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# Prevalence, cultural and pathogenic characterization of *Zymoseptoria tritici*, agent of wheat septoria leaf blotch, in Algeria

**Wahiba HARRAT[1,2*] and Zouaoui BOUZNAD[2]**

[1]Unité de Recherche de Constantine (URC), Institut National de Recherche Agronomique (INRAA), Algeria.
[2]Département de botanique, Laboratoire de Phytopathologie et Biologie moléculaire Ecole Nationale Supérieure d'Agronomie (ENSA), El-Harrach, Algeria.

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**Wheat is the 2nd most important culture in the world. Septoria leaf blotch is one of the most important wheat diseases. It is caused by *Mycosphaerella graminicola* (an: *Zymoseptoria tritici*). The aim of this study is to evaluate the presence and the importance of this disease in Algeria. A collection of 625 isolates was made through the years (2010, 2011, and 2013). Isolates were collected from the Algerian zones of cereal production. Phenotypic and genetic characterization via morphological, cultural and pathogenic analysis showed the presence of Septoria leaf blotch in 72 fields (from 122) in 20 departments (counties). In some fields the disease is highly frequent with an index of 99 according to double digit scale. Two main types of isolates were found; but the yeast-like form dominated with 94.08%. 26 isolates sampled from 25 fields were tested to evaluate isolates aggressiveness variability. Only 2 isolates from 26 inoculated were able to produce symptoms on three cereal species (triticale, durum and bread wheat).**

**Key words:** *Zymoseptoria tritici*, diversity, prevalence, virulence, wheat.

## INTRODUCTION

Wheat is the one of the most important crops worldwide. The Septoria leaf blotch (SLB) is one of the most devastating diseases of this culture (Fones and Gurr, 2015). It is caused by *Mycosphaerella graminicola* (Fuckel) J. Schröt., in Cohn (anamorph *Zymoseptoria tritici* (Desm.) Quaedvlieg and Crous) (Quaedvlieg et al., 2011). It is a heterothallic pathogen of *Dothideomycetes* class. Serious epidemics can reduce the yields on wheat from 35 to 50% in particular in Mediterranean regions (Ponomarenko et al., 2011).

In the northern half of Africa, the SLB is considered a serious threat on wheat; in Algeria it is widely present in all the northern region of the country (Sayoud et al., 1999; Zahri et al., 2013; Berraies, 2014; Teferi and Gebreslassie, 2015). Fungicides are widely used in intensive production systems. However, better yields are easily achieved by the combination of adequate cultural practices and the use of resistant varieties in the disease

*Corresponding author. E-mail: w.harrat@yahoo.fr.Tel: +213 559 460 635.

(Eyal, 1999). But the specificity of the *M. graminicola*–wheat pathosystem, frequently observed, can interfere with the use of resistant varieties (Saadaoui, 1987; Kema et al., 1996; Kema and van Silfhout, 1997; Kema et al., 2000; Brading et al., 2002; Grieger et al., 2005; Ware, 2006; Ronny et al., 2014). Several hypotheses were emitted on the gene for gene interaction between *Z. tritici* and wheat (Kema et al., 2000; Brading et al., 2002; Goodwin, 2007). Until now 18 *Stb* identified genes confer the resistance of cultivars to the pathogen (Arraiano et al., 2007; Goodwin, 2007; Chartrain et al., 2009; Tabib Ghaffary et al., 2011).

Recently, Allioui et al. (2014) and Ayad et al. (2014) demonstrated that both idiomorphs (MAT1-1 and MAT1-2) exist in Algeria and were scored at similar frequencies. Teleomorph has been identified in Algeria for the first time by Harrat et al. (2017). Other research works were made concerning the virulence, parasitic specialization and the heritability of wheat resistance to Z. tritici (Kema et al., 1996; Benkorteby, 2004; Allioui et al., 2014; Ayad et al. 2014). Of this fact, a preliminary knowledge of this disease, its distribution and diversity by a cultural and pathogenic characterization are essential to establish an adequate control approach of SLB. The objectives of the present study were to evaluate disease distribution and its importance in various cereal regions in Algeria, to realize cultural characterization and evaluate *Z. tritici* isolates aggressiveness, obtained from the Eastern regions of country, through a set of varieties constituted by wheat and triticale cultivated in Algeria.

