

## PATHOTYPE AND MOLECULAR CHARACTERIZATION OF *MYCOSPHAERELLA GRAMINICOLA* ISOLATES COLLECTED FROM TUNISIA, ALGERIA, AND CANADA

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### SUMMARY

Septoria leaf blotch, caused by *Mycosphaerella graminicola*, is one of the most devastating diseases of wheat worldwide. Forty two isolates of *M. graminicola*, collected from Tunisia, Algeria, and western Canada were characterized according to their virulence behavior and their molecular patterns. Virulence was assessed through the inoculation of four durum wheat and four bread wheat cultivars. Isolates were differentiated into eight pathotypes. Pathotype 1 and 2 were common to Algerian and Canadian isolates. Pathotype 7 was found in Tunisia and in Algeria. Pathotype 8 was specific to Tunisian isolates. Pathotypes 3, 4, 5 and 6 were found only in Algeria. The seven primer pairs used in amplified fragment length polymorphism (AFLP) analysis yielded a total of 194 polymorphic bands. Analysis of AFLP data revealed that genetic diversity within populations ( $H_S=0.315$ ) accounted for 87.5% of the total genetic diversity ( $H_T=0.360$ ). The proportion of the total genetic diversity attributable to the population differentiation ( $G_{ST}$ ) was 0.125, reflecting a moderate genetic differentiation between the three populations of *M. graminicola*. Tunisian and Canadian populations from the Old and the New World displayed almost equal levels of genetic diversity. Algerian population showed the highest genetic diversity possessing 5 private alleles which suggests that it could be a source population where diversity was maintained through cultivation of a broad spectrum of wheat cultivars. The hierarchical classification based on the AFLP analysis separated Tunisian and Canadian isolates whereas the Algerian isolates were distributed in all groups.

Key words: *Mycosphaerella graminicola*, wheat, virulence, AFLP, genetic variability.

### INTRODUCTION

Septoria leaf blotch is a major disease of bread and durum wheat (*Triticum aestivum* and *T. durum* subsp.

*durum*, respectively) in all wheat-growing areas of the world. Under favourable conditions, this disease, caused by *Mycosphaerella graminicola* (Fuckel) J. Schröt. in Cohn (anamorph: *Septoria tritici* Roberge in Desmaz.), can cause yield losses from 25 to 50% in susceptible wheat cultivars (King *et al.*, 1983). The sexual form of the fungus has been reported from many parts of the world (Garcia and Marshall 1992; Sanderson 1972; Scott *et al.*, 1988).

The existence of specificity in the wheat-*M. graminicola* pathosystem has been hypothesized since the first report of physiological specialization (Eyal *et al.*, 1973). The most obvious differential interaction reported so far occurred between bread and durum wheat and fungal isolates obtained from these species. Kema *et al.* (1996) found a highly significant interaction within each of these systems through analysis of restricted data matrices, either for bread wheat cultivars and bread wheat-adapted *M. graminicola* isolates, or durum wheat cultivars and durum wheat-adapted isolates. Recently, gene-for-gene interaction within the bread wheat system enabled the analysis of genetic control of avirulence (Kema *et al.*, 2000) and mapping of the avirulence gene (Kema *et al.*, 2002). Furthermore, isolates of *M. graminicola* from Western Canada were differentiated into two races based on the reaction of the hexaploid wheat line 'ST6' (Grieger *et al.*, 2005).

The genetic structure of *M. graminicola* populations has been studied for over decade (Zhan *et al.*, 2003; Jurgens *et al.*, 2006). Through the use of molecular techniques (RFLP, DNA fingerprints, and DNA sequencing), much knowledge has accumulated on the genetic structure of the populations and evolutionary potential of this fungus (Boeger *et al.*, 1993; Chen *et al.*, 1994; McDonald *et al.*, 1996; Zhan *et al.*, 1998, 2000, 2001, 2002a; Banke and McDonald, 2004). Moreover, the relative significance of each evolutionary force on population dynamics has been evaluated (Zhan and McDonald 2004; Banke and Mc Donald, 2005). However, most of these studies analyzed molecular polymorphism without considering the *M. graminicola*-wheat pathosystem. Recently, Zhan *et al.* (2005) compared the genetic variation and population differentiation at RFLP marker loci with quantitative characters including fungicide resist-

ance, pycnidial density, pycnidia colony size, percentage of leaves covered by lesions and percentage of leaves covered by pycnidia. The authors found a positive and significant correlation between genetic variation in RFLP loci and quantitative traits at the multitrait scale.

