doi:10.14720/aas.2022.118.3.2279 Original research article / izvirni znanstveni članek

Field resistance phenotyping of durum wheat to fusarium head blight in Algeria

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Received July 21, 2021; accepted September 06, 2022.
Delo je prispelo 21. julija 2021, sprejeto 6. septembra 2022

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Abstract: In Algeria, several research studies point to the importance of the causative agents of fusarium head blight. Indeed, our research aims to study the phenotyping of the resistance of some durum wheat genotypes for their behavior to fusarium head blight, caused by four isolates of *Fusarium culmorum* (Wm.G.Sm.) Sacc.. For this purpose, the disease assessment is carried out in the field. The different evaluation criteria are: incubation period, measurement of the mass of a thousand grains and AUDPC (Area Under the Disease Progression Curve). The results obtained revealed that the varieties and lines resulting from crosses had a quite different level of susceptibility with regard to the four isolates studied and no genotype showed complete resistance (immunity) under our growing conditions. Among the tested material, the lines showed higher resistance than their parents. The reasons for this phenomenon is that crosses between genotypes implicated cultivars from Europe and Western Asia (Syria), where wheat domestication has occurred very early (between 12 000 and 10 000 years BP), which may be promising sources of resistance to fusarium head blight. The results also show a slight variability in behavior, also linked to the aggressiveness of the *Fusarium* species studied in this work.

Key words: durum wheat; phenotyping; fusarium head blight; resistance; susceptibility; aggressiveness

Ugotavljanje odpornosti trde pšenice na fuzariozo klasov na prostem v Alžiriji

Izvleček: Številne raziskave so poudarile pomen fuzarioz pšeničnih klasov v Alžiriji. Namen te raziskave je bil ugotoviti fenotipsko odpornost nekaterih genotipov trde pšenice na fuzarioze, ki jih povzročajo štiri izolati glive *Fusarium culmorum* (Wm.G.Sm.) Sacc.. Za oceno bolezni je bil izveden poljski poskus. Za oceno okužbe so bili uporabljeni naslednji kriteriji: inkubacijsko obdobje, meritev mase tisočih zrn in AUDPC (Območje pod naraščajočo krivuljo bolezni). Rezultati so pokazali, da so imele sorte in linije, ki so nastale s križanji zelo različno občutljivost na štiri v raziskavi uporabljene isolate glive, vendar ni imel noben genotip popolne odpornosti (imunosti) v razmerah potekanja poskusa. Med testiranimi vzorci pšenice so imele linije večjo odpornost kot njihovi starši. Razlog za ta fenomen je ta, da so bila križanja med genotipi sort iz Evrope in Zahodne Azije (Sirija), kjer je bila trda pšenica udomačena že zelo zgodaj (med 12 000 in 10 000 let pred sedanjostjo, t.j od začetka datiranja starosti na osnovi radioaktivnega ogljika), kar bi lahko bil obetajoč vir odpornosti na fuzarioze klasov. Izsledki so pokazali tudi manjšo variabilnost v odzivnosti med genotipi analizirane pšenice, kar je lahko povezano z različno ogresivnostjo v raziskavi uporabljenih sevov glive iz rodu *Fusarium*.

Ključne besede: trda pšenica; fenotipsko določanje; fuzarioza klasov; odpornost; občutljivost; agresivnost

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1 INTRODUCTION

Durum wheat (*Triticum durum* Desf.) is one of the oldest and the most important cultivated cereal species in the world (Royo et al., 2009; Tidiane et al., 2019; Bouanaka et al., 2021). It is of great importance in the cereal-growing areas of the Mediterranean basin and North America, where most of the world production of this crop is concentrated (USDA, 2005; Xynias et al., 2020). However, durum wheat is no longer just a staple crop for food security, but has also become a major cash crop. Africa as a whole spends more than 4 billion euros per year for import of durum wheat to provide the raw material for its food industry (Tidiane et al., 2019). In Algeria, wheat consumption (both durum wheat and soft wheat) is far greater than its real production capacity. Consequently, the domestic market has been dependent on a significant level of imports in recent years. In addition, yields are quite low for locally grown wheat and should be improved (Touati-Hattab et al., 2016). Various reasons are at the origin of this situation such as precipitation and biotic (pests and diseases) and abiotic stresses (drought, sunshine, cold and salinity) (Xynias et al., 2020). Among the biotic constraints to wheat production, fusarium head blight (FHB) (Ghimire et al., 2020).

