Molecular mapping of septoria tritici blotch resistance in
hexaploid wheat (*Triticum aestivum* L.)

by

RICHARD DOUGLAS CUTHBERT

A Thesis submitted to the Faculty of Graduate Studies of
The University of Manitoba
in partial fulfilment of the requirements of the degree of

DOCTORATE OF PHILOSOPHY

Department of Plant Science
University of Manitoba
Winnipeg

Acknowledgements

This project could not have been completed without the support of many people. I would like to sincerely thank all that have contributed to this thesis:

Table of Contents

List of Tables

List of Figures

Abstract

Cuthbert, Richard Douglas. Ph.D., The University of Manitoba, 2011. Molecular mapping of septoria tritici blotch resistance in hexaploid wheat (*Triticum aestivum* L.). Major Professor: Dr. Anita. L. Brûlé-Babel, Department of Plant Science, University of Manitoba.

Septoria tritici blotch (Stb) is a major foliar disease of wheat worldwide caused by the fungal pathogen *Mycosphaerella graminicola*. This project mapped the chromosomal locations of Stb resistance, which will be useful for wheat cultivar enhancement. In the first study of this project, Stb resistance and grain yield quantitative trait loci (QTL) were identified in a winter wheat doubled-haploid (DH) population produced from the cultivars Pastiche (resistant) and Torfrida (susceptible). A genetic map of the population was constructed using 104 microsatellite and 202 DArT markers. Separate disease and yield field trials were conducted in north-western Europe to measure natural Stb infection and grain yield, respectively. A resistance QTL from Pastiche was identified (*QStb.jic-2A*) that reduced flag leaf pycnidial density by 31.2% compared to the population mean. A second QTL from Pastiche (*QYld.jic-2A*) co-localized with *QStb.jic-2A*, and conferred an increase in grain yield of approximately 2.0% (0.19 t ha^{-1}).

The second study determined the map location of the third multiple-isolate resistance gene in the hexaploid landrace Salamouni. A population was developed using the resistant DH line 98S05B*13 (Salamouni/Katepwa) crossed to the susceptible cultivar Katepwa. The parent 98S05B*13 was found to contain the resistance gene *Stb14* on chromosome 3BS, therefore, to minimize the effect of this gene, individual plants of the F$_2$ population were selected for self-pollination based on presence or absence of *Stb14* using flanking microsatellite markers. The *Stb14(+)* F$_{2:3}$ families were screened with *M. graminicola* isolate MG96-36. Bulked segregant analysis identified a possible linkage to the gene of interest on chromosome 3AS. Polymorphic microsatellite markers on 3AS were used to construct a linkage group. The markers barc321 and barc12 were found to flank the resistance gene at genetic distances of 1.9 and 2.5 cM, respectively. This position on 3AS has not been previously linked to Stb resistance and this gene will be designated *StbSm3*.

The third population was developed to fine map the isolate-specific resistance gene *Stb14* located on chromosome 3B of Salamouni. A large population of 84 fixed recombinant F_4 families was developed using the co-dominant microsatellite markers flanking *Stb14*. Attempts to identify clearly polymorphic molecular markers within the interval containing *Stb14* were unsuccessful.

Foreword

This thesis follows the manuscript style outlined by the Department of Plant Science, University of Manitoba. The manuscripts follow the style recommended by Theoretical and Applied Genetics. The thesis is presented as three manuscripts, each containing an abstract, introduction, materials and methods, results, and discussion section. A literature review precedes the manuscripts and a general discussion follows the manuscripts.

1.0 Introduction

Common or bread wheat (*Triticum aestivum* L.) and durum wheat (*Triticum turgidum* L. ssp. *durum*) together are one of the three (with rice and maize) largest crops produced globally. In 2008, world wheat production was over 683 million metric tonnes (MT) and was valued at approximately 87 billion dollars (FAOSTAT 2010). Canada accounted for about 4.2% of the total production and about 5.1% of the total value. In 2009, 14 million MT of the wheat production in Canada was exported as grain or flour (Canadian Grain Commission 2009).

Many biotic and abiotic stresses impact global wheat production. Septoria tritici blotch is a biotic stress and is one of the most economically devastating foliar diseases of wheat. Septoria tritici blotch is caused by the residue-borne fungal pathogen *Mycosphaerella graminicola* (Fückl) J. Schröt. in Cohn (anamorph *Septoria tritici* Roberge in Desmaz.). *Mycosphaerella graminicola* is a hemibiotrophic pathogen that reproduces both sexually and asexually (Palmer and Skinner 2002). The sexual spores are ascospores which are air-borne and are also the primary inoculum source in regions where the sexual stage occurs. The sexual stage of this disease was first found in New Zealand by Sanderson (1972). Since this time, the sexual stage has been reported in Australia (Brown et al. 1978), the United Kingdom (Scott et al. 1988), the United States (Garcia and Marshall 1992), France (Halama 1996), and Canada (Hoorne et al. 2002). Asexual spores are pycnidiospores which can travel short distances through rain splash.

During severe septoria tritici blotch epidemics, grain yield reductions of 25 to 50% have been reported (Eyal et al. 1985; Eyal et al. 1987; King et al. 1983). Often the seed produced when the crop is infected is shrivelled and not suitable for milling purposes. Due to the polycyclic nature of septoria tritici blotch, grain yield losses in winter wheat-producing regions such as Europe can be higher than areas such as Canada where spring wheat is predominant. Septoria tritici blotch has been reported to cause economic losses of about £35.5 million in the UK (Hardwick et al. 2001). Annual grain yield reduction due to septoria tritici blotch and stagonospora nodorum blotch (previously septoria nodorum blotch) averages about 1% in the United States (Coakley et al. 1985).

Economic losses have not been reported for Canada.

Cultural control practices which include diverse crop rotations, tillage, and cultivar mixtures can be used to help control this disease. Fungicides have been used to control this disease in regions where it has been economically feasible. Excessive use of two common classes of fungicides, the strobilurins and azoles, has led to widespread selection for fungicide-resistant *M. graminicola* isolates (Cools and Fraaije 2008; Fraaije et al. 2005). Fungicides mixtures are now being used to control septoria tritici blotch in European countries. In Canada, isolates of *M. graminicola* have not yet been reported to be resistant to fungicides, however a high selection pressure is being exerted by extensive application of azole fungicides such as tebuconazole (Folicur, Bayer CropScience Inc., Canada) to control fusarium head blight.

While cultural control practices and foliar fungicides have helped control losses due to septoria tritici blotch, the most economical and environmentally sound method for producers to reduce losses is to plant cultivars with durable genetic resistance. Genetic resistance to septoria tritici blotch has been reported to be both qualitative and quantitative (Narvaez and Caldwell 1957). Studies have shown that resistance can be controlled by dominant genes (Brading et al. 2002; McCartney et al. 2002; Rosielle and Brown 1979; Somasco et al. 1996; Wilson 1979), by recessive genes (Rosielle and Brown 1979), or by several genes with dominance and additive effects (Jlibene et al. 1994; Simon and Cordo 1998; van Ginkel and Scharen 1988a; Zhang et al. 2001). Disease-escape traits such as plant height, internode length, leaf morphology and heading date are known to contribute to septoria tritici blotch resistance (Arraiano et al. 2009). Recently, the cultivars Pastiche and Exsept have been reported to have genetic resistance not accounted for by disease-escape traits or the known hexaploid resistance genes (Arraiano et al. 2009). Septoria tritici blotch resistance gene molecular mapping has been extensive over the last decade. To date, the chromosomal locations are known for fifteen resistance genes in hexaploid wheat, however, knowledge of the structure and function of these genes remains unknown. Lack of genetic resources for the large, complex hexaploid wheat genome as well as difficulties in phenotyping the host response to *M. graminicola* are a challenge for researchers (Goodwin 2007).

In western Canada, two distinct virulence groups (race 1 and race 2) have been identified based on the differential reaction of wheat line ST6 (Grieger et al. 2005). Previous studies on the inheritance of genetic resistance in the highly resistant hexaploid landrace Salamouni determined that three incompletely dominant and independent genes control resistance to the two races of *M. graminicola* found in western Canada (McCartney et al. 2002). These three genes confer resistance to *M. graminicola* isolate MG96-36 (representative of race 1) while only two of the three confer resistance to isolate MG2 (representative of race 2). Cowling (2006) was later able to map the chromosomal locations of two Stb resistance genes in Salamouni, which have been designated *Stb13* and *Stb14*. The resistance gene *Stb13* was found to confer resistance to both *M. graminicola* races and was mapped to chromosome 7BL, while *Stb14* was found to confer resistance to only race 2 and was mapped to chromosome 3BS.

The future of wheat production is dependent on creating new wheat cultivars with improvements in all important traits including better agronomics, biotic stress resistance, abiotic stress tolerance, better end-use quality, and improved grain yield. By increasing durable resistance to biotic stresses and incorporating tolerance to abiotic stresses, the risk for producers will be reduced through decreased dependence on chemical inputs and energy. Marker-assisted selection (MAS) during germplasm enhancement has the potential to help deliver new cultivars to producers sooner than using conventional breeding alone. Marker-assisted selection is the process of indirectly selecting a trait by selecting for molecular markers in genomic regions that are tightly linked to gene(s) of interest in segregating populations (Ribaut and Hoisington 1998). The usefulness MAS for a plant breeder depends on three main requirements: the availability of a marker(s) that co-segregate(s) or are tightly linked to the gene of interest (≤ 1 cM); efficient procedures to screen large populations with reproducible results; and a marker screening technique that is economical and user friendly (Mohan et al. 1997).

The objectives of this research were to:
1. Conduct a genetic analysis of the winter wheat cross Pastiche/Torfrida and identify quantitative trait loci associated with septoria tritci blotch resistance,

grain yield and grain yield components

2. Identify the chromosomal location of the third locus conferring resistance to multiple Canadian isolates of *M. graminicola* in the hexaploid wheat landrace 'Salamouni'

3. Fine map *Stb14*, an isolate-specific septoria tritici blotch resistance gene in the hexaploid wheat landrace 'Salamouni' located on chromosome 3BS

2.0 Literature Review

2.1 Wheat

2.1.1 Importance of wheat and production statistics

Wheat is one of the most important crop species grown in the world. In 2008, over 683 million metric tonnes (MT) of wheat were produced globally. Canada ranked fifth in terms of production value and accounted for about 4.2% of the total production (Table 2.1) (FAOSTAT 2010). Wheat was the third-largest crop produced in 2008 next to maize (over 826 million MT) and rice (over 686 million MT) (FAOSTAT 2010).

Wheat, including bread wheat (*Triticum aestivum* L. em. Thell.) and durum wheat (*Triticum turgidum* L. ssp. *durum*), is one of the two most valuable crops grown in Canada with over 9.5 million hectares seeded and harvested in 2009 (Statistics Canada 2010). Between the years 1908-2007, an average of 96% of Canadian wheat production occurred in the prairie provinces of Manitoba, Alberta, and Saskatchewan (McCallum and DePauw 2008). Compared to the principal field crops grown in Canada, wheat occupied about 37% of the total land seeded. Production of wheat in Canada in 2009 was estimated to be 26.5 million tonnes (Statistics Canada 2010). Spring wheat accounted for 68.4% of production, while winter and durum wheat accounted for 11.3% and 20.3%, respectively.

Wheat is used to make products such as leavened and unleavened breads, pasta, noodles, cookies, animal feeds and, more recently, biofuel. The end-use determines the class of wheat grown by producers. Wheat grown in Canada can be divided into eight wheat milling classes (Canadian Grain Commission 2010). Canada Western Red Spring (CWRS) is the largest milling class of wheat grown in Canada. This class has been bred to meet strict grain quality requirements including milling properties and seed protein content (>13.0%) (McCallum and DePauw 2008). Recently, the Canada Western General Purpose class, which does not need to meet milling standards, was created to allow high-grain-yield wheat to be grown in Canada mainly for the ethanol fuel markets and animal feed (Canadian Grain Commission 2010). Regardless of the market class, new Canadian wheat cultivars must pass minimum standards for disease resistance,

agronomic performance and match the quality parameters outlined for the targeted market class (Prairie Grain Development Committee 2010).

Winter wheat is not grown on as large a scale as spring wheat in Canada mainly due to the harsh temperatures during the Canadian winter which can reduce winter survival of the crop (DePauw and Hunt 2001). In countries where winter temperatures are not as severe, such as the United Kingdom (UK), winter wheat dominates the seeded wheat area (Fischer and Edmeades 2010). Recently, annual grain yields across the UK have averaged over 8 t ha^{-1} grown over 2 million ha. When the ratio of grain value to grain production is compared between Canada and the UK (Table 2.1), it is apparent that Canadian wheat has a higher value ($156 per tonne vs. $97 per tonne). This is due to Canadian wheat being of higher quality (seed protein content) and also because western Canadian wheat is marketed using the single-desk marketing system.

Table 2.1: Wheat production statistics (2008) for the top 20 valued wheat-producing countries (FAOSTAT 2010)

Rank	Country	Production value[A] (Int $1,000)	Production (MT)
1	China	15,805,966	112,463,296
2	India	11,671,546	78,570,200
3	United States of America	9,301,602	68,016,100
4	Russian Federation	6,670,506	63,765,140
5	Canada	4,462,759	28,611,100
6	France	4,388,762	39,001,700
7	Pakistan	3,023,994	20,958,800
8	Australia	2,653,403	21,420,177
9	Ukraine	2,618,186	25,885,400
10	Turkey	2,428,920	17,782,000
11	Germany	2,315,299	25,988,565
12	United Kingdom	1,666,334	17,227,000
13	Kazakhstan	1,378,582	12,538,200
14	Argentina	1,234,294	8,508,156
15	Egypt	1,012,186	7,977,051
16	Italy	897,733	8,855,440
17	Romania	895,241	7,180,980
18	Brazil	877,289	6,027,131
19	Poland	811,707	9,274,920
20	Iran (Islamic Republic of)	800,547	7,956,647
	Global total	**87,069,055**	**683,406,527**

[A] - Production value measured in the international dollar. The international dollar is equivalent in purchasing power to the United States dollar.

2.1.2 Origin, evolution and genetics

Wheat was domesticated over 10,000 years ago in Transcaucasia (Sleper and Poehlman 2006). Bread wheat is an allohexaploid consisting of three distinct, but related genomes each consisting of seven pairs of homoeologous chromosomes for a total haploid chromosome number of 21. There is strong evidence to suggest that current bread wheat (2n=6X=42) evolved from the hybridization of the diploid *Triticum monococcum* (AA) with an unknown diploid progenitor believed to be *Aegilops speltoides* (BB), resulting in the tetraploid *Triticum turgidum* (2n=4X=28, AABB) (Daud and Gustafson 1996). *T. turgidum* then hybridized with the diploid *Triticum tauschii* (DD).