## MATERIALS AND METHODS

### Study areas

Prospecting was realized during April–May of the years 2010, 2011 and 2013 in various wheat-producing areas of the country (Figure 1). In 2010, the survey took place on the West of the country and some localities of Eastern regions. In 2011, they were generalized in the Central region and some localities of the Western region. In 2013 the survey was particularly dedicated to the Eastern region. Surveys were realized between blooming and maturity stage of the wheat. The sampling was regularly made all 15 to 20 km through the cereal-producing regions. For every wheat field, the inspection was made according to the X-shaped method of Campbell and Madden (1990).

### Diagnostic and disease assessment in the field

Diagnosis of the disease on wheat is based on the observation of the typical symptom caused by *Z. tritici*. The SLB was identified by lengthened necrosis and bounded by the nervures (Sayoud et al., 1999; Duncan and Howard, 2000). Necrosis is very often lengthened, strewed with many pycnidia (Rapilly et al., 1971). Where disease symptoms are detected, an assessment was realized on 10 plants according to double digit scale (00-99) described by Eyal et al. (1987); the first digit represents the vertical progress of the disease according to the scale 0-9 of Saari and Prescott (Eyal et al., 1987); the second digit indicates the severity of the disease according to the scale 0-9 which every digit corresponds to a percentage of foliar surfaces covered by the disease.

### Morphological and cultural study *in vitro*

In laboratory, the diagnosis of the disease and the isolation of pathogen were realized from small fragments of limbs presenting characteristic pycnidia according to Eyal et al. (1987). The isolations were made only on 25 fields, from the year 2013, in East region of country (Annaba [01 field], Sétif [03 field], Constantine [18 field] and Mila [03 field]) (Table 1). Five infected leaves were randomly sampled from every field. From a lesion, five isolates were randomly retained after isolation (a lesion by leaf). In all, 625 isolates were retained for the morphologic and cultural characterizations; among them, 26 isolates were tested for the pathogenic characterization (Table 1). Morphological characterization of the *Z. tritici* isolates was realized according to tint scale described by Siah et al. (2008).

### Evaluation of isolates aggressiveness variability

Pathogenicity test was led under greenhouse according to a plan in split plot with three repetitions. 26 isolates sampled from 25 fields (Table 2) were tested on a differential range of 16 varieties (five Bread wheat [Ain Abid, Arz, Anapo, Anforeta and HD1220], ten Durum wheat [Boussalem, Cirta, Colosseo, Cote, GTA-Dur, Ofanto, Simeto, Vitron, Waha et Wahebi] and one Triticale [Juanillo]) approved in Algeria.

Inoculum was prepared from 7 days old *Z. tritici* cultured in 18°C on YMA medium. The conidial suspension was adjusted to $10^8$ spore $ml^{-1}$, adding to it a droplet of Tween 20. The inoculation was executed by pulverizing the conidial suspension at seedling stage (3 leaves) and the humidity was maintained during 48 h according to the modified method described by Zuckerman et al. (1997). After 21 days, the number of infected leaves (NIL) was estimated on the first three leaves and the percentage of *Z. tritici* pycnidial covering (%PC) was estimated according to the scale described by Ziv and Eyal (1978). Statistical analyses were made by variance analysis (ANOVA) and hierarchical classification in dendrogram.

## RESULTS AND DISCUSSION

### Prevalence of septoria leaf blotch in Algeria

Characteristic symptoms of SLB observed on fields were necrosis more or less lengthened, which can cover, in certain cases, the majority of the foliar surface and strewed with pycnidia, the percentage of covering was variable. During the 2010 campaign, 12 fields from 18 (66.67%) presented typical symptoms of the SLB. For 2011 and 2013 campaigns, in the 104 prospected fields, the disease was present in 60 fields (57.67%). On a total of 122 prospected fields, during three years, 72 fields present the SLB disease (Table 2 and Figure 1), where 59% of the prospected fields were infected. The SLB is present in the majority of wheat-producing areas.