Understanding the genetic variability and the virulence pattern of the pathogen's populations represents a prerequisite for the development of a rational strategy of resistance gene deployment. A traditional pathogen race survey should be based on the differential reactions of several varieties, thereby generating detailed pictures of the virulence structure. This kind of analysis only reveals pathogen characters related to the host selection pressure on the pathogen populations (Kolmer *et al.*, 1995), but remains the ultimate reference of virulence analysis.

The objective of our study was to analyze and compare the pathotype and genetic variability of *M. graminicola* isolates collected from North Africa (Tunisia and Algeria) and Manitoba (Canada) which are considered to be part of the Old and New World areas, respectively. In North Africa, wheat has been grown for thousands of years, but in Canada for only hundreds of years. In addition, different agro-ecosystems are adopted in these countries and different wheat types are grown. Bread wheat is widely grown in Manitoba, whereas in Tunisia durum wheat represents the major wheat type. In Algeria durum and bread wheat are equally distributed. This study, which combines the analysis of virulence and molecular polymorphism in relation to the cultivated wheat type, was aimed at determining whether the distribution of the different wheat type and cultivars could influence the pathotype variability and genetic diversity of *M. graminicola* in these countries.

## MATERIALS AND METHODS

**Fungal isolates.** Infected wheat leaf tissues were collected randomly in 2000 from different wheat fields at North Africa (Tunisia and Algeria) and Canada (Manitoba), representing two geographically distinct regions which vary considerably in climate and agricultural practices (Table 1). Tunisia and Algeria share the same climate but differ considerably in agricultural practices. *M. graminicola* isolates were recovered from bread and durum wheat cultivars. In total, 13 single-spore isolates were obtained from Tunisia, 15 from Algeria, and 14 from Canada (Table 1). Inoculum was prepared by incubating 50 ml of liquid yeast-glucose medium in 100 ml Erlenmeyer flasks with fresh *M. graminicola* colonies from PDA plates. Flasks were incubated for 7 days in a shaker at room temperature. The resultant spore suspension was pelleted by centrifuging at 10 000 rpm for 10 min and used for virulence assessment and molecular analysis. Spores were stored for long term in glycerol at -80°C.

**Plant materials.** Four hexaploid wheat cultivars (Salamouni, Erik, ST6, and Amazon) and four tetraploid cultivars/lines (Coulter, Scepter, 4B160, and 4B1149) were used to differentiate *M. graminicola* virulence types (McCartney *et al.*, 2002; Greiger *et al.*, 2005). The experiment was conducted in three replicates. In each replicate, 10 seeds of each variety were sown in clay pots (15 cm in diameter) filled with 2:1:1 (soil/sand/peat) soil mix. Seedlings were maintained in a controlled climate chamber at 22/18°C (day/night) with a 16 h photoperiod at a light intensity of 250  $\mu\text{E}/\text{m}^2/\text{s}$ . Plants were fertilized weekly and watered as required. In each pot, five uniform seedlings at the growth stage (GS) 21 (Zadocks *et al.*, 1974) were retained for inoculation.

**Inoculation.** Inoculation experiments were conducted as described by Greiger *et al.* (2005). Due to limited space in the growth chamber, inoculation was partitioned into 11 experiments. Each experiment consisted on the inoculation with a maximum of 5 isolates including the isolate MG2 (a representative of race 2 found in Canada; McCartney *et al.*, 2002) as a control to test the reproducibility between experiments. The isolate MG2 showed similar virulence pattern between experiments. In fact, it always attacked cvs Erik, Amazon and 4B1149. A set of non inoculated differential varieties was added in each experiment. Spores of each isolate were resuspended in deionized water, adjusted to a density of  $10^7$  conidia/ml and treated with two drops of Tween 20 per 50 ml of inoculum. The suspension was sprayed on each pot until run-off using a DeVilbiss-type sprayer connected to an air line at a pressure of approximately 67 Kpa. The sprayer, hoses, and benches were thoroughly cleaned with 75% alcohol and rinsed with sterile distilled water between sprays of different isolates. After inoculation, seedlings were allowed to air-dry before being placed for 72 h under mist in a chamber consisting of a polyvinyl frame [1.75 m x 1 m x 1 m (length x width x height)] covered with a clear plastic and located in a walk-in growth room. Continuous leaf wetness was provided by two ultrasonic humidifiers mounted on each side of the chamber and filled with distilled water. To prevent cross-contamination of isolates in the chamber, plastic boards were used to separate each set of wheat genotypes inoculated with the same isolate. Pots were distributed randomly within each set and sets were placed randomly in the chamber. Plants were then allowed to dry, removed from the chamber, and placed on a walk-in growth room bench at 21/19°C (day/night) with a 16 h photoperiod (390  $\mu\text{E}/\text{m}^2/\text{s}$ ) and relative humidity between 70 and 80%. Plants were fertilized weekly with a 20-20-20 soluble fertilizer solution (5  $\text{gl}^{-1}$ ) given with a watering can.