The genome of hexaploid wheat is very large with a haploid DNA content of about 1.6×10^{10} bp (Arumuganathan and Earle 1991) and an average DNA content of 762 Mbp per chromosome. Renaturation kinetic studies of the wheat genome have determined that non-repeated and repeated DNA accounts for 25% and 50-65% of the genome, respectively (Flavell and Smith 1976). The average wheat chromosome is 25 times longer than the average rice chromosome (Gupta et al. 1999). Due to polyploidy, wheat is able to tolerate changes in structure and number of chromosomes for multiple generations. This has led to the development of complex cytogenetic stocks (deletion lines, nulli-tetrasomics and ditelosomics) using a wheat genotype named "Chinese Spring" as a recurrent parent (Endo and Gill 1996). These cytogenetic stocks have been useful in the physical mapping of loci to specific chromosomes.

2.1.3 Wheat production challenges

Crop production can be challenging in all growing regions due to continually evolving biotic and abiotic stresses (DePauw and Hunt 2001). Abiotic stresses such as drought, flooding, and nutrient-poor growing conditions are serious problems for many producers globally (Rajaram 2001). Biotic stresses that have become major limiting factors in wheat production are losses due to plant diseases and insect damage. Some of the biotic stresses that can hinder wheat production include wheat diseases such as

fusarium head blight (FHB), leaf and stem rust, and the leaf spot complex (septoria tritici blotch, tan spot, spot blotch, stagonospora nodorum blotch, and septoria avenae blotch) (Kolmer et al. 2009; Marshall 2009). Other biotic stresses in the form of insect damage from wheat stem sawfly, and orange blossom wheat midge are also serious problems for many producers (DePauw and Hunt 2001).

2.1.4 Wheat breeding

Common wheat has one of the most complex genetic structures of all field crops making genetic analysis and improvement very difficult (Gupta et al. 1999; Röder et al. 1998). Wheat breeding aims to develop new cultivars that have improved agronomics, better resistance to biotic stresses, tolerance to abiotic stresses, and better end-use quality traits. Until recently, wheat improvement has been limited to classical plant breeding techniques to make small incremental improvements to wheat cultivars. Wheat is a self-pollinating crop; therefore, the breeding schemes used are pedigree selection, single seed descent, bulk population, doubled haploid, backcross, and recurrent selection (Sleper and Poehlman 2006). Backcross breeding is commonly used to introduce a simply inherited trait such as disease resistance from a non-adapted source to adapted germplasm. Bulk populations, pedigree, and single seed descent breeding schemes apply selection in segregating populations after hybridizing parental germplasm. Recurrent selection relies on increasing the frequency of desired genes through repeated cycles of selection. Traditional plant breeding schemes are fundamentally based on selection of plants or lines by phenotypic assessment of targeted traits (Sleper and Poehlman 2006).

2.2 Septoria tritici blotch

Mycosphaerella graminicola (Fuckl) J. Schröt. in Cohn (anamorph *Septoria tritici* Roberge in Desmaz.) is the causal agent of septoria tritici blotch, an economically important foliar disease of wheat with worldwide distribution (Shipton et al. 1971). *Mycosphaerella graminicola* is able to infect diploid, tetraploid, and hexaploid wheat species. Septoria tritici blotch is considered to be the most economically important of the cereal pathogens classified in the genus *Septoria* (Sprague 1950). Two other *Septoria*

species, *Septoria passerinii* Sacc., the cause of speckled leaf spot of barley, and *Septoria secalis* Prill. and Delacr., cause of leaf spot of rye, are considered minor cereal pathogens (Sprague 1950). Some cereal pathogens that were initially classified in the *Septoria* genus have been recently reassigned to the genus *Stagonospora* (Cunfer and Ueng 1999).

The impact of Septoria tritici blotch on wheat production has become a serious concern for wheat producers in the temperate, high-rainfall wheat-production areas. Epidemics of septoria tritici blotch are capable of reducing yields by 25 to 50% (Eyal et al. 1985; Eyal et al. 1987; King et al. 1983). Often infected crops produce fewer seeds per spike and grain with reduced weight (Ziv and Eyal 1978). Septoria tritici blotch is normally found in temperate climates, however, McDonald et al. (1999) reported the likely origin of this disease is the Middle East. The pathogen has become an important disease of wheat in Europe (Hardwick et al. 2001), western Australia (Loughman and Thomas 1992), USA (Garcia and Marshall 1992; Mundt et al. 1999), and Canada (Chungu et al. 2001; Hoorne et al. 2002).

Septoria tritici blotch is one of five residue-borne, leaf-spotting diseases of wheat that have become prevalent across western Canada over the last three decades. The other four diseases includes stagonospora nodorum blotch (*Phaeosphaeria nodorum*), septoria avenae blotch (*Phaeosphaeria avenaria*), tan spot (*Pyrenophora tritici-repentis*), and spot blotch (*Cochliobolus sativus*) (Gilbert et al. 1998).

Occurrence and prevalence of the leaf-spot disease is heavily dependent on environmental conditions (Gilbert et al. 1998; Pedersen and Hughes 1993). Septoria tritici blotch development is generally favoured by moderate temperature (20-25°C) and high amounts of rainfall (Eyal et al. 1987; Shaner 1981; Shaner and Finney 1976).

2.2.1 The pathogen

The genus *Septoria* is classified in the class Deuteromycetes (imperfect fungi) and the order *Sphaeropsidales*. In New Zealand, Sanderson (1972) was the first to identify the sexual state of *S. tritici* (*M. graminicola*). *Mycosphaerella graminicola* is classified in the class Loculoascomycetes (filamentous fungi), and order *Dothideales* (Palmer and Skinner 2002).

Prior to the identification of the teleomorph state by Sanderson (1972), it was hypothesized that the pathogen propagated solely through the production of asexual pycnidiospores (Eyal et al. 1987). The asexual stage produces slender and elongated pycnidiospores within a pycnidium. The pycnidia are visible and formed in the epidermal and mesophyll leaf tissue. Two forms of pycnidiospores are produced by *S. tritici*, macropycnidiospores (35-98 x 1-3 µm) with 3-5 septa and micropycnidiospores (8-10.5 x 0.8-1 µm) with no septa. Both types of pycnidiospores are equally successful in their ability to infect wheat (Shipton et al. 1971). Pycnidiospore size has been reported to be heavily influenced by environmental factors such as temperature, leaf wetness, and age of the host leaf tissue (Shearer and Wilcoxson 1978). High temperature (>18°C) and increased wetness was found to decrease the spore size.

During the sexual stage, *M. graminicola* produces sub-epidermal pseudothecia (sexual fruiting bodies) which are 70-100 µm in diameter, globose and dark brown in colour. Within the pseudothecia, many asci are produced. Asci are obpyriform, bitunicate and range in size from 30-55 x 10-20 µm. Each ascus contains eight randomly orientated ascospores (Scott et al. 1988). Ascospores are elliptical, hyaline and 10-15 x 2-3 µm with two cells of unequal size (Eyal et al. 1987; Sanderson 1972).

2.2.2 Disease symptoms

Septoria tritici blotch symptoms almost always appear on wheat leaves and culms, but can also develop and appear on the glumes and rachis. The first visible symptoms of the disease are small chlorotic areas on the tips of the lower leaves. These chlorotic areas later develop into extended light-brown necrotic lesions. If environmental conditions are ideal, the necrotic lesions will coalesce and the leaf tissue will appear light gray in colour (King et al. 1983).

Pycnidia develop within the necrotic lesions and are light to dark brown in colour (Figure 2.1). They are distributed throughout the necrotic lesions on both the adaxial and abaxial surfaces of the leaf (Eyal et al. 1987). Pycnidia size can be dependent on the host genotype and has been reported to be inversely related to pycnidial density (Eyal and Brown 1976).
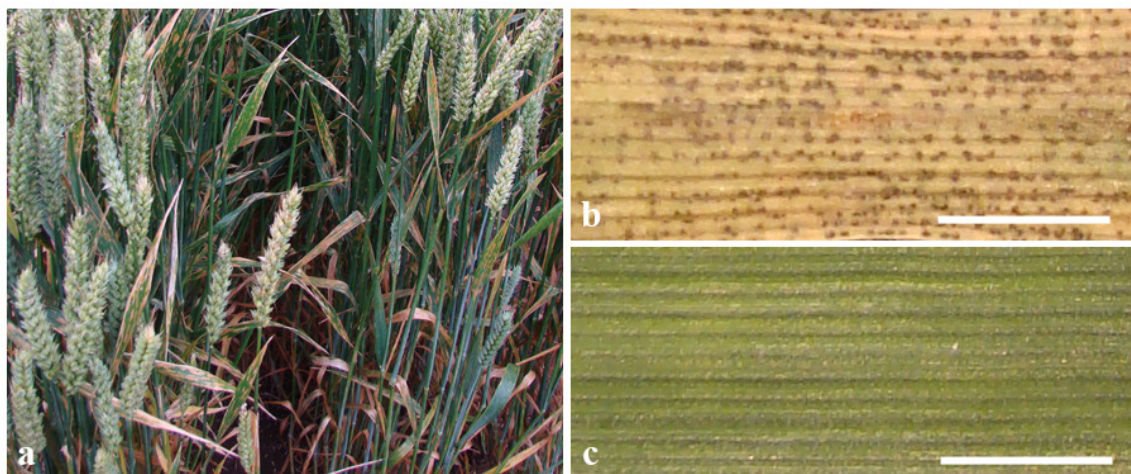
Figure 2.1: Symptoms of *Mycosphaerella graminicola* infected wheat. **(a)** Photograph of a winter wheat cultivar in a disease trial in Spalding, Lincolnshire, UK. Disease infection caused by natural populations of *M. graminicola*. **(b-c)** Photographs of wheat lines infected with *M. graminicola* isolate MG2 from Canada. Photographs taken 24 days after inoculation. **(b)** Susceptible wheat line **(c)** Resistant wheat line. Bars: (b, c), 5 mm.

2.2.3 Life cycle and epidemiology of *Mycosphaerella graminicola*

A graphical representation of the hemibiotrophic life cycle of *M. graminicola* is shown in Figure 2.2. Infected crop residue and volunteer wheat plants are primary inoculum sources for septoria tritici blotch epidemics (Eyal et al. 1987; Shipton et al. 1971). It was previously thought that ascospores were only released from overwintering stubble or volunteer plants, however, Hunter et al. (1999) more recently reported that sexual ascospores can be released from ascocarps produced on infected leaves during a wheat crop growing season. This is consistent with the finding by Kema et al. (1996c) that the pathogen is able to complete the sexual phase within five weeks. Analysis of field samples determined that ascospores were released from not only wheat crop residue but also from wheat plants during the fall, spring, and summer. These results indicate that *M. graminicola* is able to complete several sexual cycles per growing season, which contributes to the polycyclic nature of the disease. Development of septoria tritici blotch after establishment is not solely dependent on splash-dispersed pycnidiospores (Royle 1994; Shaw and Royle 1993), but also on air-borne ascospores produced throughout the growing season (Kema et al. 1996c).

Wheat crop
seedlings

Wind-blown
ascospores

Rain-splashed
pycnidiospores

Rain activates
spore dispersal

Perithecia

Pycnidia

Rain-splash spread of
pycnidiospores within crop

Secondary
cycles

Wheat crop stubble
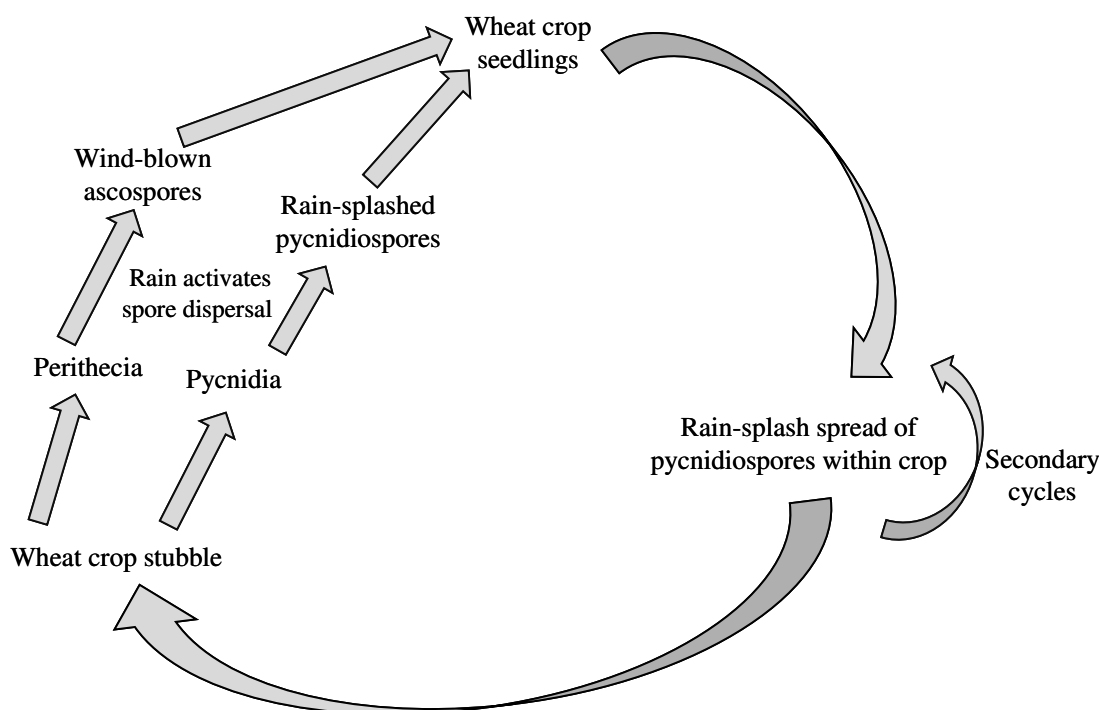
Figure 2.2: Life cycle of the wheat pathogen *Mycosphaerella graminicola.* The primary source of inoculum is wind-blown sexual ascospores and the secondary inoculum source is from rain splash of asexual pycnidiospores (Eyal et al. 1987).

The secondary inoculum source is pycnidiospores, which can remain viable for several months at temperatures from 2 to 10°C. The pycnidiospores are secreted in ooze (cirrus) which helps in their germination and protects them from radiation and desiccation. Conidia are produced during periods of wetness and are dispersed by moisture, usually by forceful rain splash. Between 40-60% of pycnidiospores are released from a pycnidium after first wetting (Eyal et al. 1987). Movement of conidia can be from crop residue or from lower leaves of a crop to the upper canopy leaves (vertical movement). Horizontal transfer can also occur through leaf-to-leaf contact.

Brokenshire (1975) reported that seed infection is possible but it is unknown if infected seed can be a source of inoculum for septoria tritici blotch epidemics. Through study of genetic diversity with molecular markers, McDonald et al. (1999) demonstrated that infected seed has been linked to the global distribution of *M. graminicola*.

