Review Article

Population Structure of Mycosphaerella graminicola and Location of Genes for Resistance to the Pathogen: Recent Advances in Argentina

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Leaf blotch of wheat (Septoria tritici Rob. ex Desm., teleomorph Mycosphaerella graminicola (Fückel) Schröt. in Cohn) causes significant losses in wheat. During the last decades studies about the genetic variability of the pathogen and location of the resistance have been intensive around the world. The knowledge about the genetic variation of M. graminicola is very important because it could allow us to determine which genotypes predominate within a geographic area. It also can be used to evaluate the germplasm resistance of wheat cultivars with isolates with high genetic differences. In addition, the knowledge of the genes conditioning resistance in different genotypes allows getting precise combination in new germplasm. The incorporation of the known genes in new cultivars could contribute to broadening the resistance to the pathogen. A paper about genetic variability of the pathogen and location of the resistance, with special emphasis in the work carried out in Argentina, is presented.

1. Importance and Biology of the Disease

Leaf blotch of wheat (Septoria tritici Rob. ex Desm., teleomorph Mycosphaerella graminicola (Fückel) Schröt. in Cohn) causes significant losses in wheat. In Argentina, yield losses from 21 to 37% [1], from 20 to 50% [2], and from 16 to 45% [3] have been found. In some other countries, yield reductions range from 31 to 54% [4], from 10 to 45% [5], and even reductions >60% have been reported [6].

Mycosphaerella graminicola is a hemibiotrophic pathogen; early infection is biotrophic, followed by a switch to necrotrophic growth just prior to symptom expression. The sexual stage is known to play a role in the disease cycle. It has been reported to cause most of the initial infection of winter wheat crops during the autumn in the UK [7] and in the USA [8]. An increase in ascospores at harvest time has been reported, suggesting that the sexual stage may be important to initiate the infection in the next growing season [9]. In Argentina, the sexual stage was also found [10].

Unburied crop residue is the major source or primary inoculum for Septoria tritici infecting wheat [8]. Ascospores are produced and released on this substrate [11]. Pseudothecia mature during winter and remain viable until early spring. Only 30 min of moistening stubble are necessary for ascospore release and dispersal [12, 13].

Different studies [9, 14] have confirmed that during spring and the beginning of summer, the severity of the epidemic was conditioned by pycnidiospores produced in the
crop; nevertheless, ascospores were present from the time the first basal leaves were infected [9]. Following stem elongation, infection of the upper leaves of a crop has been thought to be due entirely to the asexual stage of the fungus, in which pycnidia give rise to pycnidiospores, which are splash dispersed from infected basal tissue to the upper leaves by rain drops. However, more recent work has shown that upward movement of inoculum can occur in the absence of splashy rainfall, being influenced by the position of developing leaves in relation to infected leaf layers [15]. Another possible means of spread within a crop during summer is by air-borne ascospores, which may play a more important role than previously recognised [16]. It has been suggested [16] that airborne ascospores play a major role in the epidemiology of the disease during the growing season and, together with splash-dispersed spores, both have implications for the forecasting of the disease.

Recently [17], the relative abundance of M. graminicola ascospores and S. tritici conidia during two growing seasons have been quantified weekly in Argentina, establishing its relationship with weather variables (including rainfall, air temperature, relative humidity, and radiation). Pycnidiospores and ascospores were released during the entire growing cycle (June to December) in both growing seasons. However, their relative abundances depended on the time of the year and the weather conditions. Coincidentally with previous reports around the world, pycnidiospores were predominant during an important period of the crop cycle (from October or November until December) and ascospores predominated in an important part of the period after harvest when the stubble was lying on the ground and at the beginning of the crop cycle (June to September). However, there were some peaks, during which the predominance of the sexual or the asexual form was related to the environmental conditions, ascospores being less dependent on rainfall than pycnidiospores. Some other researchers [15, 16] also mentioned that ascospores are released at two peak times of the year: the first one establishes primary infections in newly sown wheat crops and the second one approximately coincides with the emergence of the upper two leaves. This makes it possible that infection occurs in the upper leaves without rainfall [15, 16].