Fusarium head blight, reported by several species of the genus *Fusarium* (Bouanaka et al., 2020; Saharan, 2020), is one of the most destructive diseases of wheat (Dweba et al., 2017; Wachowska et al., 2020), particularly affecting durum wheat (Moreno-Amores et al., 2020) and thus leading to significant reductions in yield and quality throughout the world (Touati-Hattab et al., 2016; Dweba et al., 2017; Saharan, 2020). In addition, FHB poses additional food and animal safety concerns due to the contamination of grains with mycotoxins (Ghimire et al., 2020). Among the most important species associated with the disease worldwide is *Fusarium culmorum*.

The cereal pathogen *Fusarium culmorum* (Wm.G.Sm.) Sacc. is a ubiquitous soil fungus (ascomycete) (Bilska et al., 2018), considered a chronic fungus of economic interest worldwide, including in African countries from the North like Algeria. This pathogen produces a wide range of mycotoxins, including the trichothecene-B type deoxynivalenol (DON) (Yekkour et al., 2015), which constitutes a potential health hazard (Bilska et al., 2018). Previous studies carried out in Algeria have shown that *Fusarium culmorum* appears to be the major pathogen associated with fusarium head blight (Yekkour et al., 2015; Touati-Hattab et al., 2016; Laraba et al., 2017).

The use of various methods to limit the development of *Fusarium* cereal ear diseases and their contamination with mycotoxins, before and after harvest, is an important part of sustainable agriculture and the production of healthy foods (Mielniczuk & Skwaryło-Bednarz, 2020). Genetic resistance is the most effective and sustainable approach to manage diseases in wheat (Ghimire et al., 2020), in particular, reducing the problem of mycotoxins in farmers' fields affected by fusarium head blight (Saharan, 2020).

Today, FHB phenotyping performed by breeders is performed by visual examination (Serre et al., 2015). In this context, the main objective of our present study is to compare the phenotypic resistance to fusarium wilt of two new durum wheat lines of Algerian origin selected against those of three parental commercial varieties.

2 MATERIALS AND METHODS

2.1 VEGETAL MATERIAL

In our experience, five durum wheat genotypes were chosen. To this end, two pedigree lines (G1 and G4) selected in Algeria and three commercialized varieties (G9, G10 and G11) were tested in the field. These lines are composed of F15 seeds resulting from simple crosses between 4 parental varieties: Saadi, Siméto, Ardente and Waha (Mekliche et al., 2013). The main characteristics of these genotypes are shown in Table 1. The aim is to compare their levels of resistance.

2.2 FUNGAL MATERIAL

During our study, four isolates of *Fusarium culmorum* (F.C.T5, F.C.T7, F.C10.11 and F.C1.12) were used (Table 2). These isolates were obtained from the ears and crowns of the 'Vitron' variety of durum wheat, showing typical symptoms of the disease. The ears and collars were harvested in the area of Oued Semar (Algeria) in northern Algeria. The preliminary identification was made on the basis of the conidial morphology according to Leslie and Summerell (2006) then confirmed by

Table 1: F15 pedigree lines and parental varieties used during the experiment

Codes	Genotypes	Origin	Precocity
G1	Saadi × Waha	ENSA, Algeria	Early
G4	Ardente × Siméto	ENSA, Algeria	Early
G9	Siméto	Italy	Semi- Early
G10	Ardente	France	Early to very early
G11	Waha	ICARDA, Syria	Early

Table 2: *Fusarium culmorum* isolates used in the study

Code	Species	Origin	Isolation organ	Variety
$F.C.T_5$	*F. culmorum*	Oued Smar	Ear	Durum wheat ('Vitron')
$F.C.T_7$	*F. culmorum*	Oued Smar	Ear	Durum wheat ('Vitron')
$F.C_{10.11}$	*F. culmorum*	Oued Smar	Ear	Durum wheat ('Vitron')
$F.C_{1.12}$	*F. culmorum*	Oued Smar	Collar	Durum wheat ('Vitron')

molecular tools thus using classical PCR (Touati-Hattab et al., 2016; Hadjout et al., 2022).