It is admitted that the development of SLB diseases is bound to weather conditions, particularly, humidity and temperature. Disease severity in sub-humid regions was particularly observed. In the counties of Algiers, Annaba and Blida the severity of the disease reached 98 and 99

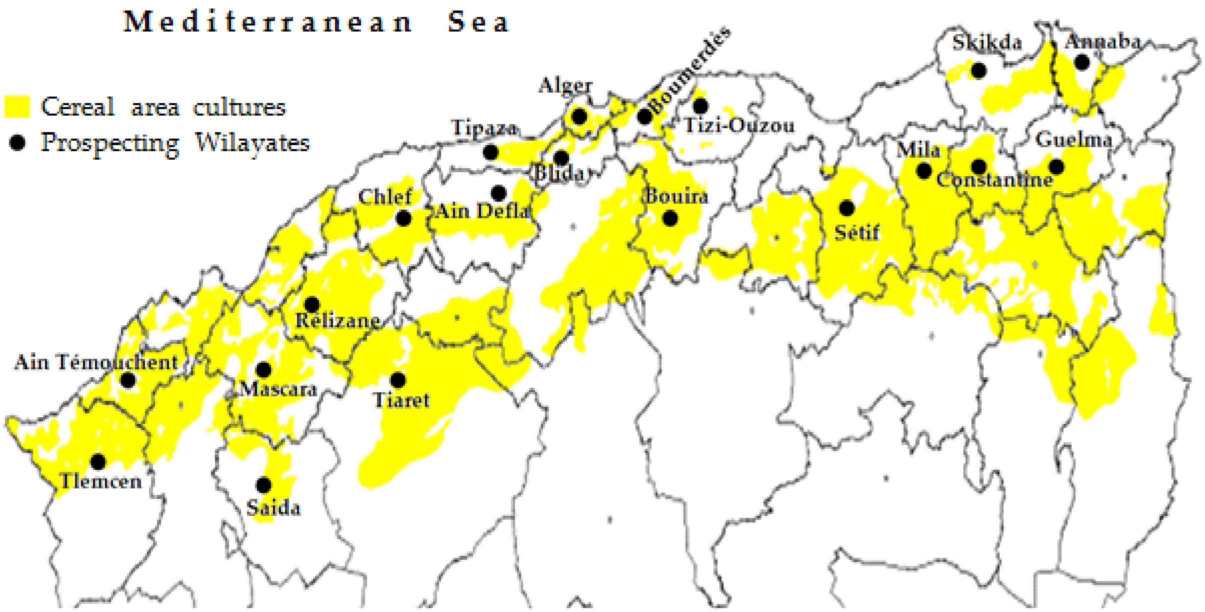


**Figure 1.** Wheat-producing areas.
Source: Algerian Ministry of Agriculture, Rural Development and Fisheries.

**Table 1.** Provenance of *Zymoseptoria tritici* isolates.

| Isolate code | Wilayats of provenance | Source isolate host |
|---|---|---|
| St1 | Constantine | Durum wheat (GTA-Dur) |
| St2 | Constantine | Bred wheat (HD1220) |
| St3 | Constantine | Durum wheat(Vitron) |
| St4 | Constantine | Durum wheat |
| St5 | Constantine | Durum wheat |
| St6 | Constantine | Bred wheat |
| St7 | Constantine | Durum wheat (Cirta) |
| St8 | Mila | Bred wheat |
| St9 | Mila | Durum wheat (GTA-Dur) |
| St10 | Sétif | Durum wheat |
| St11 | Constantine | Durum wheat (Gta-Dur) |
| St12 | Sétif | Durum wheat |
| St13 | Constantine | Durum wheat (Cirta) |
| St14 | Mila | Durum wheat |
| St15 | Sétif | Durum wheat |
| St16 | Mila | Bred wheat (Arz) |
| St17 | Mila | Durum wheat |
| St18 | Constantine | Bred wheat |
| St19 | Mila | Durum wheat |
| St20 | Mila | Durum wheat |
| St21 | Constantine | Bred wheat (HD1220) |
| St22 | Constantine | Durum wheat (Gta-Dur) |
| St23 | Constantine | Bred wheat |
| St24 | Constantine | Bred wheat |
| St25 | Constantine | Bred wheat |
| St26 | Annaba | Durum wheat (Gta-Dur) |