2. Genetic Variability of the Mycosphaerella graminicola Population and Pathogenicity Test

The pathogen has a high variability, partially caused by the presence of both asexual and sexual reproduction. Genetic evidences [18–21] showed that sexual fruit bodies of M. graminicola undergo recombination both during and between growing seasons. Therefore, ascospores served as primary inoculum to initiate the epidemic of S. tritici leaf blotch, and they also contributed to secondary infection on the upper leaves during the growing season [18].

Consideration of genetic variation of M. graminicola population is essential to understand the virulence on the different cultivars. Differences in the population around the world could be attributed to variation in regular recombination, different migration patterns, and presence and importance of the sexual form. Sexual reproduction creates large numbers of genetically diverse isolates. Populations in this fungus are in genetic equilibrium as well as in drift migration equilibrium [22] attributed to a high rate of sexual recombination.

Genetic structure of M. graminicola populations have been studied over the last decade all around the world [21, 23]. Many studies using different molecular markers showed that there was a high level of genetic variability within them and that populations were composed of many different genotypes.

Czembor and Arseniuk [24] studied different species of Septoria (S. avenae f. sp. triticea; S. nodorum, and S. tritici) and found that SSR and ISSR markers were the most sensitive techniques for the detection of DNA polymorphisms. Extensive population genetic analyses of M. graminicola have also been conducted with RFLP markers [1]. Schneider et al. [23] used AFLP markers to analyse one population of M. graminicola from Germany. They observed high within-population diversity, and the significant migration between populations prevented genetic isolation and differentiation of putative geographically separated populations. Razavi and Hughes [25] worked on a total of 90 isolates of M. graminicola from western Canada using RAPD and they detected a high degree of DNA polymorphism with many different molecular phenotypes in this population. The genetic structure of the Kansas populations of M. graminicola was evaluated at different spatial scales (microplot, macroplot, and statewide) using AFLP. Genetic identities among populations were >98%. Tests for population subdivision revealed that 98% of the genetic variability occurred within populations [26]. In addition, Gurung et al. [27] determined that there was a small but statistically significant level of genetic differentiation between populations from spring versus winter wheat. Spring and winter wheat are exposed to differences in environmental conditions and resistance sources used in wheat breeding programs; however, most of the genetic variation (>98%) occurred within spring and winter wheat regions, while <2% was due to genetic differentiation between these regions. The authors assumed that those results indicated that sexual recombination occurs frequently in the M. graminicola populations and that most populations are genetically differentiated over the major spring and winter wheat growing regions of USA.

Medini and Hamza [28], using AFLP analysis, revealed a high level of genetic diversity in populations of M. graminicola isolates, no clones were obtained and each isolate showed a unique haplotype. Abrinbana et al. [29] found that five populations from five major wheat-growing provinces in Iran showed intermediate to high genotypic diversity. Low levels of gene flow and high genetic differentiation were observed among populations and different clustering methods revealed five genetically distinct groups in accordance with the sampling areas, indicating a population structure of the pathogen contrasting to that of most other countries studied.

Recently, Goodwin et al. [30] analysed a database of 30,137 EST (expressed sequence tag) sequences from M.
**graminicola** and identified 38 di- and 71 trinucleotide microsatellites with repeat numbers of six or more. Microsatellites that showed polymorphism between the parents of the **M. graminicola** mapping population were integrated into the existing genetic linkage map [31]. The EST database provided an excellent source of new, highly polymorphic microsatellite markers that can be multiplexed for high-throughput genetic analyses of **M. graminicola** and related species. The complete genome of *Mycosphaerella graminicola* was recently sequenced. It contains 21 chromosomes, eight of which could be lost with no visible effect on the fungus and thus are dispensable. This eight-chromosome dispensosome is dynamic in field and progeny isolates, is different from the core genome in gene and repeat content, and appears to have originated by ancient horizontal transfer from an unknown donor [32].