**Disease assessment.** Plants were evaluated for reac-

**Table 1.** Code, origin, and pathotypes of *M. graminicola* isolates.

Isolate	Pathotype	Country	Location	Host	Isolate	Pathotype	Country	Location/reference	Host
Alg1-1	2	Algeria	Azzaba	Bread wheat	Tun64-5	7	Tunisia	Beja	Durum wheat
Alg1-5	2	Algeria	Azzaba	Bread wheat	Tun66-1	8	Tunisia	Ksar Mezouar	Durum wheat
Alg1-6	1	Algeria	Azzaba	Bread wheat	Tun66-3	8	Tunisia	Ksar Mezouar	Durum wheat
Alg1-8	2	Algeria	Azzaba	Bread wheat	Tun66-5	8	Tunisia	Ksar Mezouar	Durum wheat
Alg2	7	Algeria	Azzaba	Bread wheat	Tun66-7	8	Tunisia	Ksar Mezouar	Durum wheat
Alg3-1	5	Algeria	Ras Djamel	Bread wheat	Tun69-1	7	Tunisia	Ksar Mezouar	Durum wheat
Alg3-2	6	Algeria	Ras Djamel	Bread wheat	Tun69-5	7	Tunisia	Ksar Mezouar	Durum wheat
Alg3-3	3	Algeria	Ras Djamel	Bread wheat	Can1	2	Canada	Manitoba/ Greiger et al.2005	Bread wheat
Alg4	7	Algeria	Ras Djamel	Durum wheat	Can2	1	Canada	Manitoba/ Greiger et al.2005	Bread wheat
Alg5	2	Algeria	Ras Djamel	Bread wheat	Can3	1	Canada	Manitoba/ Greiger et al.2005	Bread wheat
Alg6	7	Algeria	El Hassar	Durum wheat	Can4	1	Canada	Manitoba/ Greiger et al.2005	Bread wheat
Alg8	2	Algeria	Annaba	Bread wheat	Can5	1	Canada	Manitoba/ Greiger et al.2005	Bread wheat
Alg9	2	Algeria	El Hadjar	Bread wheat	Can6	2	Canada	Manitoba/ Greiger et al.2005	Bread wheat
Alg10	1	Algeria	Vitron	Durum wheat	Can7	1	Canada	Manitoba/ Greiger et al.2005	Bread wheat
Alg15	4	Algeria	Vitron	Durum wheat	Can8	2	Canada	Manitoba/ Greiger et al.2005	Bread wheat
Tun22-3	7	Tunisia	Siliana	Durum wheat	Can9	1	Canada	Manitoba/ Greiger et al.2005	Bread wheat
Tun26-4	7	Tunisia	Mornag	Durum wheat	Can10	2	Canada	Manitoba/ Greiger et al.2005	Bread wheat
Tun33-1	7	Tunisia	Solimane	Durum wheat	Can11	2	Canada	Manitoba/ Greiger et al.2005	Bread wheat
Tun61-5	7	Tunisia	Oued Zarga	Durum wheat	Can12	2	Canada	Manitoba/ Greiger et al.2005	Bread wheat
Tun61-7	7	Tunisia	Oued Zarga	Durum wheat	Can13	2	Canada	Manitoba/ Greiger et al.2005	Bread wheat
Tun62-3	7	Tunisia	Beja	Durum wheat	Can14	1	Canada	Manitoba/ Greiger et al.2005	Bread wheat

tion to *M. graminicola* isolates at 17 days after inoculation, i.e. when differentiation between the reactions of resistant and susceptible controls is maximum. Pathotypes were classified based on a 0-5 rating scale developed by Rosielle (1972) and slightly modified by McCartney *et al.* (2002), where 0 to 2 values were considered as resistance and 3 to 5 values were considered as susceptibility.