2.3 INOCULUM PREPARATION

Fusarium isolates are cultured in Petri dishes containing PDA medium. They are then incubated in the dark and at a temperature of 25 °C until sporulation. After 20 days of incubation, a layer of sterile distilled water of 1 to 2 mm is placed on the colony contained in each Petri dish and then poured into a container. After counting in the Malassez cell, the inoculum is prepared from a suspension of conidia in water, adjusted to 5.10^4 spores per milliliter, prepared extemporaneously (Hadjout et al., 2017).

2.4 ARTIFICIAL FIELD INOCULATION METHOD

Inoculation in the field was done by spraying the ears of each genotype until the inoculum begins to run-off, approximately 200 ml m^{-2}. The inoculations were carried out at the flowering stage corresponding to a minimum of 10 % of the ears from which the stamens have emerged. The controls consist of plots where no artificial inoculation was carried out. In the field, the inoculations were carried out in the evening, after sprinkling irrigation for about 20 min before inoculation and then 10 min after, in order to maintain sufficient humidity on the plants during the night, but also to promote adhesion and the germination of conidia. Depending on climatic conditions, the plots are then irrigated regularly in the evening.

2.5 FIELD EXPERIMENTAL SET-UP

The experiment was carried out in the field, with the installation of five tests: a control test and four tests inoculated with the four isolates of *Fusarium culmorum* mentioned above. The experimental set-up was of the complete random block type, with three repetitions (Fig. 1). The spacing between the blocks was 1 m. The area of each microplot was 1 m^2, consisting of 5 lines of 1 meter (linear meter) 20 cm apart. The distance between each microplot was 50 cm. Lines of triticale were sown between trials to avoid cross-contamination.

2.6 FIELD DISEASE ASSESSMENT

2.6.1 Incubation period

The incubation period corresponds to the period between artificial inoculation and the appearance of a fusarium blighted spikelet in the plot.

2.6.2 Symptom scoring

In the case of our study, disease severity scoring was performed 21, 26 and 31 days after inoculation. The observation unit consisted of 25 ears selected at random from each microplot. On these spikes, the total number of spikelets per spike and the number of *Fusarium* colonized spikelets were counted. The proportion of spikelets showing symptoms is assessed using a logarithmic rating scale described by Michel (2001), ranging from 0 (no symptoms) to 9 (completely dead ear, generalized drying out).

2.6.3 Calculation of the area under the disease progression curve (AUDPC)

The AUDPC is calculated on the number of fused spikelets for all scoring dates according to the formula described by Shaner and Finney (1977):

$$\text{Standardized AUDPC} = \sum_i^n \left[\left(\frac{Xi + Xi{-}1}{2}\right)\right](t_i - t_{i-1})$$

Where: n: total number of observations; x_i: number of fusarium infected spikelets in 25 heads at each observation; $(t_i - t_{i-1})$: time separating two consecutive observations.

Fig. 1: Diagram of the open-field experimental set-up for each of the five trials

2.6.4 Evaluation of the Thousand Grains Mass (TGM) at harvest

TGM was measured to assess the impact of the disease on yield, all 25 heads were threshed using a threshing machine with poor ventilation.

2.7 STATISTICAL ANALYSIS OF DATA

The statistical analysis of the results in the field is carried out using statgraphics software version 15.1.0. Next, a multiple comparison of the means was performed using the ppds (least significant difference) test to determine the groups homogeneous at the 5 % significance level.

3 RESULTS AND DISCUSSION

3.1 MANIFESTATION OF THE DISEASE

Fusarium head blight of wheat was observed in the field. In fact, the inoculated plots showed symptoms of the disease, the attacks of which on wheat ears by this disease most often result in the scalding of certain groups of spikelets, part or all of the ear. Symptoms are manifested by the presence of one or more discolored spikelets on the green spikes (Fig. 2. a, b, c, d). The ripe kernels harvested were scalded, light, chalky white or sometimes pink (this is referred to as mummified or damaged kernels, fusarious kernels) (Fig. 2.e). It should be noted that the amount of symptoms depends on the stage of the plant at the time of inoculation; the peak of sensitivity

Fig. 2: Characteristic symptoms of fusarium head blight in durum wheat (personal photos)
a, b and c: fusarious ears, the orange tint denotes the presence of the pathogenic fungus
d: Hard wheat field almost completely fused; e: fusarium grains

corresponds to the flowering of the varieties. Burrows et al. (2008) report that the initial infection is characterized as a discolored lesion at the base of the glume and the rachis which then spreads in both directions of the ear. Previous data were obtained using a spray inoculation method, frequently used to screen for resistance to fusarium head blight in wheat (Prat et al. 2014). According to Miedaner et al. (2003), spray inoculation, compared to single flower inoculation, is more adequate to reproduce the natural conditions of infection.