Septoria tritici blotch epidemics are highly dependent on environmental conditions and are favoured by temperatures between 15 and 20°C (Wiese 1987). Moisture has been identified as an important factor during all stages of the infection cycle. High relative humidity after initial infection favours pycnidial formation and lesion growth (Hess and Shaner 1987; Magboul et al. 1992; Shaw 1991; Wainshilbaum and Lipps 1991). The maximum number of lesions per unit area was observed at 20°C and a fixed leaf wetness period of 96 hours (Magboul et al. 1992). The number of lesions per leaf area measurement was reduced with a temperature higher or lower than 20°C.

Initial symptoms in winter wheat appear early in the spring on the lower leaves close to the soil surface (Shaner and Buechley 1995). This finding indicates that the primary infection occurs during the fall prior to cold weather. Shaner and Buechley (1995) found that disease severity was low until later in the growing season when all leaves were expanded. After flag leaf emergence, disease severity rapidly increased and, under ideal environmental conditions, symptoms of leaf blotch were observed within 25 to 35 days after heading.

2.2.4 Host range of *M. graminicola*

Common bread wheat (*Triticum aestivum* L.) and durum wheat (*Triticum turgidum* subsp. *durum* L.) are economically important crops and both are hosts of *M. graminicola*. Other species can serve as alternative hosts such as species of the genera *Agropyron*, *Agrostis*, *Brachypodium*, *Bromus*, *Dactylis*, *Festuca*, *Hordeum*, *Glyceria*, *Poa*, *Secale*, and wild *Triticum* (wild emmer) (Eyal 1999a). *M. graminicola* has been reported to infect the weed species *Vulpia bromoides* (brome fescue) (Brokenshire 1975). The potential of these alternative hosts to serve as sources of primary inoculum for epidemics is not known (Eyal 1999b).

2.2.5 Infection process and histology of *M. graminicola*

Both the sexual ascospores and the asexual pycnidiospores can, under ideal environmental conditions, germinate and penetrate host tissue after landing on a leaf (Palmer and Skinner 2002). Unlike other plant-pathogenic fungi that penetrate leaves

directly through appressoria formation or production of cell-wall degrading enzymes, penetration by *M. graminicola* is indirect through stomata (Cohen and Eyal 1993; Kema et al. 1996d). Kema et al. (1996d) concluded that infection is a random process after observing germ tubes cross stomata without penetration occurring. After penetration, the fungus colonizes the host mesophyll leaf tissue by intercellular growth. *Mycosphaerella graminicola* does not produce specialized feeding structures such as haustoria (Palmer and Skinner 2002). Biomass increases steadily within the mesophyll over the first ten days of infection without visible symptoms. If conditions are ideal (temperature is between 20-25°C and high relative humidity) the fungal infection process switches from biotrophic to necrotrophic growth and host mesophyll cells collapse. At this stage chlorotic and necrotic lesions are visible on the host leaf (Kema et al. 1996d). Pycnidia appear within the necrotic lesions 14 to 21 days after inoculation. Pycnidia formation depends on the wheat cultivar, *M. graminicola* isolate, and environmental conditions (moisture, temperature and light).

2.2.6 Physiological specialization of *M. graminicola*

For many years, researchers have studied virulence and pathogenicity of *M. graminicola* isolates. Some research indicates that there are differences in virulence, however, other reports indicate that the difference is in the degree of pathogenicity or aggressiveness.

The first differences in virulence were reported in the 1970s (Eyal et al. 1973). By the 1980s, studies on virulence patterns were conducted. Eyal et al. (1985) was the first to conduct a virulence pattern study using 97 *M. graminicola* isolates collected from 22 countries to test reactions on seedlings of wheat and triticale cultivars. This study concluded that there was significant host cultivar x pathogen isolate interaction, which indicated that there were cultivar-specific virulence genes among isolates. Another study by Saadaoui (1987) used isolates of *M. graminicola* gathered from wheat fields in Morocco and inoculated these isolates on a set of seven differential wheat lines. This study determined that the collection of *M. graminicola* isolates could be assigned to three distinct physiological races.

In contrast to the studies on virulence, Marshall (1985) studied the variability of *M. graminicola* on winter and spring wheat cultivars using field and greenhouse conditions to determine differences in isolate aggressiveness or degree of pathogenicity. Field experiments were conducted at a total of sixteen locations over ten US states. This study reported populations of *M. graminicola* tend to be more aggressive in northern regions of Indiana and Ohio and also central and north central California, while populations from northern Alabama, Mississippi, eastern Arkansas, southern Nebraska and central Kansas were less aggressive (Marshall 1985). A later study tested thirty-four *M. graminicola* isolates from seven countries on tetraploid durum lines and a hexaploid bread wheat accession (van Ginkel and Scharen 1988b). This study concluded that cultivars varied in race non-specific resistance and isolate aggressiveness varied. Under natural field conditions, van Ginkel and Rajaram (1995) believed that the differences between pathogen populations were due to aggressiveness and not virulence.

Ballantyne (1989) later suggested that Australian isolates could be grouped in true physiological races and also that these isolates varied for pathogenicity or aggressiveness. Kema et al. (1995) reported significant cultivar x isolate interaction at seedling and adult plant stages when they studied virulence variation for 78 isolates of *M. graminicola* from 16 countries. The isolates were inoculated on a collection of differential wheat cultivars. Kema et al. (1995) concluded that cultivar specificity and virulence did vary among isolates. Ackermann et al. (1995) confirmed these results when they studied pathogenic variability of South African *M. graminicola* isolates and found that interaction between cultivars and isolates existed.

Specialization at the host species level has been investigated between tetraploid durum wheat and hexaploid bread wheat. Two experiments were conducted in Morocco to study the wheat and *M. graminicola* interaction (Jlibene et al. 1995). The first experiment included bread and durum wheat cultivars inoculated with isolates of *M. graminicola* collected from bread and durum wheat fields. In the second experiment a differential set of bread wheat lines was inoculated with isolates collected from bread wheat fields. They reported some specialization in *M. graminicola* isolates at the species level. Durum wheat isolates were specific to durum wheat lines, while bread wheat

isolates were specific to bread wheat. Bread wheat isolates showed differential virulence response to the bread wheat genotypes and the twenty-seven isolates could be assigned to eight classes.

Kema et al. (1996a) studied specialization at the host species level by seedling tests of virulence variation using a total of 63 isolates of *M. graminicola* from thirteen countries. The first experiment focused on *M. graminicola* isolates isolated from bread wheat, while the second experiment focused on *M. graminicola* isolates isolated from durum wheat. These experiments confirmed that significant cultivar x isolate interaction was present and did not agree with the findings of van Ginkel and Scharen (1988b). In 1997, Kema and van Silfhout reported a significant interaction between cultivars and isolates when tested at both the seedling and adult plant stages. Grieger et al (2005) differentiated isolates of *M. graminicola* collected from wheat fields in western Canada into two races based on the differential reaction of the wheat line ST6. Race 1 isolates (MG96-36) were virulent on ST6 while race 2 isolates (MG2) were avirulent on ST6.

These findings of significant isolate x cultivar interaction led to the suggestion that a gene-for-gene relationship existed in the wheat-*M. graminicola* pathosystem (Kema et al. 1996a; Kema et al. 1996b). Studies by Brading et al. (2002) and McCartney et al. (2002) further confirmed that isolate-specific resistance in wheat to septoria tritici blotch follows the gene-for-gene relationship similar to that originally proposed by Flor (1946). A complex avirulence locus from the *M. graminicola* isolate IPO323 was identified and mapped by Kema et al. (2000), which also supports the gene-for-gene hypothesis.

If *M. graminicola* isolates have specific interactions with host cultivars, incorporating resistance into new wheat cultivars will be a major challenge for plant breeders (Cowger et al. 2000). Physiological specialization implies that resistance can be overcome when widely released in wheat-producing regions. This has been observed for the genetic resistance (major resistance gene *Stb4*) incorporated in the cultivars Heron and Robin in Australia (Ballantyne and Thomson 1995) and Gene in Oregon (Cowger et al. 2000). The resistance was reported to have broken down in these regions in relatively short periods of time due to high selection pressure being exerted on the *M. graminicola*

population by the resistant wheat cultivars. Isolates of the population that mutate at the targeted avirulence locus will be able to overcome the host cultivar resistance and increase rapidly. This process is generally referred to as the boom and bust cycle and has been reported for many plant pathogens such as leaf and stem rust (Hulbert et al. 2001).

2.3 Methods to control septoria tritici blotch

Septoria tritici blotch can be controlled by managing cultural practices, applying foliar fungicides during the growing season, and/or by planting resistant cultivars.

2.3.1 Disease assessment

Methods to assess septoria tritici blotch severity are important to apply disease-control methods, study the pathogen, and evaluate germplasm for resistance. Disease rating is typically based on leaf necrosis area, pycnidial density, or a combination of leaf necrosis and pycnidial coverage. Leaf necrosis is a visible symptom of host tissue death or apoptosis (Cohen and Eyal 1993). Necrosis during *M. graminicola* infection may be due to growth and colonization of the pathogen, fungal toxin production, or a diffuse host response. Studies have found that necrosis and pycnidia formation may not be linked (Kema et al. 1996a; Rosielle 1972). Extensive necrosis phenotypes with minimal pycnidia formation were found by histological analysis to have minimal colonization by the pathogen (Kema et al. 1996a). Pycnidia presence is therefore generally used to measure resistant and susceptible reactions to *M. graminicola*.

Qualitative and quantitative rating scales have been used to measure the reaction of wheat genotypes to *M. graminicola*. A number of different visual rating scales have been developed and used, however, the most common rating scale is a quantitative scale and measures percent leaf area bearing pycnidia (Arraiano et al. 2009; Arraiano and Brown 2006, 2007; Arraiano et al. 2007; Arraiano et al. 2001b; Chartrain et al. 2004a; Chartrain et al. 2009; Jlibene et al. 1994; Kema et al. 1996a). James (1971) developed assessment diagrams for the percentage of leaf affected area using an electronic scanner, while Eyal and Brown (1976) developed a scale to quantify the pycnidial density on leaves using a television scanner. Several studies have used this scale to estimate pycnidial coverage on four to six upper leaves during soft dough development stage

(Baltazar et al. 1990; Danon and Eyal 1990; Danon et al. 1982). Saari and Prescott (1975) developed a scale ranging from 0-9 for evaluating the severity of foliar diseases (except rusts) on wheat, barley and triticale. Later this scale was changed to a double-digit scale (00-99) (Eyal et al. 1987). The first digit is a measurement of the height of the disease on the plant and the second digit is a rating of the severity of the disease in percentage (0 = 0% and 9 = 90%) (Eyal et al. 1987). This scale is particularly useful for plant breeding programs that screen large populations in disease trials and need to rate quickly.

The first well-defined qualitative septoria tritici blotch rating scale was developed by Rosielle (1972) (Appendix 8.5). The scale ranges from 0 to 5 and is based on rating the extent of hypersensitive flecking, coalescence of lesions, and pycnidial density. The relationship between pycnidial density and necrosis is not factored into the Rosielle scale. Since phenotypes with extensive necrosis and light pycnidial formation are not covered in this scale, the addition of an 'X' after the rating number (0-5) was added. Another drawback of this scale is the lack of differentiation between chlorotic and necrotic lesions. This difference is very important since chlorosis is attributed to a resistant response (Eyal et al. 1973) while necrosis is associated with a susceptible response. Often heterozygous plants will have expanded chlorotic lesions, therefore, McCartney et al. (2002) made a modification to the original Rosielle scale by considering ratings 0-3 as resistant and ratings 4 and 5 as susceptible.

Modern techniques to assess the severity of the disease in controlled environment conditions have been reported. A detached-leaf testing method was developed by Arraiano et al. (2001a) to study resistance to *M. graminicola* in wheat. The results from the detached-leaf test were found to be similar to those of seedling and field trials in a wide range of environments. The method developed by Arraiano et al. (2001a) differed from previous research in that the inoculum was applied to the entire seedling by spraying instead of placing inoculum on the surface of the detached leaf. To increase the frequency of penetration and the infection efficiency of *M. graminicola* isolates, detached leaves were kept in the dark for 48 h. Previous attempts with detached-leaf tests resulted in senescence of leaf segments during the long incubation time required for symptom

development. To overcome this problem, Arraiano et al. (2001a) used water agar supplemented with benzimidazole. The ends of each freshly cut leaf segment were inserted in the agar and the middle of the leaf segment was suspended in air. Leaf segments remained green for over 30 days. Studies have used the detached-leaf screening method to assess the reaction of a large number of cultivars to global isolates of *M. graminicola* (Arraiano and Brown 2006). Mapping resistance to septoria tritici blotch has also relied on the detached-leaf method (Chartrain et al. 2009). Detached-leaf disease reactions usually are reported as Area Under the Disease Progress Curve (AUDPC) by rating the leaf segments every two days over a period of 10-14 days (Arraiano and Brown 2006).

One of the biggest challenges in assessing septoria tritici blotch disease severity is keeping inoculated leaves from senescing and free from other plant diseases or pests. The specific environmental conditions required for infection and the long latent period (14-18 days) after inoculation requires screening to be completed in controlled environments with appropriate light intensity, temperature, and relative humidity. Generally, these conditions are also appropriate for other plant diseases such as powdery mildew (*Blumeria graminis* f. sp. *tritici*). Plants must be kept isolated and free from contamination throughout the entire screening process, which can be a challenge. Another disadvantage to using controlled-environment disease screening is the cost of the equipment. A method that has been recently studied to overcome these challenges is the quantification of fungal biomass growth on inoculated leaves following *M. graminicola* inoculation using real-time PCR (Adhikari et al. 2004a; Goodwin 2007). It was previously thought that the pathogen rapidly increased in biomass about 3 days post inoculation in susceptible cultivars; however, Adhikari et al. (2004a) found that real-time PCR was unable to detect differences in resistant and susceptible genotypes for the fungal gene marker β-tubulin until day 16. They concluded that the use of real-time PCR to discriminate between resistant and susceptible phenotypes was not economical.

2.3.2 Cultural practices affecting septoria tritici blotch

Septoria tritici blotch is a residue-borne leaf-spotting disease; therefore,

management of infected crop residue is important in reducing carryover of the pathogen. One of the major cultural practices that affected wheat residue decomposition is tillage. Deep ploughing of fields and burning of stubble have been reported to be effective in reducing disease severity (Brown and Rosielle 1980); however, due to environmental concerns these types of cultural practices are not recommended. Zero or minimal tillage practices leave more crop residue on the surface of the field and recently have become more popular. This practice can help minimize soil erosion and reduce the number of field passes with equipment, which decreases fuel usage and production costs. Zero or minimal tillage has also been used to conserve soil moisture. Tillage reduction across Canada over the past thirty years may have contributed to the increase in septoria tritici blotch incidence and severity in wheat fields. Conservation tillage practices allow crop residue to remain on the soil surface and decompose more slowly than buried residue (Bailey and Duczek 1996). Increasing the decomposition of wheat residue should lower the severity of septoria tritici blotch; however, reports have found that the pathogen is found more frequently under conventional tillage practices when compared to conservation tillage in Ontario winter wheat and Manitoba spring wheat crops (Gilbert and Woods 2001; Sutton and Vyn 1990).