**Table 2.** Prevalence and severity of septoria leaf blotch of wheat in Algeria.

| Bioclimatic stage | Wilayats | Prospected fields between 2010-2013 | Infected fields with *Zymoseptoria tritici* | Double digit 00-99 (vertical progression and disease severity) |
|---|---|---|---|---|
| **Humid** | Skikda | 1 | 1 | 98 |
| Sub-humid | Ain Defla | 4 | 3 | 33 - 55 |
| | Alger | 6 | 5 | 53 - 99 |
| | Annaba | 1 | 1 | 98 |
| | Blida | 4 | 2 | 31 - 98 |
| | Boumerdès | 8 | 5 | 51 - 75 |
| | Guelma | 1 | 1 | 75 |
| | Mila | 14 | 7 | 75 |
| | Tipaza | 4 | 1 | 31 |
| | Tizi-Ouzou | 2 | 2 | 31 |
| Sub - Total 1 | | 44 | 27/44 (**61%**) | - |
| Semi-arid | Ain Témouchent | 6 | 4 | 53 - 98 |
| | Bouira | 6 | 2 | 11 - 31 |
| | Chlef | 4 | 2 | 31 - 73 |
| | Constantine | 30 | 22 | 75 |
| | Mascara | 5 | 1 | 11 |
| | Rélizane | 3 | 2 | 53 |
| | Saida | 2 | 1 | 11 |
| | Sétif | 10 | 3 | 51 - 53 |
| | Tiaret | 9 | 6 | 75 - 98 |
| | Tlemcen | 2 | 1 | 51 |
| Sub – Total 2 | | 77 | 44/77 **(57%)** | - |
| **Totals** | | **122 fields** | **72/122 fields** | **-** |

according to "double digit" scale because of climatic conditions (according to data from ONM). For example, during 2010 the annual pluviometry in Annaba was 596 mm, and that for the first four months was 237 mm, which represent period for the disease development.

The year 2011 was characterized by a sum of 673 mm pluviometry in Algiers, 314 mm registered during the first four months (ONM source) and the average temperatures between 17 and 20°C; the meteorological conditions were favorable for the dissemination of SLB. However, we also noticed that in some Wilayate of the semi-arid regions, particularly, Constantine and Tiaret, where an important severity of the disease was observed. It can be explained by the special agricultural features of Algeria, where the cereals cultivation is in intense monoculture system in semi-arid regions rather than diversified as cultivated in the sub-humid regions.

In the regions where the drought caused damage during the 2011 campaign, the disease was observed only on the first leaves. Indeed, the results indicate that the severity of the disease does not exceed 11 according to the "double digit" scale. It was the case of Saïda and Mascara where the sum of precipitation of the first four months did not exceed 130 mm (ONM source). According to Danon et al. (1982) and Cowger et al. (2000), the SLB engenders major losses of yields, in particular, when the spring rains persist, after the emergence of the flag leaf. These losses vary with weather conditions, cultivated varieties and precocity of attacks (Devale et al., 2000).

## Morphologic and cultural characterizations

Phenotypic observations, of colonies stemming, from isolates of *Z. tritici* of 10 days on solid YMA (Yeast Malt Agar) medium show a big diversity of texture and color. The isolates of pinkish color have a creamy texture (Yeast Like), which can cover completely the culture medium or in the form of colonies which follow the lines of sowing. The isolates of dark color are solid and compact. It is noticed that the pink color is the most dominant (darkened 42.88%, clear 24.16% and very clear 27.04%), whereas the dark brown occupied 5.92% group cultures. These results corresponding to those of Bentata et al. (2011) and Ayad et al. (2014) which realized a cultural characterization of the Moroccan and Algerian isolates of *Z. tritici*; also, those of Siah et al. (2008) on the distribution of “mating type” according to the colonies phenotype. According to Quaedvlieg et al., (2011) variants of *Z. tritici* can appear on culture medium.

