently at ENSA EL Harrach and tested in 2013 showed interesting properties. The line G1 is issued from a cross between an introduced variety and a local variety. These two lines showed low disease index and ergosterol index when inoculated with the NIV producing strain T 5 06. In addition they showed a low level of nivalenol accumulation and had by far the lowest NIV/EI ratio. These two lines have been retained among various line issues for their correct behaviour when grown in Algerian climatic conditions. However, these results are preliminary as these new lines have been tested for resistance only once and with only one strain of *F. culmorum*. In addition, the test has been performed using spray inoculation which mainly evaluates type I resistance to FHB that operates against initial infection but does not really evaluate the progression of the fungus within the host (type II resistance) (Schroeder and Christensen 1963; Boutigny et al. 2008). These lines must be tested in various location and with different strains of *Fusarium* and different methods of inoculation to correctly assess their tolerance to FHB, but this result is very encouraging and shows that it would be possible to select for durum wheat material adapted to arid or semi-arid climatic conditions that exhibit an improved tolerance to FHB.

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