Crop rotations are important for reducing the incidence and severity of diseases. Krupinsky (1999) recommended crop rotation as a means to increase decomposition of the infected crop residue while non-host crops are grown. Crop rotations may not eliminate the disease; however, they will reduce the inoculum level of the pathogen. Pederson and Hughes (1992) found that under ideal environmental conditions a crop rotation of at least two years between wheat crops was required to achieve adequate control of the septoria disease complex, while a one-year break between wheat crops was effective in reducing disease severity under conditions that were unfavourable for the pathogen . In Israel, Eyal (1981) reported a three- to five-year crop rotation reduced the incidence of septoria tritici blotch, while even longer rotations of six to eight years were insufficient to eliminate the disease. In south-eastern Saskatchewan, a spring wheat rotation of two years followed by two years of a non-cereal crop and summer fallow has been recommended to reduce leaf-spotting disease severity (Fernandez et al. 1998).

Sutton and Vyn (1990) determined that one year breaks from wheat in conservation tillage systems reduced the number of lesions per leaf in winter wheat. These studies indicate that crop rotations can help reduce incidence and severity of septoria tritici blotch but may not be adequate to effectively control of this disease on their own.

Another production practice that can reduce disease severity is planting wheat cultivar mixtures. Mundt et al. (1995) determined that cultivar mixtures on average reduced septoria tritici blotch severity from 9 to 27% in the field when compared to the pure stands of the component wheat cultivars. The progress of the epidemic was reduced in mixtures of susceptible and moderately resistant cultivars. A later study by Cowger and Mundt in 2002 was unable to determine if the cultivar mixtures altered pathogen populations with either a fitness advantage or liability. Cultivar mixtures can be difficult to implement on a large scale due to differences in agronomic and quality traits between the pure wheat lines.

Inter-cropping has also been suggested as a method to reduce inoculum movement within a wheat field. A study conducted by Bannon and Cooke (1998) found that under laboratory conditions, a wheat-clover intercrop system reduced horizontal *S. tritici* pycnidiospore movement by 33% and vertical movement by 63% when compared to a wheat monoculture. However, this study was unable to determine if the wheat-clover intercrop was able to reliably reduce septoria tritici blotch at the field level.

The effect of synthetic fertilizer on the severity of septoria tritici blotch has been studied with conflicting results. High rates of nitrogen have been reported to increase disease severity (Shipton et al. 1971). The increase in crop canopy from high nitrogen rates is thought to create a favourable microclimate for pathogen infection. A dense crop canopy reduces air movement which extends the period of leaf wetness and slows drying of leaves following precipitation or dew. Tompkins et al. (1993) found that in Saskatchewan, higher disease severity was correlated with low nitrogen rates and concluded that lesion development might be encouraged by nitrogen deficiency. A later study found that nitrogen deficiency in dry years increased the severity of the leaf-spotting disease complex (Fernandez et al. 1998).

2.3.3 Chemical control

Chemicals have been used effectively and economically to control septoria tritici blotch in regions where substantial grain yield losses occur. Cook (1999) reported that over 95% of the wheat crop in the United Kingdom is sprayed with foliar fungicides annually. Over 70% of the fungicides applied to wheat crops in Europe are to combat septoria tritici blotch (Goodwin 2007). However, foliar fungicides may not be the most desirable method to control the disease since they are expensive and may damage the environment by affecting non-target beneficial organisms.

Both contact and systemic fungicides are available for control of this disease. The dithiocarbamate fungicides (Metiram, Mancozeb) as a group are contact fungicides that are effective in controlling septoria tritici blotch; however, they require multiple applications at 10-14 day intervals (Eyal et al. 1987). Systemic azole fungicides such as propiconazole (Tilt, Syngenta Crop Protection Canada, Inc.; triazole) and pyraclostrobin (Headline EC, BASF Canada Inc.; strobilurin) protect the crop for longer periods of time compared to dithiocarbamate (Eyal et al. 1987). Sanderson and Gaunt (1980) reported that a single application of the systemic fungicide benomyl (Benlate) on wheat at the 3-4-leaf growth stage was adequate to control septoria tritici blotch. Higher grain yield and larger seed size have been observed when propiconazole (Tilt) fungicides are applied at Zadok's growth stage 52-59 (Entz et al. 1990).

Overuse and dependence on two of the most effective fungicide classes, azoles and strobilurins, have resulted in a very high selection pressure in favour of resistant septoria tritici blotch isolates across Europe. Isolates of septoria tritici blotch resistant to azole fungicides have a mutated *CYP51* gene (Cools and Fraaije 2008). Resistance to quinone outside inhibitors (QoIs) or the strobilurin class of fungicide has been studied in European populations of *M. graminicola*. It has been reported that QoI resistance emerged independently at least four times across Europe (Torriani et al. 2009). It is imperative for producers to rotate classes of fungicides to avoid losing the effectiveness of chemical control. To help extend the effectiveness of foliar fungicides, the Home Grown Cereal Authority has developed an internet-based interactive tool for producers in the UK to aid in choosing effective fungicides and determining correct spray timing

(HGCA 2010). Mixtures of azole fungicides are now being used in the UK to achieve adequate control of septoria tritici blotch. Fungicide resistance in Canadian *M. graminicola* isolates has not yet been reported.

2.3.4 Genetic resistance

The most economical and environmentally sound control method for a producer to reduce losses associated with septoria tritici blotch is to plant cultivars with durable genetic resistance. Host plant resistance is the ability of the host to hinder the growth and/or development of the pathogen (Parlevliet 1979; Robinson 1969). A number of review articles have been published over the past four decades on the genetics of resistance and breeding of wheat for resistance to septoria tritici blotch (Eyal 1999b; Goodwin 2007; King et al. 1983; Shipton et al. 1971).

Identifying disease resistance sources is one of the most important objectives for resistance-breeding programs. Mann et al. (1985) reported that the best sources of resistance to septoria tritici blotch are from Russian winter wheat cultivars (Aurora, Bezostaya 1, Kavkaz), wheat lines from Argentina, Brazil and Uruguay, as well as lines from the United States. High levels of resistance to *M. graminicola* have been found by developing synthetic hexaploid lines through crosses between durum wheat and accessions of *Aegilops squarrosa* (syn. *Ae. squarrosa* L., syn. *Triticum tauschii*) (van Ginkel and Rajaram 1999). *Triticale* and the *Triticum* species (*T. dicoccum*, *T. carthicum*, *T. polonicum*, *T. pyramidale*, and *T. turgidum spp. durum*) have been reported to have high-level resistance to *M. graminicola* (King et al. 1983; Rosielle 1972).

Although high levels of resistance to *M. graminicola* have been identified; such resistance was attributed to tall plant stature and late maturity (Bahat et al. 1980; Baltazar et al. 1990; Danon et al. 1982; Eyal 1981; Jlibene et al. 1992; Rosielle and Brown 1979). Taller wheat lines tended to have more widely spaced leaves (longer internodes) which reduced vertical spore spread (secondary infection cycles) from the lower to the upper leaves. Semi-dwarf cultivars (plant height between 70-90 cm) have shorter internodes. Dubin and Rajaram (1996) reported that the linkage was broken and intermediate-maturing, high-yielding semi dwarfs with high resistance to septoria tritici blotch were

developed. Arraiano et al. (2009) studied the contributions of the disease-escape traits (plant height, leaf spacing, leaf morphology and heading date) using 226 wheat lines spanning the time since scientific wheat breeding began (1860s) and found that two cultivars, Pastiche and Exsept, had resistance that could not be accounted for by the measured disease-escape traits or known isolate-specific septoria tritici blotch resistance genes.

Genetic resistance to *M. graminicola* has been reported to be monogenic, oligogenic or polygenic (Chartrain et al. 2004a). Examples of spring wheat accessions with resistance to septoria tritici blotch controlled by multiple genes with additive effects are Bobwhite "S" and Kavkaz/K4500 L.6.A.4 both of which are from CIMMYT (Centro Internacional de Mejoramiento de Maíz y Trigo / International Maize and Wheat Improvement Centre) in Mexico (Dubin and Rajaram 1996; Jlibene et al. 1994). The wheat lines/cultivars Tadinia (Somasco et al. 1996), Bulgaria 88 (Rillo and Caldwell 1966), Israel 493, Veranopolis (Wilson 1979) and ST6 (McCartney et al. 2002) have resistance to septoria tritici blotch that is controlled by single major dominant genes. Septoria tritici blotch resistance has been found to be controlled by recessive genes in wheat cultivars such as Colotana, and Klein Titan (Danon and Eyal 1990). McCartney et al. (2002) used isolates of *M. graminicola* from western Canada to determine that the high-level resistance of the wheat landrace Salamouni was controlled by three independent and incompletely dominant genes.

 To date, sixteen major resistance genes have been identified in wheat cultivars/lines and their chromosomal locations mapped (Table 2.2). Two of these genes have been used directly in wheat improvement (Goodwin 2007) . The resistance gene *Stb1* was used in the Indiana soft red winter cultivars Oasis and Sullivan (Patterson et al. 1975; Patterson et al. 1979). This gene has conferred long-term resistance to septoria tritici blotch in Indiana and parts of the Midwestern US. *Stb4* was incorporated into the cultivar Tadinia and provided resistance for about 15 years (Somasco et al. 1996). The resistance gene *Stb4* was overcome by *M. graminicola* around the year 2000 and today all cultivars with this gene are considered susceptible.

Of the sixteen mapped septoria tritici blotch resistance genes, none have been

cloned and further characterized; the basic structure and function of these resistance genes is unknown. Further marker development is needed in hexaploid wheat for fine-mapping work to be completed. Mapping resistance in diploid *Triticum* species is a possibility to help determine the function of host resistance genes in the wheat-*M. graminicola* pathosystem. To date, molecular mapping of septoria tritici blotch resistance has not been reported in tetraploid wheat, but Jing et al. (2008) recently published the first *Triticum monococcum* septoria tritici blotch resistance gene which was named *TmStb1* (accession MDR043). This resistance gene is effective against the *M. graminicola* isolate IPO323. Since *T. monococcum* is a diploid progenitor modern bread wheat and has a genome that is roughly two-thirds smaller, it should be easier to clone and study the structure and mechanisms of host resistance to septoria tritici blotch. These findings will help plant breeders identify resistance genes that are more durable for introgression into new cultivars.

Table 2.2: Chromosomal locations and molecular markers associated with sixteen major septoria tritici blotch resistance genes in wheat. Adapted and updated from Cowling (2006) and Goodwin (2007).

Gene	Original cultivar source	Chromosome location	Nearest molecular marker (cM)[A]	Nearest flanking marker (cM)	Reference
Stb1	Bulgaria 88	5BL	Xbarc74 (2.8)	AGC/M-CTA-1 (8.4)	(Adhikari et al. 2004d)
Stb2	Veranopolis	3BS	Xgwm533 (0.9)	Xgwm493 (3.7)	(Adhikari et al. 2004c)
Stb3[B]	Israel 398	7AS[B]	Xwmc83 (0.0)	Xbarc222 (2.1)	(Goodwin et al. 2008)
Stb4	Tadorna	7DS	Xgwm111 (0.7)	AGC/CAT10 (4.0)	(Adhikari et al. 2004b)
Stb5	Synthetic 6X	7DS	Xgwm44 (7.0)	Xpsr490 (21.9)	(Arraiano et al. 2001b)
Stb6	Flame, Hereward	3AS	Xgwm389 (2.0)	-[C]	(Brading et al. 2002)
Stb7	ST6 (Estanzuela Federal)	4AL	Xwmc313 (0.5)	-[C]	(McCartney et al. 2003)
Stb8	Synthetic W7984	7BL	Xgwm146 (3.5)	Xgwm577 (5.3)	(Adhikari et al. 2003)
Stb9	Courtot, Tonic	2BL	Xfbb226 (3.6)	XksuF1b (9)	(Chartrain et al. 2009)
Stb10	Kavkaz-K4500 l.6.A.4	1D	Xgwm848[D]	-[C]	(Chartrain et al. 2005a)
Stb11	TE9111	1BS	Xbarc8[D]	-[C]	(Chartrain et al. 2005c)
Stb12	Kavkaz-K4500 l.6.A.4	4AL	Xwmc219[D]	-[C]	(Chartrain et al. 2005a)
Stb13	Salamouni	7BL	Xwmc396 (7.0)	-[C]	(Cowling 2006)
Stb14	Salamouni	3BS	Xwmc500 (2.0)	Xwmc623 (5.0)	(Cowling 2006)
Stb15	Arina	6AS	Xpsr904 (14)	-[C]	(Arraiano et al. 2007)
TmStb1	MDR043 (*T. monococcum*)	7A[m]	Xbarc174 (23.5)	-[C]	(Jing et al. 2008)

[A]- Genetic distances reported in centiMorgans (cM) for the closest molecular marker indicated.

[B]- The location of *Stb3* was originally reported to be 6DS. Recently *Stb3* was reported to be on chromosome 7AS.

[C]- No flanking marker identified

[D]- *Stb10*, *Stb11*, and *Stb12* mapped as quantitative trait loci

2.4 Molecular mapping of traits in wheat

2.4.1 Molecular markers

Molecular markers are representations of heritable differences between individuals. These differences, which are also referred to as polymorphisms, can be due to simple sequence repeats (SSRs), insertions or deletions (InDels), single nucleotide polymorphisms (SNPs), or rearrangements (translocations or inversions). Differences in DNA sequence can be detected by use of restriction endonuclease, nucleic acid hybridization, or DNA sequence amplification. Amplification-based markers use the polymerase chain reaction (PCR) (Saiki et al. 1988). The invention of PCR has allowed development of new marker types that amplify specific DNA fragments such as random amplified polymorphic DNA (RAPD) (Williams et al. 1990), amplified fragment length polymorphism (AFLP) (Vos et al. 1995), and simple-sequence repeats (SSR) (Lagercrantz et al. 1993).

The decision to use a marker system in a breeding program depends on the plant species, resources available and the objective of the study. In field crop research, there are three main uses of molecular markers: (i) genetic diversity and variation assessment for germplasm; (ii) determining genomic regions associated with qualitative or quantitative traits; and (iii) marker-assisted selection for traits or reconstitution of the recurrent parent in backcrossing (Ribaut and Hoisington 1998; Ribaut et al. 2002).