**DNA extraction.** Mycelia of *M. graminicola* isolates were collected by vacuum filtration on sterile Whatman filter discs and washed thoroughly with 2 vol. of sterile water. Samples were then frozen in liquid nitrogen and ground to a fine powder with mortar and pestle. DNA was then extracted with a Wizard genomic DNA Purification Kit from Promega (Promega, Madison, WI, USA). The DNA concentration was estimated by fluorimetry and by visual comparison with known concentrations of standard bacteriophage lambda DNA by agarose gel electrophoresis.

**AFLP analysis.** AFLP analysis was conducted according to a method modified from Vos *et al.* (1995). The AFLP protocol consisted of digestion of total genomic DNA with the restriction enzymes *EcoRI* and *MseI*. Adapter molecules specific to the restriction fragment ends were then ligated to the mixture of restriction fragments. The adapter molecules and nucleotides adjacent to the site of ligation served as a template for the annealing of specific oligonucleotide primers used for the polymerase chain reaction (PCR). Each fragment may differ internally in the nucleotides immediately adjacent to the restriction sites, and this property is the ba-

sis of the selective amplification characteristic of the AFLP method. An initial amplification was carried out with one selective nucleotide, in this case, C for *EcoRI* and A for *MseI*. The preamplification step was followed by a second selective amplification step with two selective nucleotides. Adapters, non-selective primers and selective primers are listed in Table 2. PCR products were visualized after electrophoresis on a 6% polyacrylamide sequencing gel using a silver staining method as described by Pillen *et al.* (2000). Dried gels were visually scored and scanned for records.

**Data analysis.** All AFLP fragments were scored as one putative locus with two alleles, one allele indicating the presence of a fragment (1) and the other indicating its absence (0). The data sets were compiled as a matrix of isolates and AFLP fragments. The allelic diversity at each locus was calculated as  $h = 1 - \sum p_i^2$ , where  $p_i$  is the frequency of  $i^{\text{th}}$  allele (Nei, 1973). Allelic diversities within each population were the mean allelic diversities over all 194 loci. Nei's  $G_{ST}$  was used as measure of genetic differentiation.  $G_{ST}$  was calculated according to the following formula (Nei 1987):

$$G_{ST} = (H_T - H_S) / H_T$$

where  $H_T$  represents the total gene diversity and  $H_S$  represents the within-population gene diversity. All genetic parameters were corrected for small, unequal sample sizes according to Nei and Chesser (1983).

A similarity matrix was constructed based on the Dice coefficient (Dice, 1945) using the SIMQUAL program in the NTSYS-pc software package (version 2.1; Exeter Software, Setauket, NY, USA). Cluster analysis was done using the unweighted pair-group method with arithmetic averages (UPGMA) using the SAHN program in NTSYS-pc 2.1 (Rohlf, 2000).

**Table 2.** Oligonucleotide sequences used in the AFLP analysis.

Oligonucleotide	Sequence
Adapters	
<i>EcoRI</i>	
Forward adapter	5' CTC GTA GAC TGC GTA CC 3'
Reverse adapter	5' AAT TGG TAC GCA GTC TAC 3'
<i>MseI</i>	
Forward adapter	5' GAC GAT GAG TCC TGA G 3'
Reverse adapter	5' TAC TCA GGA CTC AT 3'
Non-selective primers	
<i>EcoRI</i> -C	5' GAC TGC GTA CCA ATT CC 3'
<i>MseI</i> -A	5' GAT GAG TCC TGA GTA AA 3'
Selective primers	
<i>EcoRI</i> -CA	5' GAC TGC GTA CCA ATT CCA 3'
<i>EcoRI</i> -CT	5' GAC TGC GTA CCA ATT CCT 3'
<i>EcoRI</i> -CC	5' GAC TGC GTA CCA ATT CCC 3'
<i>MseI</i> -AA	5' GAT GAG TCC TGA GTA AAA 3'
<i>MseI</i> -AT	5' GAT GAG TCC TGA GTA AAT 3'
<i>MseI</i> -AC	5' GAT GAG TCC TGA GTA AAC 3'
<i>MseI</i> -AG	5' GAT GAG TCC TGA GTA AAG 3'