**Table 3.** Classification in homogeneous groups of the 26 studied isolates of *Z. tritici* according to their aggressiveness.

| ***Z. tritici* isolate** | **NIL** | **%PC** |
|---|---|---|
| ST1 | 0.03[cde] | 1.87[f] |
| ST2 | 0.11[cde] | 8.75[bcdef] |
| ST3 | 0.02[de] | 4.37[def] |
| ST4 | 0.27[abc] | 21.25[ab] |
| ST5 | 0.10[cde] | 5.93[cdef] |
| ST6 | 0.22[abcde] | 10.62[abcdef] |
| ST7 | 0.27[abc] | 17.50[abcd] |
| ST8 | 0.02[de] | 0.31[f] |
| ST9 | **0.39[a]** | 16.87[abcde] |
| ST10 | 0.01[e] | 1.25[f] |
| ST11 | 0.26[abcd] | 10.62[abcdef] |
| ST12 | 0.00[e] | 0.00[f] |
| ST13 | 0.04[cde] | 1.56[f] |
| ST14 | 0.06[cde] | 8.75[bcdef] |
| ST15 | 0.02[de] | 2.50[f] |
| ST16 | 0.02[de] | 3.43[ef] |
| ST17 | 0.37[ab] | **22.5[a]** |
| ST18 | 0.04[cde] | 3.12[f] |
| ST19 | 0.22[abcde] | 18.43[abc] |
| ST20 | 0.13[bcde] | 9.37[abcdef] |
| ST21 | **0.41[a]** | 21.25[ab] |
| ST22 | 0.13[bcde] | 11.87[abcdef] |
| ST23 | 0.02[de] | 4.37[def] |
| ST24 | 0.01[e] | 1.25[f] |
| ST25 | 0.01[e] | 1.25[f] |
| ST26 | 0.03[cde] | 3.75[ef] |

**NIL:** Number of infected leaves; **PC%**: Pycnidial covering. Letters in superscript represent statistically different homogeneous groups.

**Isolates aggressiveness and evaluation of the varietal assessment**

Variance analysis of the infected leaves number (NIL) and of the pycnidial coverage percentage (%PC) of the 26 isolates tested on the differential set varieties shows a very highly significant difference for both parameters (Table 3). Five pathotypes were distinguished for NIL parameter. The most virulent isolates, according to this parameter, were ST9, ST17 and ST21. For the %PC, six pathotypes were detected. The most virulent isolates, according to this parameter, were ST4, ST17 and ST21 (Figure 2). We noticed that the area from where the isolates are sampled did not influence systematically the level of aggressiveness. Both isolates ST19 and ST20 arise from the same field and have a different behavior towards the studied wheat and triticale varieties. However, isolates ST17 and ST21 were obtained from two different fields and belong to the same pathotype. From 26, 10 isolates showed a physiological specificity for the Bread wheat or the Durum wheat, only ST9 and ST20 presented symptoms on three studied host species.

Statistical study of the 16 host varieties (Durum wheat, Bread wheat and Triticale) comportment (resistance or sensibility) towards the range of isolates showed a very highly significant difference. Four homogeneous groups for both parameters ILN and %PC are observed. The most sensitive varieties were HD1220 and Waha, whereas, the most resistant varieties, with no symptom were Ain Abid, Colosseo and Simeto (Figure 2; Table 4). A qualitative variation of *M. graminicola* virulence was indicated in certain studies (Eyal et al., 1973; Saadaoui, 1987; Kema et al., 1996). Brading et al. (2002) and Kema et al. (2000)'s works brought the proof of gene-for-gene relation between wheat and *M. graminicola*.