2.4.1.1 Restriction Fragment Length Polymorphisms (RFLP)

Restriction fragment length polymorphisms (RFLP) are differences in DNA fragment lengths due SNPs or InDels detected by digestion with restriction endonucleases and hybridization with probes. This type of marker is highly reproducible and co-dominant (Gupta et al. 1999). The RFLP marker technology has been used extensively in plant genome mapping (Tanksley et al. 1989) and has been extensively reviewed (Botstein et al. 1980). The basis of the RFLP analysis is to hybridize labelled cDNA probes to a Southern blot (Southern 1975) of DNA digested with different restriction enzymes. The fragments are visualized by the hybridized probes. Alleles are

identified in samples by size differences of fragments and banding patterns.

The first whole-genome genetic maps were constructed using RFLP technology for several crop species such as maize (Helentjaris et al. 1986), tomato (Bernatzky and Tanksley 1986), and rice (McCouch et al. 1988). Comparative genomics of plant species were facilitated by use of RFLPs (Gale and Devos 1998; Paterson et al. 2000). The RFLP marker technology is of limited use in wheat since only 10% of RFLP loci have been reported to be polymorphic (Röder et al. 1998). The low frequency of polymorphism can be attributed to the polyploid nature of the crop, the large genome size, and high proportion of repetitive DNA. One advantage of RFLP technology is that PCR-based markers can be developed easily from probe sequences.

A number of limitations reduces widespread adoption of RFLPs. Initial development of RFLP probe sets and markers is a multi-step process which is labour intensive. The RFLP analysis requires a large amount of high-quality DNA per reaction (1 – 10 µg) which can be difficult to extract for large populations or when plant material is limited. The procedure also does not adapt well to automation or multiplexing and is therefore not ideal for high-throughput genotyping. Probes used for RFLP analysis must be physically maintained and are difficult to transfer between laboratories.

2.4.1.2 Microsatellites (SSRs)

Microsatellites or Simple-Sequence Repeats (SSRs) are usually co-dominant markers composed of tandemly repeated nucleotide motifs (di-, tri-, tetra-, penta-, and hexa-) flanked by unique sequence. SSRs can arise in the genome through DNA polymerase slippage during DNA replication. Simple di-nucleotide repeats are the most common type of SSR found in genomes, however, tri- and tetra-nucleotide repeats are found to a lesser extent (Lagercrantz et al. 1993). SSRs are ubiquitous, highly polymorphic and randomly dispersed throughout repetitive DNA (Lagercrantz et al. 1993; Tautz and Renz 1984). Studies have found that SSRs tend to be locus specific and co-dominant which makes them ideal for use in polyploid wheat research (Somers 2004). SSRs can be found by searching expressed sequence tag (EST) databases, or from

genomic libraries/sequences (Gupta et al. 2003; Thiel et al. 2003).

SSRs are PCR based and are commonly used in wheat molecular mapping. The size difference of amplified fragments between individuals can be very small (2 bp), therefore, high-resolution electrophoresis such as polyacrylamide gel or capillary electrophoresis is needed to visualize the fragments. The main limitations of SSR markers are the costs for development and for the high-resolution gel equipment required for high-throughput genotyping. Multiplexing SSRs is possible, however, the development of the multiplex assay can be labour intensive (Hayden et al. 2008). SSRs are generally multiplexed by amplifying each microsatellite separately and pooling the PCR fragments either based on size or fluorescent label prior to electrophoresis.

2.4.1.3 Single Nucleotide Polymorphisms (SNPs)

A new type of molecular marker is based on single nucleotide polymorphisms (SNPs). SNPs are single point mutations between individuals within a given DNA sequence and are the most common DNA sequence polymorphism in genomes (Sachidanandam et al. 2001).

The best method to identify SNPs has been DNA sequencing, especially the Sanger sequencing method (Sanger et al. 1977). This method can be difficult for large-scale SNP detection in complex plants such as common wheat due to its polyploid genome. A major resource in SNP detection is the large number of ESTs that have been identified in hexaploid wheat (Qi et al. 2004). Sequence information from ESTs can be used to develop forward and reverse primers to amplify specific DNA fragments. These amplified fragments can be sequenced between genotypes using DNA sequencing with SNPs identified based on single point mutations.

Gel-based methods have been developed to simplify SNP screening. The methods include cleaved amplified polymorphic sequence (CAPS), and single stranded conformation polymorphism (SSCP) (Konieczny and Ausubel 1993; Prosser 1993). Agarose or polyacrylamide gels are used to separate the PCR products to visualize polymorphic SNPs. Since gel electrophoresis is required, these types of SNP assays are

not suited to high-throughput genotyping.

Melting curve analysis using real-time PCR has been used successfully to identify SNPs between individuals (Akey et al. 2001). The theory of the RT-PCR method is based on differences in denaturing temperatures for the targeted DNA sequences due to differences in the GC content. The PCR product with a higher GC content will denature at a higher temperature when compared to a PCR product with a lower GC content. Melting curve analysis does not require gel equipment, but relies on differences in the double stranded DNA melting point which is detected with a 'dissociation step' PCR to measure fluorescence intensity of the PCR product to be measured in a linear denaturation step from 60-95°C. Fluorescence is created by the addition of SYBR Green 1 dye (Roche Diagnostics, Mannheim, Germany) to the PCR reaction mix. Insertion-site based polymorphism (ISBP) markers have used melting curve analysis to successfully screen SNPs (Paux et al. 2010).

High-throughput assays have been developed for SNP-based markers; however, most assays still require marker-specific primers (Invader), probes (Taqman), or oligonucleotides (single feature polymorphism arrays). The development cost for marker discovery and assay development makes SNP analysis difficult to implement in many crop species.

2.4.1.4 Diversity ARrays Technology (DArT)

Diversity ARrays Technology (DArT) has been developed to overcome some of the limitations of the previously described marker systems (Jaccoud et al. 2001). DArT is hybridization-based and is a cost-effective method for DNA fingerprinting that uses procedures similar to microarray technology. The DArT assay simultaneously tests for potentially thousands of SNP and InDel polymorphisms. The DArT procedure can be broken down to five basic steps: (i) genomic library construction, (ii) microarray construction using the genomic library, (iii) labelling genomic representations, (iv) hybridization of labelled samples to the microarray followed by slide washing, and (v) microarray reading and data processing/analysis (Akbari et al. 2006).

DArT markers depend on accurate genotype scoring through array image reading and post processing (Kilian et al. 2005). Wenzl et al. (2004) developed software (DArTsoft) which can analyze large amounts of data generated from each DArT experiment and scores genotypes. Triticarte Pty Ltd (http://www.triticarte.com) has been established to provide a high-throughput genotyping service to plant breeders worldwide.

Most marker technologies are limited since they are dependent on gel electrophoresis which is time and labour intensive. While polyacrylamide gels can separate about 50-150 fragments at one time, array-based markers such as DArT have the potential to provide information on more than 10,000 markers at one time (Jaccoud et al. 2001). An additional benefit to DArT markers is that, in contrast to SSR or SNP markers, sequence information is not required for development. This is an important consideration when selecting a marker system for use in complex polyploidy crops such as common wheat.

2.4.2 Genetic map construction

DNA-based molecular markers have been used extensively to develop genetic linkage maps for different crop species. The main use of the genetic maps has been to identify genomic regions associated with simply inherited traits (single major gene) or complex traits controlled by several genes (polygenic) (Langridge et al. 2001). The positions of molecular markers are indicated on genetic maps relative to other molecular markers along a chromosome. Genetic mapping is based on segregation of genes and markers through chromosome recombination during meiosis. The degree of linkage is measured as the frequency of recombinant genotypes in progeny of a segregating population. Genetic distance will be small between genes or markers in areas of a chromosome where recombination is low. Large genetic distances indicate chromosomal regions of high recombination. Genes or markers with low recombination frequency are tightly linked and will more frequently be transmitted together from parents to progeny. Genetic maps are constructed by studying the segregation of many polymorphic markers across many individuals.

Constructing genetic maps using large populations and high numbers of markers is simplified by using computer software packages such as JoinMap 4.0 (van Ooijen 2006) or MAPMAKER (Lander et al. 1987). The accuracy of genetic distances and marker order of a genetic map is related to the number of individuals and the number of polymorphic markers across the plant genome. Young (1994) reported that a mapping population should ideally consist of a minimum of 50 individuals.

Often markers are not evenly distributed across a genome and appear clustered in chromosomal areas of low recombination. Recent studies on the recombination frequency along the 1-Gb chromosome 3B in wheat reported that 90% of crossovers occurred in the distal subtelomeric region and very few crossovers were observed around the centromere region (Saintenac et al. 2009). They also found that the physical distance of large genetic distances can be very small. Environmental stresses, such as extreme temperatures, during plant development have been linked to altered recombination frequency (increased or decreased chiasma) in certain crop species (Wijnker and de Jong 2008), therefore, growing conditions for producing mapping population can be very important.

The limitations in genetic map construction have been the lack of available genetic markers and the polyploid nature of many important crop species. The first genetic maps constructed for plant species (rice, maize, tomato and soybeans) only used RFLP markers (Tanksley et al. 1989). Similarly, the first genetic maps of common wheat were constructed using RFLP markers (Chao et al. 1989). Wheat has a lower rate of polymorphism compared to other cereal crops and requires more molecular markers for good coverage of its hexaploid genome. Further more, the level of polymorphism of wheat is not consistent among the three genomes, the D genome tends to be more conserved which makes mapping more difficult. Since 1989, a number of genetic maps have been constructed for bread wheat (Gupta et al. 1999; Röder et al. 1998; Song et al. 2005). A high-density microsatellite map of bread wheat was developed by Somers et al. (2004) by combining four individual genetic maps by consensus. The consensus map includes 1,235 microsatellite marker loci covering 2,569 cM with an average interval

distance of 2.2 cM (Somers et al. 2004). Molecular mapping of many important traits in wheat has been facilitated by the development of the bread wheat microsatellite consensus map.

2.4.3 Mapping populations

A number of population types can be used in genetic map construction such as doubled haploid (DH), recombinant inbred lines (RILs), F_2 and backcross populations. Two of the most common populations used for mapping are DH lines and RILs. These populations have a number of benefits such as being homozygous at all or the majority of loci. They are also immortal populations that will produce identical progeny after selfing each line. In comparison, F_2 populations have a high degree of genetic variation (50% heterozygous) and are ephemeral (Varshney et al. 2004). DH lines from F_1 plants can be developed quickly in wheat using the wheat-maize pollination system followed by embryo rescue and chromosome doubling of the haploid plants with colchicine (Sleper and Poehlman 2006). Recombinant inbred lines are easier to develop since less labour is required, however, a number of generations of selfing are required which is time consuming. The single seed descent (SSD) breeding scheme is used in the development of RILs. Usually six generations of selfing are required for a RIL population to achieve about 98.3% homozygosity (Sleper and Poehlman 2006), although it has been reported that some genomic regions tend to remain heterozygous longer than expected due to low levels of recombination (Burr and Burr 1991). The level of recombination is much higher in a RIL population compared to a DH population due to more generations of meiosis which can result in a higher-resolution genetic map. Generally DHs are the favoured population type for extensive mapping projects since they are immortal, should be homozygous at all loci and can be produced relatively quickly (Varshney et al. 2004). A larger number of DH lines would be required for mapping to achieve a genetic map of the same resolution as a RIL mapping population.

2.4.4 Mapping traits

The approaches used to map the chromosomal location(s) controlling a trait of interest are dependent on genetic inheritance.

2.4.4.1 Bulked Segregant Analysis (BSA)

Qualitative traits vary in kind and are easily classified into categories. Qualitative traits are controlled by single or a few major genes (Sleper and Poehlman 2006). Disease resistance is an example of a trait that can considered qualitative. Bulked segregant analysis (BSA) was developed by Michelmore et al. (1991) as a method to identify molecular markers linked to qualitative traits. The BSA strategy involves creating and comparing two pools (bulks) of individuals from a segregating population produced from a bi-parental cross. The theory is that the individuals combined in the bulk will be identical for the trait and target locus but random for the remainder of the loci. Michelmore et al. (1991) was able to identify RAPD markers tightly linked to downy mildew (*Peronospora parasitica*) resistance in lettuce (*Lactuca sativa*) from F_2 progeny from a bi-parental cross. In theory, it is possible to identify markers with association to the trait up to 25 cM away from the targeted locus (Michelmore et al. 1991).

The number of polymorphic markers and the distribution of the polymorphic markers across the genome limit application of BSA. Bulked segregant analysis can also be used to identify quantitatively inherited traits, however, RILs or DH populations should be used to increase the probability of finding markers linked to the locus/loci controlling the trait (Quarrie et al. 1999).

2.4.4.2 Quantitative trait loci (QTL)

Complex traits with continuous distribution are quantitatively inherited and controlled by many genes at several loci with varying degrees of contribution (Tanksley 1993). These traits are generally influenced by the environment. Compared to mapping a qualitative trait, mapping a quantitative trait locus (QTL) can be much more challenging. Identifying a QTL is useful for marker-assisted selection and germplasm

enhancement and is helpful in determining the genetic basis of complex traits (Asins 2002).

Sax (1923) was the first researcher to identify the principle of QTL analysis when it was reported that a linkage existed between a major seed coat pigmentation gene and the quantitative trait seed weight in beans (*Phaseolous vulgaris*). At that time, QTL analysis was not feasible on a large scale due to lack of genetic markers. With the invention of methods to genotype a species and construct genetic maps using DNA-based markers such as RFLPs, more QTL studies were completed (Botstein et al. 1980).

The principle of QTL analysis involves a systematic search for linkage disequilibrium between molecular markers and QTL. Mapping a quantitative trait can be more difficult and involve more complex analyses since the genes controlling the trait may also be epistatic and be influenced by the environment (Mackay 2001).

Many QTL mapping methods have been reported with different levels of complexity. The simplest QTL analysis method is referred to as single-factor analysis (SFA; also referred to as single-marker analysis) and involves testing the association between single molecular markers and the trait of interest (Edwards et al. 1987). Differences between genotype means can be tested using a one-way analysis of variance (ANOVA), t-test or linear regression (Liu 1998). This method is limited by not providing a map position for the QTL. Single-factor analysis is most useful for fast QTL detection.

A second method of QTL detection is interval mapping (simple interval mapping; SIM) developed by Lander and Botstein (1989). Simple interval mapping requires a complete genetic map and assumes only one QTL is present. This method involves testing association between a trait and marker positions within marker intervals by calculating a QTL likelihood map. The likelihood for a segregating QTL is calculated for each position of the genome. The likelihood ratio is equal to the logarithm of the odds (LOD) score. Population type (DH, RIL, F_2), population size, genome size, marker density, marker type and missing data will impact the threshold LOD value (Hackett and Broadfoot 2003). Generally, a QTL is declared when the LOD score is greater than 3.0, which is approximately odds of 1000:1 (Lander and Botstein 1989). Permutation tests

have often been used to calculate the significance threshold (Churchill and Doerge 1994). To quickly identify QTL, the LOD scores can be plotted against the linkage groups and the LOD threshold can be indicated at a constant Y value. When the LOD score is larger than the LOD threshold, a QTL is declared. The maximum peak of a QTL is the estimated position of the QTL on the linkage group. Intervals of one and two-LOD are fitted around the QTL as position error.