## RESULTS

**Virulence assessment.** The reactions of the differential lines to the different pathotypes are summarized in Table 3. The eight wheat genotypes differentiated the 42 isolates of *M. graminicola* into 8 pathotypes that differed from each other by the reaction to at least one of the genotypes. The Canadian isolates were virulent on bread and durum wheat cultivars and were differentiated by the line ST6 onto two pathotypes, referred to as pathotype 2 and pathotype 1 (Table 3). The seven pathotype 2 isolates were avirulent on ST6, whereas the remaining isolates were virulent on this wheat cultivar and were attributed to pathotype 1 (Greiger *et al.*, 2005). The Tunisian isolates were virulent only on durum wheat cultivars and were differentiated onto two pathotypes, 7 and 8. Both pathotypes were virulent on Scepter and Coulter, whereas pathotype 8 isolates were

**Table 3.** Reaction of wheat differential lines to the different pathotypes of *M. graminicola*.

Cultivar	Pathotype							
	1	2	3	4	5	6	7	8
Erik	+	+	+	+	+	+	-	-
4B1149	+	+	+	+	+	+	-	-
Amazon	+	+	-	+	+	+	-	-
ST6	+	-	+	+	+	-	-	-
Sceptre	-	-	-	+	+	-	+	+
Coulter	-	-	-	-	+	+	+	+
4B160	-	-	-	-	-	-	-	+
Salamouni	-	-	-	-	-	+	-	-

differentiated by their virulence on 4B160. Despite the low number of the Algerian isolates, they were characterized by their high pathotype variability. In fact, 7 of the 8 pathotypes reported in the present study were identified in the Algerian collection. In addition to pathotypes 1 and 2 found in Canada and pathotype 8 found in Tunisia, the Algerian population possessed four specific pathotypes (3, 4, 5, and 6). Pathotype 5, which was virulent on six wheat differentials (Erik, Amazon, ST6, 4B1149, Sceptre, and Coulter), was the most virulent. Pathotype 4 isolates were virulent on the same set of cultivars except for Coulter. Pathotype 3 isolates were virulent on Erik, 4B1149, and ST6. Pathotype 6 was virulent on five cultivars and was the only one virulent on Salamouni.

**AFLP analysis.** AFLP analysis was conducted on DNA prepared from the same set of *M. graminicola* isolates using seven primer pair combinations. The number of DNA fragments detected varied with the primer pair and the isolate used. A summary of the number of bands observed and the number of polymorphic bands obtained with each primer pair is presented in Table 4. The seven primer pair combinations yielded a total of 340 AFLP bands among which 194 (57%) were polymorphic. The primer pair *EcoRI-CT/MseI-AT* present-

**Table 4.** List of primer pair combinations and number of total and polymorphic bands and percentage of polymorphism obtained in the AFLP analysis.

Primer pair	Total bands (No.)	Polymorphic bands (No.)	Polymorphic bands (%)
<i>EcoRI-CA/MseI-AA</i>	43	25	58.1
<i>EcoRI-CA/MseI-AC</i>	36	11	30.5
<i>EcoRI-CT/MseI-AC</i>	40	27	67.5
<i>EcoRI-CA/MseI-AG</i>	32	18	56.2
<i>EcoRI-CT/MseI-AT</i>	88	53	60.2
<i>EcoRI-CC/MseI-AT</i>	53	33	62.2
<i>EcoRI-CT/MseI-AA</i>	48	27	56.2
Total	340	194	57.0