According to Touati-Hattab et al. (2016) and Laraba et al. (2017), *F. culmorum* is the main fungal pathogen associated with fusarium head blight in Algeria. In addition, *F. culmorum*, the causative agent of various diseases of the ear and crown of cereals, is considered a chronic fungus of economic concern worldwide, including North African countries such as Algeria. (Yekkour et al., 2015).

3.2 SSESSMENT OF GENOTYPE BEHAVIOUR BY INCUBATION PERIOD

The results obtained show that the appearance of the first symptoms on the ears estimated by the incubation time varies according to the genotype / isolate interaction (Fig. 3). Analysis of variance for all four trials revealed a significant difference for genotypes ($p < 0.001$) and for treatments (isolates) ($p < 0.001$); on the other hand, the interaction (genotypes / treatments) has no significant effect ($p > 0.05$) (Fig. 3.a, b). In our trials, we were able to characterize the behavior of genotypes with respect to the incubation period. This criterion allowed us to observe that the G1 line ranked well compared to other genotypes, due to the long incubation period recorded, 18 days after contamination. This reflects a good level of type I resistance for this line, linked to a cellular mechanism that slows the expression of the first symptom and therefore the onset of the disease. In contrast, varieties G9 and G11 recorded a shorter incubation period, approximately 10 and 11 days respectively after contamination. They are considered to be the sensitive controls chosen during our experiments; G4 and G10 genotypes had a very comparable average incubation period, an average of 13 days after contamination. It should be noted that the resistant behavior of G1 line expressed by a longer incubation period is in agreement with the work of Trottet and Saur (1994) who also used this parameter. From these results, we can say that the period of onset of symptom onset is directly related to the level of resistance of the genotypes, but also to the aggressiveness of the isolates. The mechanisms of resistance in plants to fusarium wilt are very complex (Mesterhazy et al., 1999). It is generally accepted that resistance to fusarium wilt is controlled by a polygenic system, which is known to slow the development of individual infections, the spread of the disease in fields, and the rate of spread of the fungus in adjacent plant tissues (Qi et al., 1999; Lindhout, 2002).

3.3 EVALUATION OF GENOTYPE BEHAVIOUR BY AUDPC VALUE OF THE NUMBER OF *FUSARIUM* INFECTED SPIKELETS

AUDCP analysis of variance for the number of *Fusarium* infected spikelets showed a significant difference for genotypes ($p < 0.001$) (Fig. 4.a) and trials ($p < 0.001$) (Fig. 4. b). On the other hand, the interaction between genotypes and trials is not significant ($p > 0.05$). Our results show that the AUDPC of the number of *Fusarium* infected spikelets for the G1 line is very low (9.75), while the two susceptible varieties (G9 and G11)