A third method of QTL detection named composite interval mapping (CIM) was created by Zeng (1994) to address some of the limitations of single-factor analysis and simple-interval mapping. Composite interval mapping has also been referred to as multiple-QTL mapping (MQM) by Jansen (1993). The CIM method is based on estimating the location of a QTL between two markers, while QTL in other genomic regions are accounted for by regression analysis. The CIM calculations are complex due to fitting of parameters for the target intervals and background markers simultaneously. Several computer programs such as MapQTL 5.0 (van Ooijen 2004) and QTL Cartographer (Wang et al. 2010) have been created along with the various QTL mapping methodologies to perform the complex algorithms.

Large populations are required for high-resolution QTL mapping, which are generally not feasible for traits which are difficult or expensive to measure (Buckler and Thornsberry 2002). In many plant species, the relationship between genetic distance and physical distance is variable due to recombination 'hot spots' and 'cold spots' (Wijnker and de Jong 2008). The physical-to-genetic distance ratio in wheat has been reported to vary from 0.02 to 16.7 megabase pairs per centiMorgan (Mb cM^{-1}) (Akhunov et al. 2003; Spielmeyer et al. 2000). It is very probable that a wheat QTL spanning only 10 cM contains hundreds of genes. Finding and testing candidate genes from such a large region is not feasible; therefore, a smaller QTL interval and a smaller number of genes must be identified through fine mapping. This process involves "Mendelizing" the identified QTL through mapping in a large population (>1000 individuals) created to localize the QTL to an interval often less than one centiMorgan (Chicaiza et al. 2006; Price 2006). Fine mapping has been used in wheat for a number of QTL such as grain protein content

(Olmos et al. 2003), grain weight (Röder et al. 2008), preharvest sprouting (Torada et al. 2008), leaf and stem rust resistance (Spielmeyer et al. 2008), and fusarium head blight resistance (Cuthbert et al. 2006). Fine mapping a QTL is generally an expensive process and should be used for a QTL with a large effect since it is difficult to assess the phenotypic differences of a small-effect QTL (Price 2006).

2.4.5 Marker-assisted selection and breeding

Marker-assisted selection (MAS) is the process of using molecular markers in genomic regions that are tightly linked to gene(s) of interest in segregating populations (Ribaut and Hoisington 1998). Using molecular markers to select for traits can be useful when a trait has a low heritability, is difficult/expensive to phenotype or is highly influenced by the environment. Progress in breeding for tolerance to abiotic stresses, resistance to certain biotic stresses, quality traits, or quantitative traits could be improved by using MAS (Mohan et al. 1997). Marker-assisted selection can be useful in disease resistance breeding by allowing gene pyramiding and selection of linked resistance genes without the need for extensive phenotypic segregation ratio testing (Young et al. 1988).

The usefulness of MAS for a plant breeder depends on three main requirements: 1) the marker and gene should co-segregate or be tightly linked (≤ 1 cM); 2) there should be efficient procedures to screen large populations with reproducible results; and 3) the marker screening technique should be economical and user friendly (Mohan et al. 1997). DNA isolation from a large number of individuals remains a challenge for adoption of MAS on a large scale in many small- to medium-sized plant breeding programs. Marker-assisted selection can be very useful for backcross breeding schemes since it allows for selection of the desired marker(s) linked to the trait of interest from the donor parent and also helps accelerate recovery of the recurrent (elite) parent genome (Visscher et al. 1996). It has been reported that trait selection and fixation of about 87% of the elite parent can be achieved in one backcross generation using MAS (Somers et al. 2005). The cost associated with MAS can be offset by the shorter cultivar development time (Dreher et al. 2003; Morris et al. 2003).

3.0 Quantitative trait loci (QTL) for septoria tritici blotch resistance and grain yield in a Pastiche/Torfrida winter wheat cross

3.1 Abstract

Quantitative trait loci (QTL) for septoria tritici blotch (Stb) resistance, grain yield and grain yield components were identified in a winter wheat doubled-haploid (DH) population of 130 lines produced from a cross between the Stb-resistant cultivar Pastiche and the Stb-susceptible cultivar Torfrida. A genetic map of the cross was constructed using 104 microsatellite markers and 202 Diversity ARrays Technology (DArT) markers. Four field trials for disease during the 2006/07 growing season, and four field trials for yield during the 2007/08 and 2008/09 growing seasons were conducted in wheat-producing regions of England and Denmark. Disease field trials were subject to natural infection by *Mycosphaerella graminicola* and lines were scored to measure flag leaf pycnidial density for septoria tritici blotch infection. The disease-escape traits height to flag leaf and heading date were also measured. Grain yield, thousand-kernel weight and test weight were measured on the DH population in the yield field trials. A total of 16 QTL was identified for Stb resistance, grain yield, and yield components. A QTL for Stb resistance (*QStb.jic-2A*) reduced flag leaf pycnidial density by 31.2% compared to the population mean when the Pastiche allele was present. A second QTL, named *QYld.jic-2A*, co-localized with *QStb.jic-2A*, and conferred an increase in grain yield of about 2.0% (0.19 t ha^{-1}). The plant height QTL identified on chromosomes 1A and 6A were observed in both disease and yield field trials. The positive alleles of these two plant height QTL co-localized with the positive alleles for thousand-kernel weight QTL. No QTL were identified for test weight.

3.2 Introduction

Bread wheat (*Triticum aestivum* L.) is the most important food crop in the world with annual global production estimated to be over 600 million tonnes in recent years (FAOSTAT 2010). The world population is growing rapidly and demand for wheat in

the year 2020 is estimated to be one billion tonnes (Rajaram 2001). To meet the projected demand, global wheat production will need to increase at a rate of 1.5% per year.

Improving bread wheat cultivars is challenging for plant breeders, due to the genome size and complexity of wheat. Bread wheat is an allopolypoid species with three homeologous genomes and a total haploid genome size of 1.6×10^{10} bp (Arumuganathan and Earle 1991). The main objectives of wheat breeding programs are to increase grain yield while maintaining or improving end-use quality traits and incorporating disease and pest resistance. The large number of residue-borne diseases has caused plant breeders difficulty in incorporating durable genetic resistance to all possible wheat pathogens.

Septoria tritici blotch (Stb), which is caused by *Mycosphaerella graminicola* (Fückel) J. Schröt. in Cohn, is an important foliar disease in many wheat-producing temperate regions with yield losses estimated to be 25 to 50% during severe epidemics (Eyal et al. 1985; King et al. 1983). Management of this devastating foliar disease is becoming more difficult with recent reports of resistance to strobilurin and triazole fungicides (Brunner et al. 2008; Fraaije et al. 2005). The most economical and sustainable method for producers to control Stb is to use wheat cultivars with durable genetic resistance. Recent research has found that producers tend to select wheat cultivars which are high yielding but susceptible to septoria tritici blotch (Arraiano 2008). The effort by wheat breeders to incorporate genetic resistance to Stb may have resulted in a cost to grain production. Studies which have located resistance loci to *M. graminicola* have not considered the effect of resistance on grain yield and yield components.

The genetic resistance mechanisms to Stb are not as well understood as for other foliar diseases of wheat such as powdery mildew and leaf rust. Both qualitative and quantitative resistance have been reported for septoria tritici blotch (Narvaez and Caldwell 1957). Qualitative genetic resistance to Stb is controlled by single major loci and follows the gene-for-gene model (Brading et al. 2002). This type of resistance confers a near-immune type response to specific isolates of the *M. graminicola* population. Quantitative or partial resistance is controlled by many genes with small

additive effects and is non-isolate specific (Brown et al. 2001).

Fifteen major genes (named *Stb1* to *Stb15*) for resistance to Stb have been identified and mapped with various types of molecular markers in hexaploid wheat (Adhikari et al. 2003; Adhikari et al. 2004b; Adhikari et al. 2004c; Adhikari et al. 2004d; Arraiano et al. 2007; Brading et al. 2002; Chartrain et al. 2005a; Chartrain et al. 2005c; Chartrain et al. 2009; Cowling 2006; Goodwin 2007; McCartney et al. 2003). Recently, the Stb resistance gene *TmStb1* was mapped in the diploid *Triticum monococcum* to the short arm of chromosome 7A^m (Jing et al. 2008). Quantitative trait loci (QTL) have been identified by Eriksen et al. (2003) for partial Stb resistance on chromosomes 2B, 3A, 3B, 6B and 7B in the wheat cv. Senat. QTL for seedling-stage and adult-plant Stb resistance have also been identified on chromosomes 1D, 2D, 3D, 6B, and 7B (Simon et al. 2004b).

Wheat breeders have successfully used major Stb resistance genes in cultivar development. The *Stb1* gene has been used effectively for over 25 years in wheat cultivars across areas of the USA prone to Stb (Adhikari et al. 2004d) and is considered to provide durable resistance. Another Stb resistance gene, *Stb4*, was only effective for about 15 years before it was overcome (Adhikari et al. 2004b). Marker-assisted selection has given plant breeders the ability to pyramid major resistance genes into new cultivars efficiently and effectively. It is hypothesized that a cultivar with two or three resistance genes to a single pathogen will help slow down the mutation of pathogen virulence. Presently, it is not known (with certainty) if the resistance to a pathogen will be prolonged by pyramiding major resistance genes. Quantitative resistance does not follow the gene-for-gene model; therefore, pyramiding QTL for disease resistance could be the best method for developing sustainable resistance to *M. graminicola* since a pathogen may not be able to overcome the resistance. Progress in this area has been slow due to the complexity in validating the small effects of resistance QTL. New methods to increase the speed of detecting disease resistance QTL are possible with the recent invention of high-throughput molecular marker technologies such as Diversity Arrays Technology (DArT) (Akbari et al. 2006).

Arraiano et al. (2009) recently identified potential new sources of partial field

resistance in the winter wheat cultivars Pastiche and Exsept that could not be accounted

for by known isolate-specific Stb resistance genes or disease-escape traits such as plant

height, leaf spacing, leaf morphology and heading date. The objective of the current

study was to construct a genetic map of a winter wheat cross Pastiche/Torfrida using

Diversity ARrays Technology (DArT) and microsatellite (SSR) markers and to determine

the quantitative trait loci (QTL) responsible for septoria tritici blotch resistance, grain

yield and yield components.

3.3 Materials and Methods

3.3.1 Population development

The Stb-resistant hexaploid winter wheat cultivar Pastiche (Pedigree:

Jena/Norman) was crossed to the genetically diverse and Stb-susceptible winter wheat

cultivar Torfrida (Pedigree: Rendezvous//Moulin/Mercia) at the John Innes Centre (JIC,

Norwich, UK). A DH population with a total of 130 lines was developed using the

standard wheat x maize hybridization and embryo rescue technique (Laurie and Bennett

1988). Presence of isolate-specific resistance genes in Pastiche and Torfrida was

determined previously by Arraiano and Brown (2006). The cultivar Pastiche was

previously identified to possess Stb resistance not accounted for by a major resistance

locus or disease-escape traits (Arraiano et al. 2009).

3.3.2 Genetic map construction

A random subset of 90 DH lines was selected for genetic map construction. DNA

was extracted from fresh two-week-old leaf tissue of two plants from each line using the

Qiagen DNeasy 96 Plant Kit (Qiagen, Mississauga, ON, Canada) according to the

manufacturer's procedures. DNA samples were diluted to 100 ng/µl and 10µl of the

diluted DNA was sent for DArT marker analysis by Triticarte Pty. Ltd. (Canberra,

Australia; http://www.triticarte.com.au) using the Wheat *Pst*I(*Taq*I) Version 2.3 DArT

array of 2500 clones. Triticarte conducted the hybridization of genomic DNA to the

DArT array, analyzed images, and scored polymorphic markers as described previously

by Akbari et al. (2006).

Microsatellite (SSR) marker genotyping data were obtained using HotStarTaq Master Mix (Qiagen) (Qiagen 2005) and M13 primer tailing (6-FAM, NED, PET or HEX labels) (Schuelke 2000). Fluorescent PCR fragments were separated using capillary electrophoresis on an ABI3700 Genetic Analyzer (Applied Biosystems, Foster, CA, USA). Approximately five SSR markers from each chromosome were selected to cover the hexaploid wheat genome adequately. Selected SSR markers were known to be polymorphic between Pastiche and Torfrida from previous association mapping studies (Dr. Lia S. Arraiano, Vilmorin SA, pers. comm.). The map positions of the selected SSR markers were found using the Komugi composite wheat map (http://shigen.lab.nig.jp/wheat/komugi/maps/markerMap.jsp). The 101 SSR primer pairs detected a total of 115 loci. SSR primer pairs were obtained from the following sources: 32 from the BARC set (Song et al. 2005), two from the CFA/CFD set (Grain Genes, http://wheat.pw.usda.gov), 43 from the GWM set (Röder et al. 1998), three from the GDM set (Pestsova et al. 2000), seven from the PSP set (Stephenson et al. 1998), 15 from the WMC set (Somers et al. 2004) and five EST-SSR with the suffix DuPw (Eujayl et al. 2002). The SSR psp3029 was found to have excessive missing data (>30%) and was excluded from linkage analysis. Microsatellite analysis revealed five DH lines with multiple heterozygous loci; therefore, these individuals were removed from genetic map construction and QTL analysis. The final mapping population had a total of 85 individuals.

DArT and SSR marker data were combined to construct a genetic map of the Pastiche/Torfrida population using the software package JoinMap 4.0 (van Ooijen 2006). Linkage between loci and the map distances were calculated using the Kosambi mapping function (Kosambi 1944). A logarithm (base 10) of the odds (LOD) score equal to or greater than 3.0 was set as the threshold to determine linked loci. The chromosomal locations of DArT markers were mostly unknown during map construction, therefore, microsatellite markers were used to anchor linkage groups to the 21 haploid chromosomes of hexaploid wheat using previously published genetic maps (Akbari et al.

2006; National Bioresource Project 2009; Röder et al. 1998; Somers et al. 2004; Song et al. 2005). The order of the SSR markers determined the orientation of linkage groups.