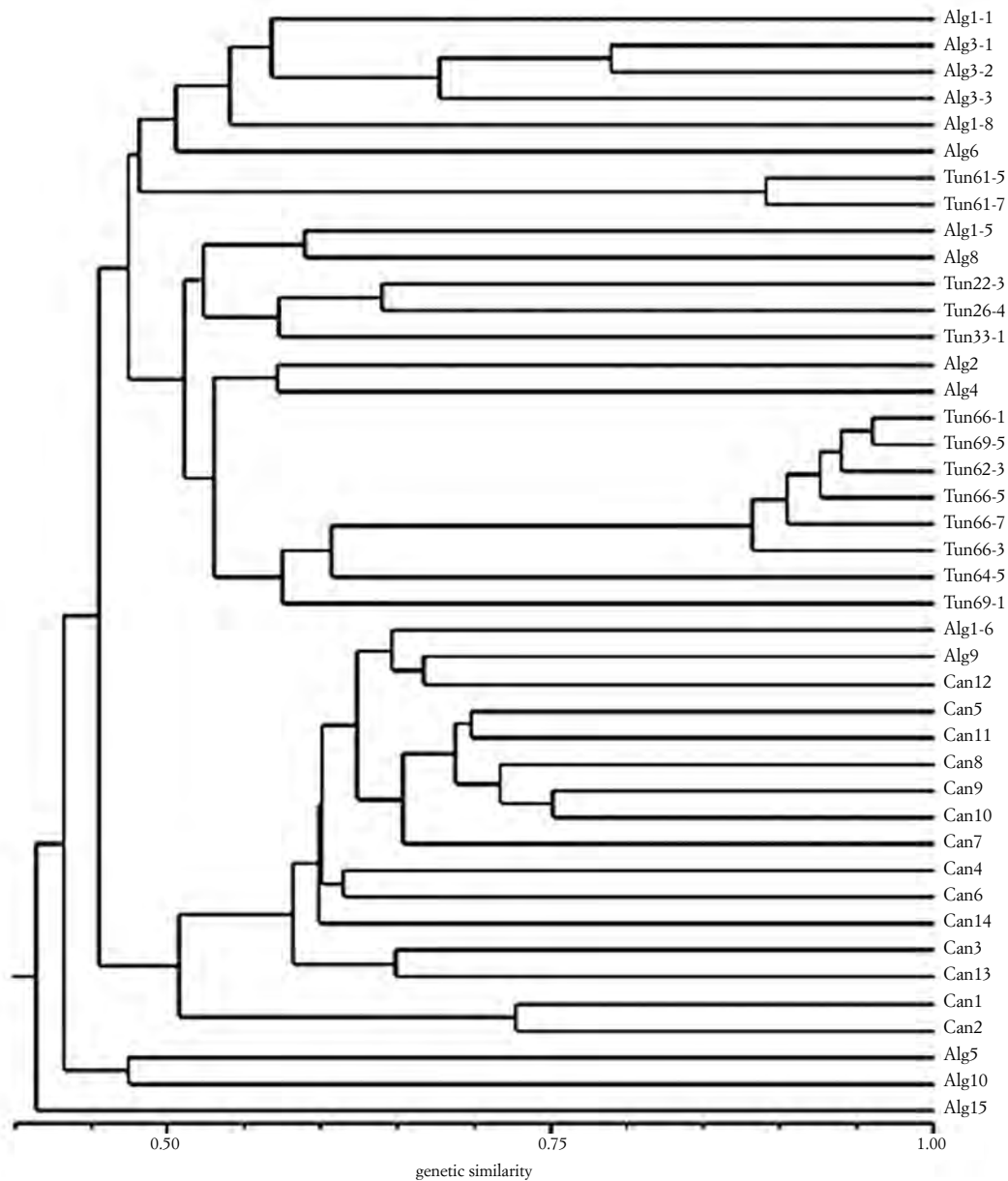
ed the highest number of total and polymorphic bands (88 and 53, respectively), while the primer pair *EcoRI-CT/MseI-AC* showed the highest percentage of polymorphic bands (67.5%). Allelic frequency for each population was determined considering that each polymorphic band was an allele. The Algerian isolates possessed the highest number of alleles (189), whereas Tunisian and Canadian isolates presented 167 and 164 alleles respectively. Moreover, the Algerian isolates presented 5 private alleles and shared 27 alleles with the Tunisian isolates and 22 alleles with the Canadian isolates. No private alleles were observed within Tunisian and Canadian isolates (Table 5).

The highest value of genetic diversity within populations ( $H_S = 0.338$ ) was obtained for the Algerian population, compared to the genetic diversity within Tunisian and Canadian populations which were almost equal (0.287 and 0.272 respectively, Table 6). Average  $H_S$  of the three populations was 0.315, accounting for 87.5% of the total genetic diversity ( $H_T = 0.360$ ). The proportion of the total genetic diversity attributed to the population differentiation ( $G_{ST}$ ) averaged 0.125, indicating a moderate genetic differentiation between populations.

Hierarchical classification based on UPGMA method clearly separated Tunisian and Canadian *M. graminicola* isolates. Algerian isolates were distributed into 4 groups; ten isolates were clustered in group I with the Tunisian isolates and two isolates in group II with Canadian isolates. The remaining isolates formed the group III and IV with two and one isolate respectively (Figure 1).