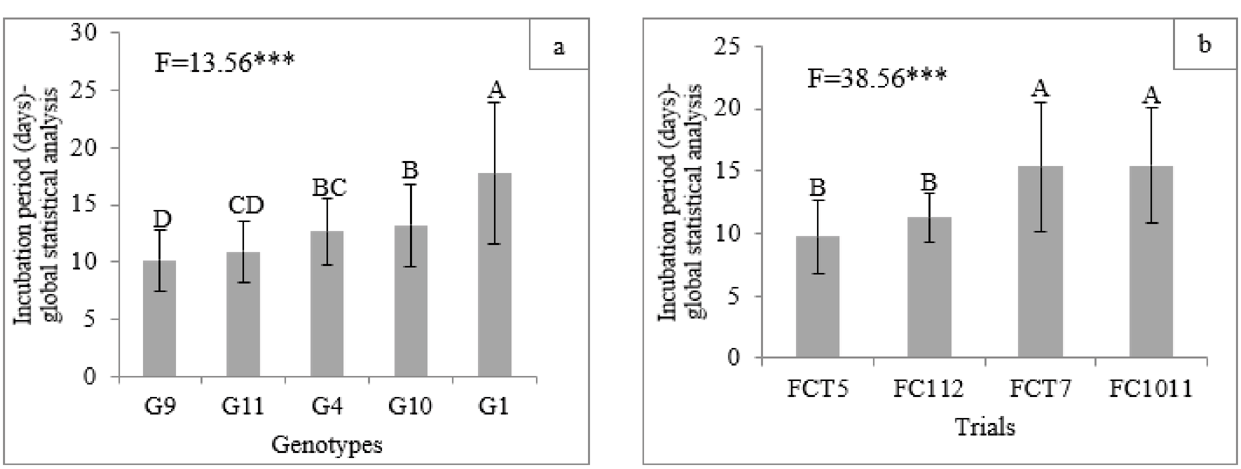

Fig. 3: Behavior of genotypes towards isolates according to incubation time

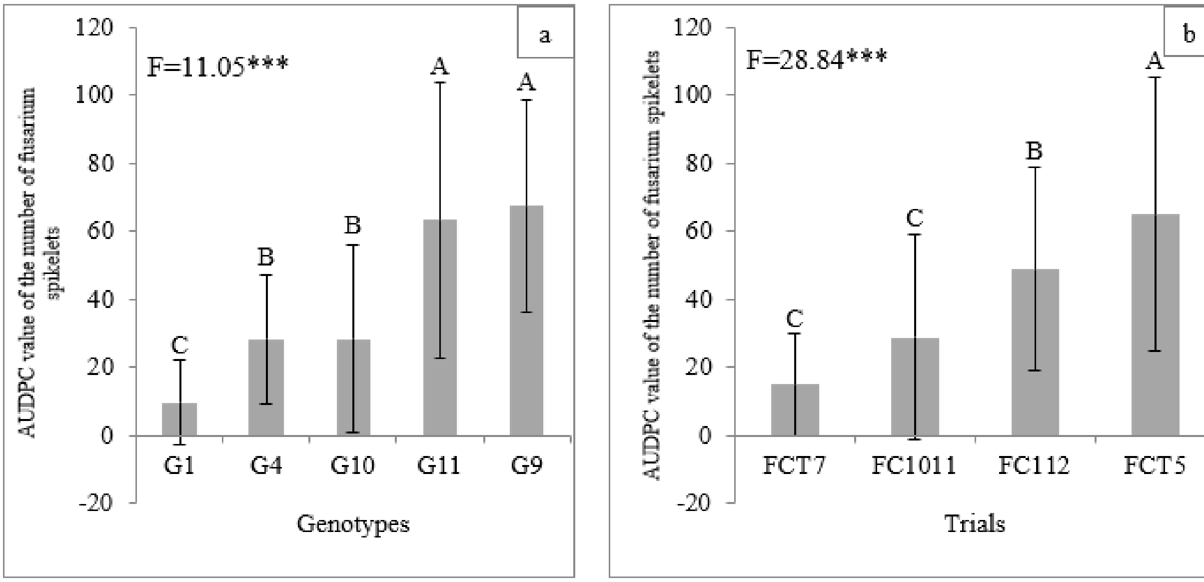

Fig. 4: Average AUDPC values of the number of *Fusarium* infected spikelets

recorded very high AUDPC values (67.57 and 63.49); the G4 and G10 genotypes marked AUDPCs intermediate between the resistant and the susceptible, namely 28.23 and 28.64 respectively. It is therefore clearly established that the G1 line behaves resistant to the progression of symptoms after inoculation. This variability in the behavior of durum wheat genotypes is most likely the result of the presence or absence of genes for resistance to this pathogen, but also the presence or absence of virulence or non-virulence genes in the pathogen. On the pathogenic side, the four isolates show different aggressiveness indicating that they differ in their pathogenicity. In many studies, the assessment of the severity of the disease in the field is essentially based on the calculation of the values of the AUDPC (Hadjout, 2013, Hadjout et al., 2017).