3.3.3 Disease field trials

Four field trials for disease evaluation were conducted during the 2006/07 growing season in wheat-producing areas of England and Denmark (Table 3.1). Plots were grown under normal crop management conditions, including applications of fertilizer, plant growth regulator, and herbicides but not fungicides (Appendix 8.1). Each trial was arranged in randomized complete blocks with two replicates. Prior to seeding, all trial sites were ploughed, disced, and harrowed. Plots in all environments were seeded late October 2006 in squares of 1.0 x 1.5 m seeded at a rate of 200 seeds m^{-2}. Natural infection of *Mycosphaerella graminicola* was scored for each plot based on percentage leaf area covered by necrotic lesions bearing pycnidia. Scoring for each disease field trial was conducted when about 30% pycnidial coverage of flag leaf was observed on the susceptible parent cultivar Torfrida. Ratings were optimal at this time since senescence was not evident on the flag leaves. For all disease field trials, Stb was scored on ten randomly selected flag leaves from culms in the middle rows of each plot. Ratings were completed on July 17 for location Adv07, July 13 for location Nic07, July 3 for location Syn07, and July 10 for location Sej07. Days to heading (Hdg) was recorded when 50% of spikes were half emerged in a plot (GS55) (Zadoks et al. 1974). Plant height to flag leaf (Ht) was measured on three representative tillers at maturity (GS85) (Zadoks et al. 1974).

3.3.4 Yield field trials

Four yield field trials were conducted in wheat-producing areas of England and Denmark. Two of the yield field trials were completed during the 2007/08 growing season and the remaining two were completed during 2008/09. Each trial was arranged in randomized complete blocks with two replicates. Prior to seeding, all trial sites were ploughed, disced, and harrowed. Geographical locations of the yield field trials are

summarized in Table 3.1. A total area of 5.3 m^{-2} for Els08, 3.2 m^{-2} for Syn08, 8.1 m^{-2} for Sej09, and 5.7 m^{-2} for SW09 was sown and harvested for each plot. All four trials were seeded at a rate of 200 seeds m^{-2}. Plots were grown under normal crop management conditions, including applications of fertilizer, plant growth regulator, herbicides, and fungicides (Appendix 8.1). Yield field trials were harvested mid-August in each year. Grain yield and moisture were measured per plot and used to calculate yield in t ha^{-1} at 13% moisture. The yield component thousand kernel weight (Tkw) was measured for all yield field trials by counting and weighing one thousand seeds randomly selected from each plot. Test weight (Twt) was measured on each plot for the trials indicated in Table 3.1using the test weight module on a FOSS Infratec 1241 near-infrared transmittance (NIT) machine.

Table 3.1: Summary of disease and agronomic data collected for the each of the disease and yield field trials conducted between 2006 and 2009 at sites in England and Denmark.

Environment[A]	Field trial site	Field trial location	Field trial type	Stb	Ht	Hdg	Twt	Tkw	Yld
Adv07	Advanta Seeds Ltd	Docking, Norfolk, UK	Disease	✓	✓	✓			
Nic07	Nickerson UK Ltd	Woolpit, Suffolk, UK	Disease	✓	✓	✓			
Syn07	Syngenta Seeds Ltd	Whittlesford, Cambridgeshire, UK	Disease	✓	✓	✓			
Sej07	Sejet Plantbreeding	Horsens, DK	Disease	✓	✓	✓			
Els08	Elsoms Seeds Ltd	Spalding, Lincolnshire, UK	Yield	✓			✓	✓	✓
Syn08	Syngenta Seeds Ltd	Whittlesford, Cambridgeshire, UK	Yield					✓	✓
Sej09	Sejet Plantbreeding	Horsens, DK	Yield	✓	✓		✓	✓	✓
SW09	SW Seed Ltd	Abbots Ripton, Cambridgeshire, UK	Yield					✓	✓

[A]- Suffixes: 07, 2006/2007; 08, 2007/2008; 09, 2008/2009.

Stb = septoria tritici blotch (*Mycosphaerella graminicola*). Ht = plant height to flag leaf. Hdg = days to heading. Twt = test weight. Tkw = thousand kernel weight. Yld = grain yield.

✓ indicates the trait was scored in that trial. A blank indicates the trait was not scored.

3.3.5 Statistical analysis

DArT markers are bi-allelic dominant markers. Each marker was scored as Present (1) or Absent (0) for each line. A marker which could not be scored reliably for a particular line was recorded as a missing value. Marker quality was estimated by calculating a percentage P value (0-100) which determined how well the two phases were separated in the sample. This study used only DArT markers with P values greater than 80.

3.3.5.1 Disease field trials

Since septoria tritici blotch (Stb) was measured using a percentage scale, the ratings were transformed using a logit-transformation. The mixed model procedure (PROC MIXED) of SAS (SAS v9.2, SAS Institute Inc., Cary, NC, USA) was used for Stb, Ht, and Hdg to generate an lsmean for each line by fitting Entry, Environment, Replicate(Environment), and Entry x Environment. The Entry term was considered fixed while all other terms were random. The correlations of Stb with Ht and Stb with Hdg were both non-significant, therefore, the Stb means were not adjusted for the effects of Ht and Hdg. Non-adjusted Stb means were used for QTL analysis. To determine if the parent cultivars Pastiche and Torfrida were significantly different for a particular trait, an unpaired t-test was calculated.

3.3.5.2 Yield field trials

All traits measured in yield field trials (Ht, Hdg, Tkw, Twt, and Yld; Table 3.1) were analyzed using the mixed model procedure (PROC MIXED) of SAS (SAS v9.2, SAS Institute Inc., Cary, NC, USA) since parent cultivars were missing from Sej09 and led to an unbalanced data set. Each environment was analyzed separately and then combined as site-years rather than by site and by year. The Entry term was considered a fixed effect while all other terms, Replication, Environment, Replicate(Environment), and Entry x Environment, were considered random in the combined model. To determine if the parent cultivars Pastiche and Torfrida were significantly different for a

particular trait, an unpaired t-test was calculated.

3.3.6 QTL analysis

QTL analysis was completed using the computer software package MAPQTL 5.0 (van Ooijen 2004). The non-parametric Krustal-Wallace procedure was performed on each trait (Stb, Hdg, Ht, Tkw, Twt and Yld) for single-factor QTL analysis. Trait association was tested against 306 mapped and 25 unmapped markers. Markers with a *P*-value for the K-statistic of 0.05 or less were selected as co-factors for automatic cofactor selection (ACS). The cofactors identified by ACS were used in multiple QTL mapping (MQM). Permutation tests were performed for each trait to determine the threshold of the LOD score using 1000 iterations. The LOD score with a relative cumulative count of at least 0.95 was selected as the LOD threshold for each trait. This value corresponds to a *P* value of 0.05 or less. Chromosome and QTL figures were generated using the software package MapChart 2.2 (Voorrips 2002).

3.4 Results

3.4.1 Genetic map

The total set of 331 polymorphic markers (113 microsatellite markers and 218 DArT) were compared using JoinMap 4.0 software (van Ooijen 2006) to construct the genetic map for the Pastiche/Torfrida winter wheat cross. Markers which were highly similar among individuals (0.99 to 1.00% similarity) were identified (gwm88, gwm130, psp3030, wPt-4637_7A, wPt-6869_4B|7B, wPt-7285_2A, wPt-9104, and wPt-9350_2B) and were excluded from locus ordering and linkage analysis. A total of seventeen markers, including eleven DArT and six microsatellite markers, did not associate to any linkage groups. The microsatellite markers were used along with previously constructed wheat genetic maps (National Bioresource Project 2009; Röder et al. 1998; Somers et al. 2004; Song et al. 2005) to anchor the linkage groups to the twenty-one haploid chromosomes of hexaploid wheat. The complete Pastiche/Torfrida genetic map was constructed with a total of 306 molecular markers, including 104 microsatellite markers

and 202 DArT markers. These markers were assigned to thirty-two linkage groups (See Appendix 8.4 for linkage groups and chromosome assignments). The total length of the Pastiche/Torfrida genetic map was 1283.2 cM with an average marker interval of 4.2 cM. The DArT and SSR marker distribution across the chromosomes of the A, B, and D genomes is summarized in Table 3.2. The DArT and SSR marker coverage of the D genome was lower when compared to the A and B genomes. Chromosome group 4 was found to be under represented with a total of four DArT and two SSR markers.

Table 3.2: Distribution of DArT and SSR molecular markers across the three genomes (A, B and D) and the seven chromosome groups for the genetic map of the Pastiche/Torfrida DH population. The number of marker loci for each chromosome and group is indicated in each column.

Chromosome group	DArT			SSR			Marker Total		
	A	B	D	A	B	D	A	B	D
1	17	13	4	10	5	5	27	18	9[A]
2	27	18	6	10	4	7	37[A]	22	13[A]
3	3	31	2	1	12	3	4	43[A]	5[A]
4	1	2	1	1	-	1	2	2	2
5	1	9	-	6	3	3	**7**	12[B]	3
6	8	14	2	9	6	1	17[B]	20	3
7	13	28	2	10	3	4	23[A]	31[A]	6
Total	70	115	17	47	33	24	117	148	41
Percent[C]	34.7	56.9	8.4	45.2	31.7	23.1	38.2	48.4	13.4

[A]- Two linkage groups contained on this chromosome
[B]- Three linkage groups contained on this chromosome
[C]- Fraction of the total of DArT markers, SSR markers, or total markers where appropriate

A chi-square test for segregation distortion was calculated for each of the molecular markers. Out of 332 markers, 23 showed significant distortion (P ≤ 0.05), ten towards the Pastiche allele and thirteen towards the Torfrida allele. Of the 23 distorted markers, 18 were DArT and 5 were SSR, which is significantly different (χ^2 = 5.92, P = 0.01*). Three clusters of marker distortion (≥ 3 loci) were identified on chromosomes 1B, 2A, and 2D. These clusters accounted for about 56% of the distorted markers. The total number of alleles scored in the Pastiche/Torfrida mapping population was large (24,802), including 12,432 from Pastiche and 12,370 from Torfrida with no significant difference

observed (χ^2 = 0.15, P = 0.70). About 4.9% of the Pastiche/Torfrida population consisted of missing alleles. Significant differences in allele ratio for the DArT markers and SSR markers as groups were not found. DArT markers had a higher amount of missing data (6.6%) when compared to SSR markers (1.7%).

The accuracy or power of QTL analysis is not increased with marker resolution less than 10 cM (Darvasi et al. 1993). Since the Pastiche/Torfrida genetic map was found to have areas of high marker clustering and a small average marker interval distance (4.2 cM), a second genetic map was constructed for QTL analysis. This map was constructed with preference given to SSR markers since this marker type had a lower amount of missing allele data and also because SSR markers can be used directly for marker-assisted selection (MAS) in wheat breeding programs. A total of 221 markers was used to construct the genetic map for QTL analysis with a total genetic distance of 1360.9 cM and an average marker interval of 6.2 cM. The localization of QTL in areas with extensive marker clustering, such as chromosome 2A, was improved with this map.

3.4.2 Quantitative traits

Frequency distributions were plotted and analyses of variance (ANOVA) were calculated for traits measured in disease field trials and yield field trials (Appendix 8.2; Appendix 8.3). The error variances of all traits were found to have normal distributions. Transgressive segregants were observed on the upper and lower ends of the frequency distribution for all phenotypic traits measured. Phenotypic trait measurements for parents and the DH population are summarized in Table 3.3. The difference between parents for flag leaf pyncidia coverage (Stb) was large with an average of 15.7% for Pastiche and 42.8% for Torfrida. The differences in Stb resistance across the DH population was wide with a range of scores from 8.2 to 64.5% and an average score of 28.2 %. Height was significantly different between the parents (67.6 vs. 60.9 cm) in the disease field trials while no difference between the parents was observed in the yield field trials. Heading date for the parents was not different (149.2 vs. 149.5 days) in the disease field trials. Pastiche and Torfrida (9.02 and 9.92 t ha^{-1} respectively) were not different for Yld at a

significance level of 5%; but were significantly different at a10% level. Pastiche (53.3 g) had a significantly higher Tkw when compared to Torfrida (48.6 g). Test weight was not different between the parents (84.8 vs 83.3 kg hL^{-1}).

Correlation coefficients were calculated for all traits measured in disease and yield field trials for the Pastiche/Torfrida DH mapping population (Table 3.4). The correlation between yield and test weight was highly significant (0.42***); while yield and thousand kernel weight were not significantly correlated (0.17 ns). The correlation of Ht measurements between the disease and yield field trials was high (0.69***). The correlation for heading between the disease and yield trials also was high (0.71***).

3.4.3 QTL mapping

QTL analysis of the DH mapping population (85 individuals) identified 16 QTL across the combined disease and yield field trials for Stb, Ht, Hdg, Tkw, and Yld (Table 3.5, Figure 3.1, Figure 3.2). No QTL were identified for Twt. One QTL was detected for Stb resistance (LOD 5.8) on the short arm of chromosome 2A and was named *QStb.jic-2A*. This QTL, which associated with the allele from Pastiche, conferred a 31.2% decrease in flag leaf pycnidia coverage when compared to the population mean (28.2%) and explained 27.8% of the phenotypic variation for this trait. No other significant QTL for Stb resistance were identified in the mapping population.

Four grain yield QTL with LOD scores ranging from 3.5 to 5.8 were detected on four chromosomes (2A, 2B, 2D, and 6D) and explained from 9.8 to 19.8% of the grain yield variation. The grain yield QTL on chromosome 2A, named *QYld.jic-2A*, co-localized with *Q.Stb.jic-2A* (Figure 3.1), and conferred a significant increase in grain yield of 0.19 t ha^{-1} compared to the population mean of 9.83 t ha^{-1} when the positive Pastiche allele was present. The three remaining Yld QTL were positively associated with the Torfrida allele.

Table 3.3: Data summary for phenotypic traits measured on the Pastiche/Torfrida DH population (130 individuals) in disease field trials and yield field trials from 2006 to 2009.

Trait	Parents		DH lines			Environment[D]
	Pastiche	Torfrida	Mean	Min	Max	
Disease field trials						
Stb[A] (%)	15.7[Y]	42.8[Z]	28.2	8.2	64.5	Adv07, Nic07, Syn07, Sej07
Ht[B] (cm)	67.6[Y]	60.9[Z]	62.8	57.2	70.7	Adv07, Nic07, Syn07, Sej07
Hdg (days)	149.2[Y]	149.5[Y]	149.7	146.9	153.6	Adv07, Nic07, Syn07, Sej07
Yield field trials						
Ht[B] (cm)	61.5[Y]	60.5[Y]	62.1	52.3	74.5	Els08, Sej09
Hdg (days)	-[C]	-[C]	152.1	149.5	154.0	Sej09
Twt (kg hL^{-1})	84.8[Y]	83.3[Y]	84.1	77.0	91.3	Els08, Sej09
Tkw (g)	53.3[Y]	48.6[Z]	48.8	42.8	55.7	Els08, Syn08, Sej09, SW09
Yld (kg ha^{-1})	9.02[Y]	9.92[Y]	9.44	8.51	10.78	Els08, Syn08, Sej09, SW09

[A] Stb scored as percent pycnidial coverage on the flag leaf

[B] Height measured from ground to the flag leaf

[C] Mean cannot be estimated because parental lines were absent in the trial

[D] Prefixes indicate trial location while suffixes indicate year trial was conducted (Table 3.1)

[Y,Z] Means followed by the same letter are not significantly different at $P \leq 0.05$

Table 3.4: Correlation coefficient matrix of least square means for all traits measured in disease and yield field trial combined environment analyses for the Pastiche/Torfrida DH mapping population (85 individuals).