In Argentina, a first study of the **M. graminicola** population was conducted with a limited set of isolates of the pathogen from some areas using RFLP. This study showed that the pathogen has a high virulence degree variation [33]. Jürgens et al. [34], using RFLP, also compared five populations from Argentina (Los Hornos, Balcarce, and Barrow) and determined that the populations from uninoculated fields in Argentina had higher gene and genotype diversities compared to those from inoculated fields.

A new study using ISSR molecular markers was carried out with isolates from several locations of the Argentinean wheat region: subregion IV (SE of Buenos Aires Province) and II South (central part of Buenos Aires Province). Samples were taken from different bread wheat (*Triticum aestivum* L.) cultivars. A total of 126 isolates were subjected to molecular analysis to compare the genetic structure of the isolates from both wheat subregions. Ten ISSR primers were used: (GACA)4; (AAC)7; (ATC)7; (AC)9; (AAG)7; (AG)9; (AGC)5; (CAG)5; (GTG)5; (GACAC)3. Eighty-four bands ranging from 200 bp to 8,000 bp were amplified. Eighty-one distinct haplotypes were identified and 43 isolates did not generate any amplification products. The highest number of polymorphic DNA fragments was produced using ISSR primers (ATC)7 and (GTG)5, which detected bands in 38 isolates. The molecular analysis revealed the existence of 81 different haplotypes among the 126 isolates studied [35]. These results revealed a high degree of genotypic diversity in the *M. graminicola* population in Argentina (100% in the subregion IV, and 94.3% in the subregion II South). Furthermore a high gene flow was found between both subregions without significant genetic differences between populations. Although the asexual pycnidiospores are dispersed by rain-splash [7], the sexual ascospores of *M. graminicola* have the potential to move at least several hundred meters, perhaps even over ten kilometers [36] indicating their potential as a source of genetic exchange between spatially distant populations.

In addition, virulence tests were conducted on nine selected Argentinean wheat cultivars and 14 foreign cultivars with some level of resistance to the pathogen inoculated with 16 different isolates molecularly characterized in the previous work, and with genetic differences, in two environments. Significant differences among isolates, cultivars, and isolates × cultivar interactions were observed. Cultivars with good levels of partial and complete resistance to some isolates were detected. From the Argentinean cultivars, “Klein Dragon”, “Buck 75 Aniversario” and “Klein Volcán” showed resistance or moderate resistance to most of the isolates probed, which could indicate the presence of partial resistance in seedlings. “Klein Volcán” and “Buck 75 Aniversario”, showed partial resistance in the adult stage. From the foreign lines tested “Tonico”, “Oasis”, “IAS 20”, “TE 9111”, and “Oasis” showed the best levels of resistance in seedlings and “TE 9111” and “IAS 20” in the adult stage [37].

These recent studies about the structure of the population in Argentina were the first step to locate *Stb* genes and QTL in Argentinean cultivars. We are actually starting the studies to determine which of the known genes are present in Argentinean wheat cultivars and also developing double haploid populations with some genotypes to identify new genes.

### 3. Location of the Resistance

During the last decade, 18 major genes conferring resistance to the pathogen have been identified. They were: *Stb1* [38], *Stb2* [39], *Stb3* [39], *Stb4* [40], *Stb5* [41], *Stb6* [42], *Stb7* [43], *Stb8* [44], *Stb9* [45], *Stb10* [46], *Stb11* [47], *Stb12* [46], *Stb13* [48], *Stb14* [48], *Stb15* [49], *Stb16* [50], *Stb17* [51], and *Stb18* [52]. The known genes, chromosomal location, sources of resistance, and closest molecular markers are indicated in Table 1.

In addition, several QTL were also found. Eriksen et al. [53] mentioned some QTL on chromosomes 2BL, 3AS, 3BL, 6B, and 7B in a doubled-haploid (DH) population of a cross between the susceptible winter wheat cultivar Savannah and the resistant cultivar Senat. Risser et al. [54] also detected QTL on chromosomes 3B and 6D from “Floret” and 4B and 6B from “Tuareg”. Furthermore, Kelm et al. [55] found that cv “Solitario” conferred resistance to a specific isolate governed by *Stb6* on chromosome 3A as well as to some other isolates by a QTL on chromosome 1BS, possibly corresponding to *Stb11* and minor QTL on chromosomes 1B, 3D, 6B, and 7D.