3.4 EFFECT OF DIFFERENT *FUSARIUM* ISOLATES AND SPECIES ON THOUSAND GRAIN MASS (TGM)

Analysis of variance integrating all trials showed the effects of genotypes and trials to be statistically significant ($p < 0.001$), while the genotypes / trials interaction showed a non-significant effect ($p > 0.05$) (Fig. 5 a, b). Analysis of the losses of the main component of yield showed that the different isolates affect all genotypes by decreasing TGW. According to our results, it is the susceptible varieties G9 and G11 which recorded the greatest losses in TGM (44.37 g and 45.30 g) followed just after by the moderately resistant variety G10 (47.41 g). The treatments affected the G1 (resistant) and G4 (moderately resistant) lines with relatively very low losses, namely 48.68 g and 54.82 g respectively, this reflects their good level of resistance, probably those of type II linked to the progression of the pathogen in the ear. The fact remains that the G10 variety showed more losses (47.41 g) than the two lines, something which was observed in previous work by Hadjout (2013). In addition, the symptoms observed explained part of the losses in TGM, this is in agreement with current knowledge on the epidemiology of fusarium head blight. The fact that the pathogen develops after the flowering stage, at the onset of the disease, the number of kernels per ear is already fixed, while the kernel filling has only just begun. The disease therefore affects this parameter and results in a large drop in TGM, especially in susceptible varieties (G9 and G11). The work of Gate et al. (1991) showed that a low TGM can be the result of end-of-life diseases (fusarium wilt), or late rains associated with high heat, and to a lesser extent with lodging. Fusarium head blight reduces grain yield and quality at the end of the crop's growth cycle, when non-diseased wheat kernels normally develop into fleshy, healthy kernels (McMullen et al., 2012).

4 CONCLUSIONS

The growing interest of the cereal sector for the sanitary quality of grains and particularly for mycotoxin contamination, strongly increases the demand for productive genotypes that accumulate few mycotoxins in their grains. In the absence of reliable information on the ability of genotypes to limit the accumulation of these

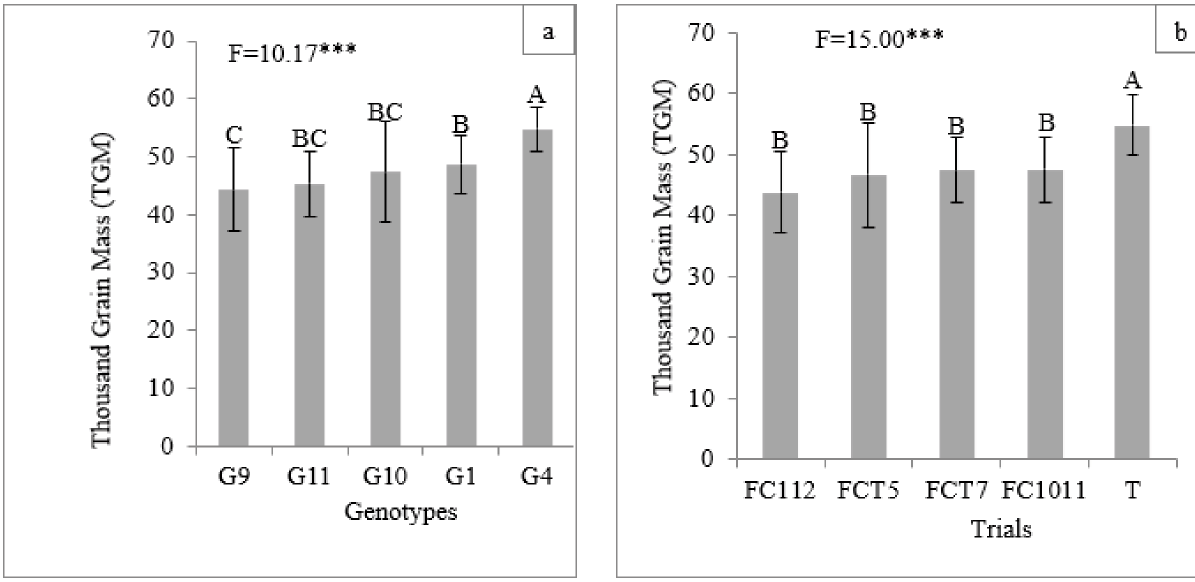

Fig. 5: Thousand grain mass for each genotype and in each trial

molecules, attention is focused on finding varieties with a good level of resistance to fusarium head blight. Indeed, the use of resistant genotypes linked to good agronomic practices remains the most satisfactory solution for farmers. Therefore, our study falls within the overall framework of the genetic control against fusarium head blight and this by the selection of genotypes resistant to the disease. To this end, the behavior of tested durum wheat genotypes with respect to fusarium head blight is evaluated under open field conditions. This behavior indicates that the G1 line exhibits longer incubation times, lower AUDPC values and thus exhibiting low disease yield losses compared to other genotypes and therefore it is of interest from a standpoint seen resistance to the appearance of the first symptoms and to the rate of spread of the fungus inside the ear.

Our results open up very important research perspectives on fusarium head blight in Algeria, in particular the search for mycotoxins as possible causes of poorly understood human diseases and the factors that contribute to their accumulation in grains.

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