Medini and Hamza (2008) study showed that the Algerian isolates have more variability, with eight pathotypes, compared with the Tunisian and Canadian isolates. The strong variability of the Algerian isolates can be associated to the agricultural practices. Indeed, both wheat cultures are important, 24.3% for bread wheat and75.7% for durum wheat according to statistical data of

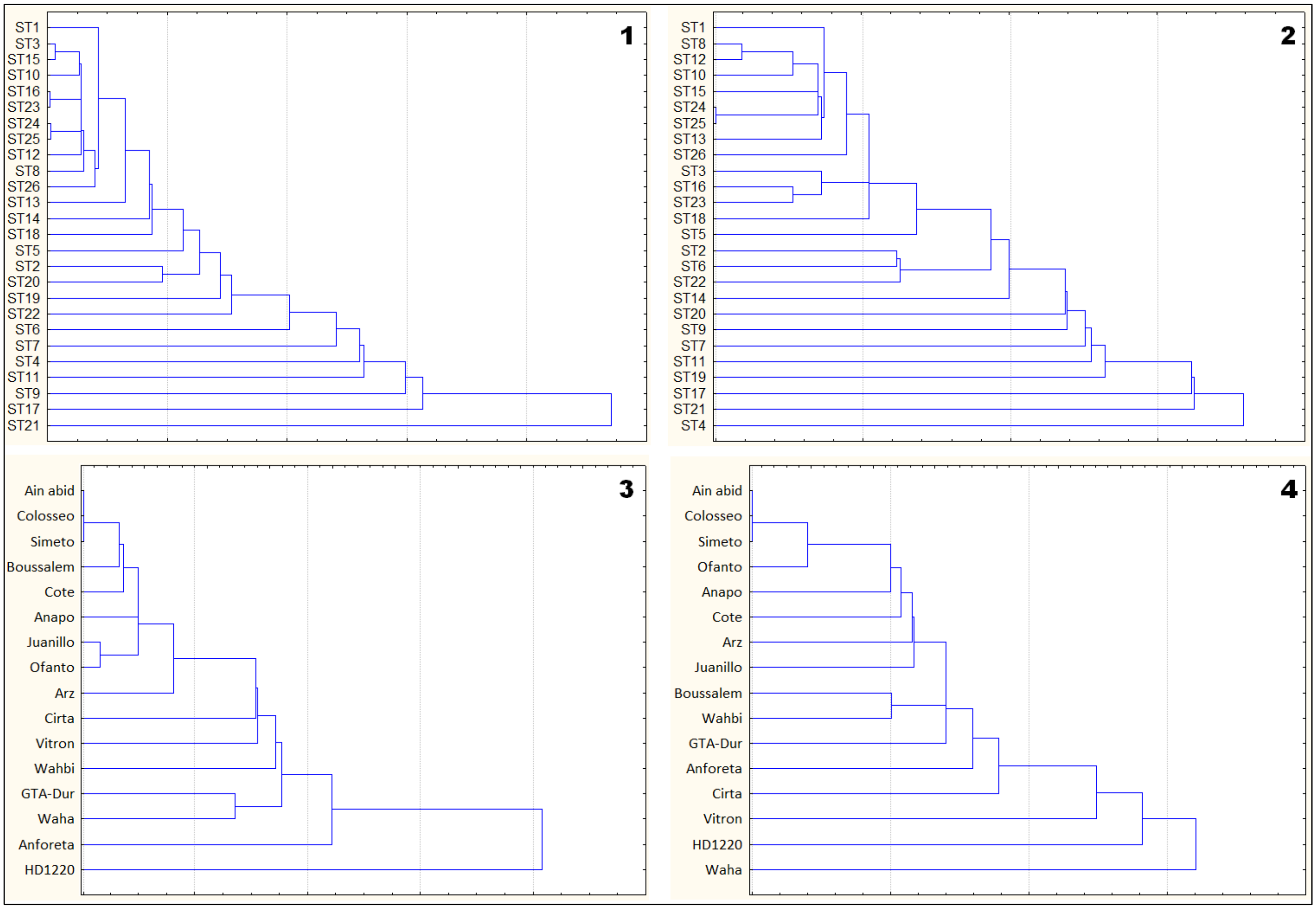


**Figure 2.** Hierarchical classification (Euclidean Distance).
1: Isolates × Infected Leaves Number per plant; 2: Isolates × Pycnidial Coverage Percentage
3: Varieties × Infected Leaves Number per plant; 4: Varieties × Pycnidial Coverage Percentage.