Resistance of Marzuka to some isolates was caused by a QTL located in a region on 4AL which harbours *Stb7* or *Stb12*. Miedaner et al. [56] detected five QTL in each of two populations (Arina/Forno, History/Rubens) amounting to an explained genotypic variance of 45–63%. Zwart et al. [57] mentioned some QTL on chromosomes 2BL, 3AS, 3BL, 6B, and 7B in a doubled-haploid population of a cross between the synthetic hexaploid CP1133872 and the bread wheat *T. aestivum*. Major QTL each for resistance to *Septoria tritici* blotch and yellow leaf spot were contributed by the synthetic hexaploid parent and linked in repulsion with the coincident Lr24/Sr24 locus carried by parent Janz. Raman et al. [58] assessing three double haploid populations derived from Chara/WW2449, Whistler/WW1842, and Krichau/WW2451 found that resistance to the pathogen was conditioned in the three populations by a single major gene designated as *Stb*WW2449, *Stb*WW1842, and *Stb*WW2451 located on the short arm of chromosome 1B.
Table 1: Major genes conditioning resistance to *Mycosphaerella graminicola* identified in hexaploid wheat.

<table>
<thead>
<tr>
<th>Stb genes</th>
<th>Cultivars source</th>
<th>Chromosomal location</th>
<th>Closest (flanking) markers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stb1</td>
<td>Bulgaria 88</td>
<td>5BL</td>
<td>Xgwm335</td>
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<tr>
<td>Stb2</td>
<td>Veranopolis</td>
<td>3BS</td>
<td>Xgwn389</td>
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<td>Stb3</td>
<td>Israel 493</td>
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<td>Not published yet</td>
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<td>Tadinia</td>
<td>7DS</td>
<td>Xgwn111</td>
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<td>Xgwm44</td>
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<tr>
<td>Stb6</td>
<td>Shafir</td>
<td>3AS</td>
<td>Xgwm369</td>
</tr>
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<td>Stb7</td>
<td>Estanzuela Federal</td>
<td>4AL</td>
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<td>4AL</td>
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<td>Salamouni</td>
<td>7BL</td>
<td>Xwmc396</td>
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<td>6AS</td>
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<tr>
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<td>Synthetic hexaploid M3</td>
<td>5AL</td>
<td>Xhbg247</td>
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<tr>
<td>Stb18</td>
<td>Balance</td>
<td>6DS</td>
<td>Xgpw5176, Xgpw3087</td>
</tr>
</tbody>
</table>

Although during the last decade, several genes have been identified and several molecular markers have been developed, the analysis of resistance gene expression and utility for plant improvement programs would be increased if the resistance genes were isolated in a common susceptible background. To address that problem Goodwin and Thompson [59] started a program to backcross resistance genes Stb1–8 into two susceptible wheat cultivars. Their work with genes Stb2, Stb3, Stb6, and Stb8 has proceeded the farthest. They are also validating molecular markers linked to the resistance genes in the backcross progeny, which would provide the materials for efficient introgression of those genes into elite germplasm. They also determined that Stb3 is dominant, while Stb2 may be recessive.

Our group determined the chromosomal location of the resistance to the pathogen in substitution lines of a “Synthetic 6x” (*T. dicoccoides × T. tauschii*, *T. spelta*) and the wheat cultivars “Cheyenne” and “Cappelle-Desprez”. Several minor gene effects were detected at the seedling stage. Only chromosome 7D of “Synthetic 6x” was found having a major effect against the two isolates inoculated (IPO 92067 and IPO 93014). When tested in the adult stage, the line carrying chromosome 7D of “Synthetic 6x” showed resistance to isolate IPO 92067 but not for isolate IPO 93014. Major gene effects effective against both isolates were found on chromosomes 5A and 5D of “Synthetic 6x”. Lines carrying chromosomes 1B, 5D, or 6D from “Cheyenne” showed major effects against isolate IPO 92064 [60, 61].