**Table 5.** Number of private and shared alleles between Algerian, Tunisian, and Canadian populations.

Populations	Private alleles (No.)	Shared alleles (No.)		
		Algeria	Tunisia	Canada
Algeria	5	189	27	22
Tunisia	0		167	13
Canada	0			164



**Fig. 1.** UPGMA cluster analysis based on AFLP data showing the genetic similarity among 42 isolates of *M. graminicola* collected from Tunisia, Algeria, and Canada (Manitoba).

## DISCUSSION

Eight pathotypes were identified in 42 *M. graminicola* isolates based on the reaction of eight wheat genotypes. These differential hosts were effective and appropriate for characterizing *M. graminicola* virulence types because they all gave clear reactions to the pathogen. This pathosystem showed specific interaction between isolate and cultivar suggesting a gene-for-gene interaction. Further efforts are needed to develop a differential set of wheat lines to survey *M. graminicola* populations for virulence/avirulence genes present in local *M. graminicola* populations. The construction of this differential set will require genetic studies to elucidate the re-

lationships between different reported resistance genes. Wilson (1985) identified three unlinked loci for septoria leaf blotch /STB/ resistance: *Stb1*, *Stb2*, and *Stb3*. Somasco *et al.* (1996) reported a fourth gene *Stb4* in the spring wheat cultivar Tadinia. Later, *Stb5* was mapped near the centromere of chromosome 7DS (Arraiano *et al.*, 2001; Simon *et al.*, 2001) and *Stb6* was mapped to the distal end of chromosome 3AS (Brading *et al.*, 2002). Recently, McCartney *et al.* (2003) identified a new gene *Stb7*, conferring resistance to one of the two races identified in western Canada. This gene is located at the distal end of chromosome 4AL. The *T. aestivum* line Salamouni has three additional *Stb* genes that are unlinked to *Stb7*, but their relationship to other *Stb*

**Table 6.** Genetic diversity within populations ( $H_s$ ), total genetic diversity ( $H_T$ ), and Genetic differentiation ( $G_{ST}$ ) between populations.

	$h_s$			$H_s$	$H_T$	$G_{ST}$
	Algerian population	Tunisian population	Canadian population			
All loci	0.338	0.277	0.263	0.315	0.360	0.125

genes still unknown (McCartney *et al.*, 2002).

Contrary to the Algerian and Canadian isolates, Tunisian isolates were specialized on durum wheat cultivars. The physiological specialization of *M. graminicola* on bread wheat and durum wheat was for a long time open to various speculations. Eyal *et al.* (1985) studied the virulence of 97 isolates of *M. graminicola* collected from 22 countries on 35 wheat varieties (28 varieties of common wheat, 4 varieties of durum wheat and 3 varieties of triticale). The authors presented good evidence for the existence of a division in the leaf blotch population, separating bread wheat from durum wheat isolates, concluding that in countries where durum wheat culture prevails, *M. graminicola* populations tend to show adaptation to durum wheat. This separation was confirmed by Kema *et al.* (1996), who showed differential interactions within each of these two populations. They even suggested the designation of two variants within *M. graminicola*, a variant adapted to durum wheat and the other adapted to bread wheat. However, comparison of the ribosomal DNA sequences of five bread wheat-derived isolates and five durum wheat-derived isolates revealed a perfect homology between the two variants (Hamza *et al.*, 1999). In addition, in the Middle East, where the two wheat species are grown and where *M. graminicola* can be found on seedlings of the spontaneous specie *Triticum turgidum* var. *dicoccoides*, the distinction between the two variants was not obvious (Eyal, 1999).

The main feature of the present study is the relative complexity of the pathotype composition of pathogen populations in Algeria since 7 of the 8 pathotypes recorded were from this country. One pathotype was common with Tunisian isolates; two pathotypes were also found in Canada and four pathotypes were unique to Algeria. The high variability of Algerian isolates may be related to agricultural practices used in this country (use of landraces, cultivation of bread and durum wheat in equal proportions). In contrast, the specialization of Tunisian isolates on durum wheat seems to be related to the predominance of this species and the use of few inbred cultivars that are genetically uniform (Medini *et al.*, 2005). In Tunisia, the area devoted to the durum wheat culture is considerably more important than that cultivated with bread wheat. In Algeria, the farming landscape is completely different. In fact, bread and durum wheat cultures share the same importance. Moreover, several landraces are still grown and occupy more

than 40% and 15% of the durum and bread wheat cultivated area, respectively (Ben Belkacem, personal communication). Consequently, *M. graminicola* is being exposed to a broad spectrum of host genotypes having various sources of resistance genes. This variability at the level of host plant may be at the origin of the high pathogen variability observed within Algerian isolates.