Ministry of Agriculture, Rural Development and Fisheries. Consequently, *M. graminicola*is is exposed to wide genotype groups having various sources of resistance genes. Kema et al. (1996) suggest the existence of two variants of *M. graminicola*, one adapted to the durum wheat and the other one to the bread wheat. The hypothesis of specificity existence in the pathosystem wheat – *M. graminicola* was emitted since the first report indicating a physiological specialization (Eyal et al., 1973).

According to several authors, the isolates of *Z. tritici* obtained from tetraploids wheat show a bigger virulence on wheat whether it was tetraploids or hexaploids (Kema et al., 1996; Van Ginkel and Scharen, 1988). It was the case of the ST21 of our study. Nevertheless, among the most virulent isolates of the tested range (ST17, ST9 and ST4) were obtained from durum wheat. Shaner and Finney (1982) identified the varietal resistance with *Z. tritici*. More than 12 main genes conferring to the host high levels of resistance were identified. Most of them were mapped in wheat genome specific regions (McCartney et al., 2002; Adhikari et al., 2004; Chartrain et al., 2005; Arraiano et al., 2007).

## CONCLUSION

Wheat septoria leaf blotch caused by *M. graminicola* is a disease, present in all the country cereal zones, of both cultivated wheat species (Durum wheat and Bread wheat). According to the importance of attacks, this disease can engender considerable yield losses, in particular, when weather conditions were favorable for pathogen development. Severity of the SLB was more significant on the regions where weather conditions were favorable and the monoculture was widely practiced. Results indicate a big phenotypic variability of the obtained colonies. The 26 tested isolates, of East Algeria

**Table 4.** Classification of homogeneous groups representing the 16 varieties sensitivity of Durum, Bred wheat and Triticale.

| Cereal species | Varieties | NIL | PC% |
|---|---|---|---|
| Bred wheat | Ain abid | $0.00^{d}$ | $0.00^{d}$ |
| | Arz (Beni Slimane) | $0.07^{cd}$ | $5.00^{cd}$ |
| | Anapo | $0.01^{d}$ | $1.92^{cd}$ |
| | Anforeta | $0.16^{bcd}$ | $6.53^{cd}$ |
| | HD1220 (Hiddab) | $\mathbf{0.62^{a}}$ | $\mathbf{32.88^{a}}$ |
| Durum wheat | Boussalem | $0.01^{d}$ | $2.69^{cd}$ |
| | Cirta | $0.17^{bcd}$ | $9.03^{cd}$ |
| | Colosseo | $0.00^{d}$ | $0.00^{d}$ |
| | Core | $0.02^{d}$ | $2.69^{cd}$ |
| | GTA-Dur | $0.27^{b}$ | $10.96^{d}$ |
| | Ofanto | $0.01^{d}$ | $0.76^{d}$ |
| | Simeto (sersou) | $0.00^{d}$ | $0.00^{d}$ |
| | Vitron (hoggar) | $0.22^{bc}$ | $21.34^{b}$ |
| | Waha | $0.27^{b}$ | $28.46^{ab}$ |
| | Wahbi | $0.11^{bcd}$ | $4.80^{cd}$ |
| **Triticale** | Juanillo (Chelia) | $0.02^{d}$ | $3.84^{cd}$ |

Letters in superscript represent statistically different homogeneous groups.

wheat-producing region, show a big variability towards the studied wheat and triticale varieties. Five pathotypes were distinguished for the infected leaves number parameter and six pathotypes for pycnidial coverage parameter. Some isolates have physiological specialization towards hosts. It would be interesting to include the varieties, which have proved resistant characters to this disease, in future wheat improvement program. For a better knowledge of pathogen, it would be useful to study more isolates by molecular markers.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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