On the basis of these results, a series of chromosome 7D introgression lines in the background of the susceptible recipient landrace “Chinese Spring” and the resistant donor (Synthetic 7D) was inoculated with the isolates IPO 92067 and IPO 93014. The resistance was effective at both the seedling and the adult stage against both isolates and the resistance locus mapped to the centromeric region of chromosome arm 7DS. On the basis of its relationship with the microsatellite marker Xgwm44, it is likely that the gene involved was Stb5, which proved to be effective against *M. graminicola* isolates originating from both Europe and South America [62].

In addition, a source of resistance has been mapped on chromosome 7D of spelt wheat, *Triticum aestivum* L. subsp. *spelta* (L.) Thell. The microsatellite-based genetic map was constructed from a set of 87 single-chromosome recombinant doubledhaploid lines bred from the cross between the landrace “Chinese Spring” and a “Chinese Spring”-based line carrying chromosome 7D from spelt wheat. Two regions of the chromosome were associated with isolate-specific QTL, one expressed at the seedling and another at the adult plant stage. The seedling resistance locus QStb.ipk-7D1 was found in the centromeric region of chromosome 7D, which corresponds to the location of the major resistance gene Stb4 originated from bread wheat cultivar “Tadina” and Stb5 originated from *Triticum tauschii*. The adult resistance locus QStb.ipk-7D2 was found on the short arm of chromosome 7D in a similar position to the locus *Lr34/Yr18* known to be effective against multiple pathogens. Composite interval mapping confirmed QStb.ipk-7D1 and QStb.ipk-7D2 to be two distinct loci [63].

Furthermore, using a mapping population of the International Triticeae Mapping Initiative (*W7984 × Opata* 85), three loci were discovered on the short arms of chromosomes 1D, 2D, and 6B at the seedling stage effective to isolates IPO 92067 and IPO 93014. At the adult plant stage, two isolate-specific QTL were found. The loci specific for isolates IPO 92067 and IPO 93014 were mapped on the long arms of chromosomes 3D and 7B, respectively [51].

Furthermore, one of the most confounding factors in selecting for resistance to *Septoria tritici* blotch could be the reported interaction between resistance and plant height or heading date. Miedaner et al. [56] found moderate and negative correlations between disease ratings and heading date in two populations, whereas correlation between disease rating and plant height was higher and negative. In our recent work, the effects of the plant height and heading date on the expression of the resistance were investigated in wheat near isogenic lines in the Mercia and Cappelle-Desprez backgrounds and differing in dwarfing genes (*Rht*) or in genes for insensitivity to photoperiod (*Ppd*). Strong associations between susceptibility and reduced height were only found in very short wheats indicating that moderately short wheats are not necessarily more susceptible to *Septoria tritici* blotch. The association between heading date and resistance was due to weather conditions [64]. In addition, experiments with 50 Argentinean wheat cultivars demonstrated no evidence of genetic associations between plant height, heading date, and resistance, indicating that selection of early and short lines.
with high levels of quantitative resistance is possible. In these materials, the association between those traits was mainly caused by environmental and epidemiological factors which indicates that management of cultivars should be optimized to minimize these association [64]. In addition, when the location of the resistance on chromosome 7D of spelt wheat, *Triticum aestivum* L. subsp. spelta was investigated, there was variation for flowering date within the mapping population, but none of this was associated with the resistance QTLs on chromosome 7D, showing that neither linkage nor pleiotropy is involved between this particular resistance and flowering date. This findings indicated that while some *septoria tritici* blotch resistance factors do suffer from this complication (association between resistance and heading date or plant height), others, like the present one, do not [51, 64, 65]. Risser et al. [54] also determined that all correlations between *Septoria tritici* blotch and heading date as well as between *Septoria tritici* blotch and plant height were low. Such a lack of correlation is encouraging from the breeding point of view, since it allows for the improvement in *septoria tritici* blotch resistance independently of flowering time.

4. Conclusions

Important and recent advances have been made on the population structure and location of the resistance to the pathogen. However, in Argentina little is known about genes conditioning resistance and how they are effective against the local population of the pathogen. In addition, there still much work to do in relation to the incorporation of the genes in new cultivars broadening the resistance to the pathogen.

References