AFLP analysis revealed a high level of genetic diversity in populations of *M. graminicola* isolates. In fact, no clones were obtained and each isolate showed a unique haplotype. Algerian *M. graminicola* isolates displayed the highest level of genetic diversity. In particular, the population of the pathogen in Algeria was highly diverse as compared to the Canadian population. These results are in agreement with previous studies showing that ancestral populations of *M. graminicola* are likely to be present in the Old World areas (Zhan *et al.*, 2003; Banke and McDonald, 2004; Stukenbrock *et al.*, 2007). However, Tunisian and Canadian isolates shared the same level of genetic diversity notwithstanding that in Tunisia wheat has been grown for several thousands of years whereas in Canada only for hundreds of years. Therefore, genetic diversity would not rely only on the age of wheat cultivation, but the presence of various sources of resistance in Algeria could have contributed to the variability of the pathogen. In fact being exposed to a high selection pressure, the Algerian population has evolved to overcome host resistance (Zhan *et al.*, 2002b). The occurrence of sexual recombination in Algeria may have facilitated this evolution (Zhan *et al.*, 2003; 2007). In fact, several mechanisms can contribute to enhance genetic diversity and the main factor would be sexual recombination (Zhan *et al.*, 1998; 2000). Moreover, estimation of allelic frequencies for all 194 AFLP markers showed that the Algerian population shared 27 alleles with the Tunisian isolates and 22 alleles with the Canadian isolates. Five alleles were specific to the Algerian population whereas the two other populations did not manifest any specific alleles. These results suggest that the Algerian population may constitute a source population from where alleles migrated and were maintained in the recipient regions by natural selection (Zhan and McDonald, 2004). Therefore, we can emphasize that Algerian population has maintained its genetic diversity because of the cultivation of different sources of resistance whereas in the neighboring Tunisia genetic diversity was reduced due to host plant uniformity.

Working on four populations of *M. graminicola* from California, Oregon, Canada, and the Middle East, McDonald *et al.* (1995) found that the latter population was characterized by a high genetic diversity at the nuclear and mitochondrial DNA level. These authors noted the presence of a high number of private alleles on RFLP loci in this population, concluding that the Middle East would be a genetic center of diversity and a potential center of origin of *M. graminicola*. Later, Zhan *et al.* (2003)

completed the work on a collection of *M. graminicola* isolates from 11 countries. The authors reported that the populations originating from Algeria, Germany, India and the Middle East showed the greatest genetic diversity. In addition, analysis of the diversity at the mitochondrial DNA level showed that the 52 Algerian isolates tested provided 6 mitochondrial haplotypes, whereas the 160 Israeli isolates and the 31 Syrian isolates showed only 3 and 4 mitochondrial haplotypes, respectively.

Cluster analysis revealed the existence of high genetic differentiation between Tunisian and Canadian populations. The different genetic background of wheat cultivated in the two countries has probably led to the differentiation of their respective *M. graminicola* populations.

Breeding programs for resistance to *M. graminicola* in Tunisia should consider the high variability of virulence present in the neighboring countries. In fact, it was suggested that the high genetic variation found in this pathogenic fungus provide optimum conditions for the emergence of new virulence and/or fungicide resistance alleles. Regular sexual reproduction provides a mechanism for this pathogen to rapidly generate novel allele combinations while asexual reproduction, gene flow, and natural selection ensure the maintenance and rapid dissemination of allele combinations with highest fitness (Zhan and McDonald, 2004). Therefore, exclusive and widespread use of major resistance genes to control septoria leaf blotch is likely to lead to the rapid emergence of new virulent strains in the pathogen's population. The control of the disease caused by *M. graminicola* would be more efficient with the deployment of moderately resistant cultivars as the Tunisian cv. Nasr. Actually, Tunisian farmers are encouraged to cultivate this variety in order to ensure durable resistance (Gharbi *et al.*, 2000).

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