Trait[A]	Disease field trials				Yield field trials			
	Ht[B]	Hdg[B]	Stb[B]		Ht[C]	Hdg[C]	Tkw[C]	Twt[C]
Hdg[B]	0.12 ns							
Stb[B]	0.03 ns	-0.02 ns						
Ht[C]	0.69 ***	0.16 ns	-0.12 ns					
Hdg[C]	-0.06 ns	0.71 ***	-0.09 ns		0.10 ns			
Tkw[C]	0.60 ***	-0.12 ns	-0.07 ns		0.61 ***	-0.25 **		
Twt[C]	0.08 ns	-0.07 ns	-0.31 **		0.21 ns	0.02 ns	0.19 ns	
Yld[C]	0.09 ns	0.01 ns	-0.09 ns		0.34 **	0.03 ns	0.17 ns	0.42 ***

[A] Ht = plant height to flag leaf. Hdg = days to heading. Stb = septoria tritici blotch (*Mycosphaerella graminicola*). Twt = test weight. Tkw = thousand kernel weight. Yld = grain yield.

[B] Trait measured in disease field trials

[C] Trait measured in yield field trials

*, **, ***, and ns indicates significant at $P<0.05$, $P<0.01$, $P<0.001$, and not-significant, respectively

Table 3.5: QTL detected in the Pastiche/Torfrida DH mapping population (85 individuals) for septoria tritici blotch resistance (Stb; *Mycosphaerella graminicola*), height to flag leaf (Ht), heading date (Hdg), thousand kernel weight (Tkw), and grain yield (Yld).

QTL (LOD threshold[A])	Marker	LOD	Positive allele	% Expl.[B]	Additive	% Effect[C]	Environment[D]
Stb (3.0)							
QStb.jic-2A	barc212	5.8	Pastiche	27.8	-8.8 %	-31.2	Adv07, Nic07, Syn07, Sej07
Ht (2.9)							
QHt.jic-1A.1	wPt-3698_1A	3.4	Torfrida	7.9	0.8 cm	1.3	Adv07, Nic07, Syn07, Sej07
QHt.jic-1B.1	wmc044	3.2	Torfrida	8.0	0.8 cm	1.3	Adv07, Nic07, Syn07, Sej07
QHt.jic-1B.2	wPt-3566_1B	3.2	Torfrida	8.0	0.8 cm	1.3	Adv07, Nic07, Syn07, Sej07
QHt.jic-2B	wPt-1127	4.8	Torfrida	11.9	0.9 cm	1.3	Adv07, Nic07, Syn07, Sej07
QHt.jic-6A.1	barc146	7.5	Pastiche	19.5	1.3 cm	2.1	Adv07, Nic07, Syn07, Sej07
QHt.jic-1A.2	gwm135	2.9	Torfrida	10.9	1.1 cm	1.8	Els08, Sej09
QHt.jic-6A.2	barc003	3.4	Pastiche	15.2	1.4 cm	2.2	Els08, Sej09
Hdg(3.1)							
QHdg.jic-2A	gwm095	3.3	Torfrida	15.3	-0.5 days	-0.33	Adv07, Nic07, Syn07, Sej07
QHdg.jic-7A	wPt-1928_7A	6.5	Torfrida	27.4	-0.4 days	-0.26	Sej09
Tkw(2.9)							
QTkw.jic-1A	gwm164	5.1	Torfrida	22.4	1.3 g	2.7	Els08, Syn08, Sej09, SW09
QTkw.jic-6A	barc171	5.5	Pastiche	22.5	1.3 g	2.7	Els08, Syn08, Sej09, SW09
Yld (2.9)							
QYld.jic-2A	wPt-8573	5.8	Pastiche	19.8	0.19 t ha^{-1}	2.0	Els08, Syn08, Sej09, SW09
QYld.jic-2B	wPt-9856	3.5	Torfrida	11.6	0.14 t ha^{-1}	1.5	Els08, Syn08, Sej09, SW09
QYld.jic-2D	wPt-1554_2D	3.4	Torfrida	9.8	0.14 t ha^{-1}	1.5	Els08, Syn08, Sej09, SW09
QYld.jic-6D	barc173	4.8	Torfrida	16.6	0.17 t ha^{-1}	1.8	Els08, Syn08, Sej09, SW09

[A] LOD thresholds were calculated by permutation test (1000 iterations) using MAPQTL 5.0 software
[B] The percentage of the variance explained by the QTL
[C] Effect (%) is the increase/decrease of the trait compared to the mean of the population
[D] Prefixes indicate trial location while suffixes indicate year trial was conducted (Table 3.1)
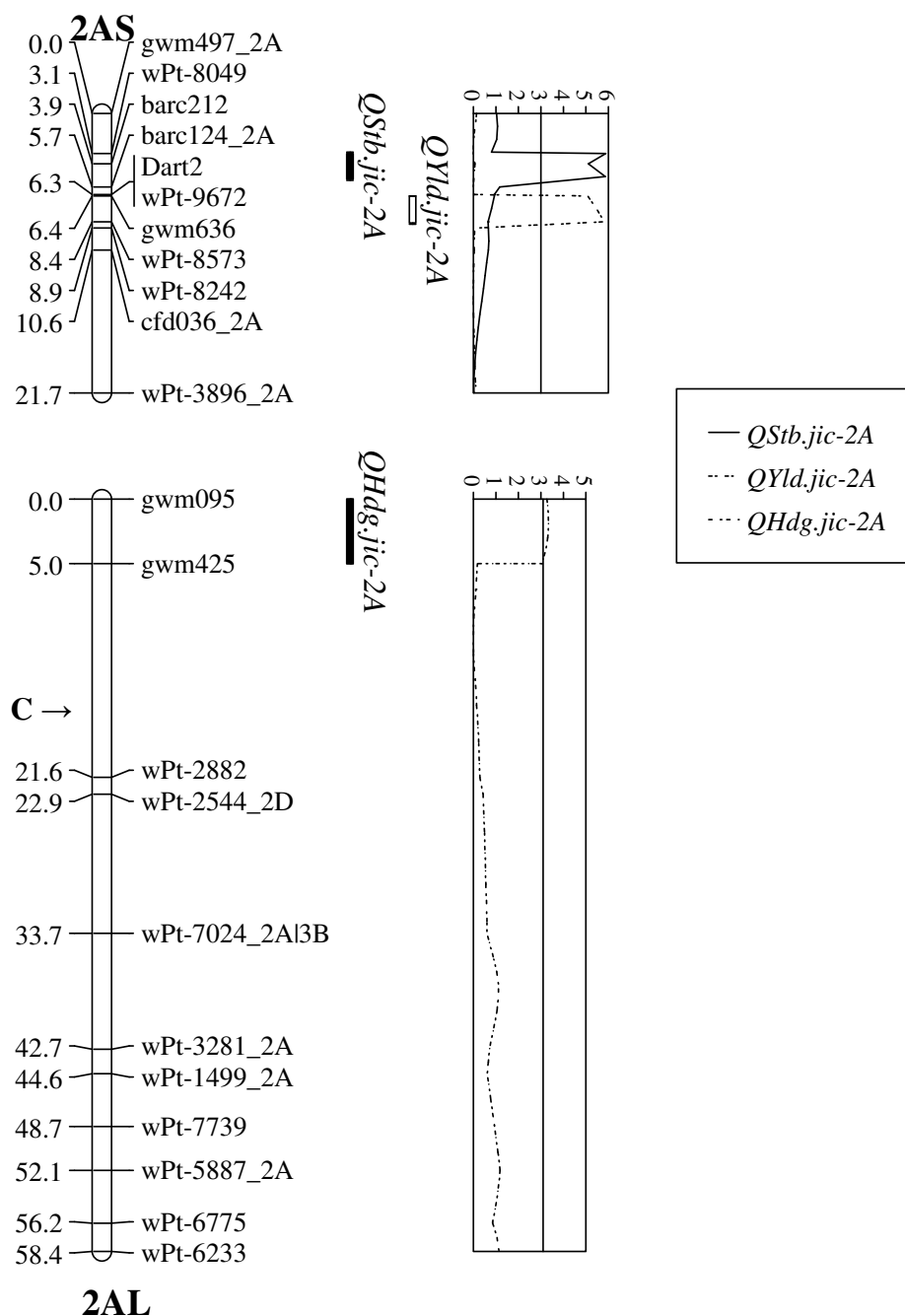
Figure 3.1: Linkage groups and LOD scans for chromosome 2A of the Pastiche/Torfrida winter wheat cross. QTL are indicated for septoria tritici blotch (Stb) resistance, days to heading (Hdg), and grain yield (Yld). QTL identified in disease trials are shown with solid bars while QTL identified in yield field trials are shown with open bars. Molecular markers are indicated to the right of the chromosome and genetic distances are indicated to the left of the chromosome in Kosambi centiMorgans. The constant line at LOD 3.1 represents a 5% significance level. The letter "C" indicates the approximate location of the centromere.
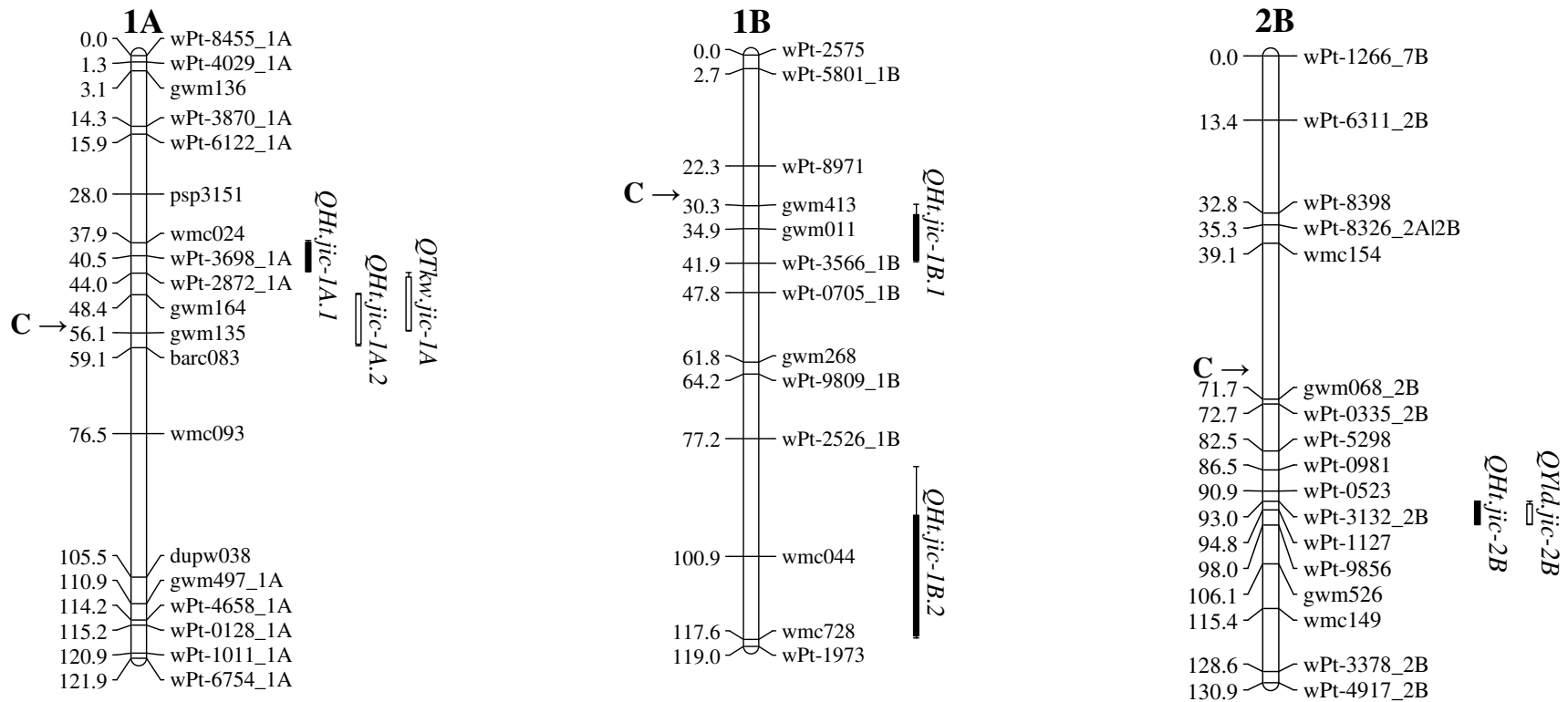
Figure 3.2: Linkage groups of the seven chromosomes with QTL indicated for septoria tritici blotch (Stb) resistance, plant height to flag leaf (Ht), days to heading (Hdg), thousand kernel weight (Tkw), and grain yield (Yld) in the Pastiche/Torfrida winter wheat cross. QTL identified in disease trials are indicated by solid bars while QTL identified in yield field trials are indicated by open bars. The length of QTL bars identifies a LOD drop of 1.0 and the outside line designates a LOD drop of 2.0. Genetic distances (Kosambi centiMorgans) and molecular markers are indicated to the left and right of the chromosomes respectively. The letter "C" indicates the approximate location of the centromere.

2D

0.0 — barc124_2D

11.9 — gdm035
15.4 — cfd036_2D

0.0 — gwm484
2.3 — barc168

C →
21.3 — wmc018

43.7 — gwm539

63.3 — wPt-1554_2D
64.3 — wPt-2781_2D
wPt-5865_2A
64.6 — wPt-2160
67.5 — wPt-3728_2D
70.5 — wPt-0638_2D

QYld.jic-2D

6A

0.0 — Dart1
wPt-4016_6A
2.5 — gwm334
3.7 — wPt-6904_6A
7.4 — wPt-8266_6A

0.0 — wPt-7599_6A|6B

C →

21.7 — psp3152
24.4 — barc146
barc171
24.6 — psp3071
25.4 — barc003
27.2 — barc107

46.3 — gwm570

0.0 — wPt-4229_6A

4.1 — wPt-0404

13.7 — dupw167
13.8 — wPt-7204_6A

QHt.jic-6A.2

QHt.jic-6A.1

QTkw.jic-6A

6D

0.0 — barc173

14.2 — wPt-7394

21.4 — wPt-6500

C →

QYld.jic-6D

7A

0.0 — wPt-1510_7A
1.6 — wPt-6034_7A

25.3 — wPt-4880_7A
26.6 — wPt-6447_7A
wPt-1179_5B

46.0 — wPt-3702

60.0 — gwm060
64.4 — barc127
68.0 — wPt-1928_7A

81.2 — wPt-7188_1B

89.5 — wPt-9857_1D

C →
101.4 — gwm068_7A
102.6 — wPt-2266_7A
102.7 — barc108
104.0 — wmc247
114.6 — Dart3
barc029
114.8 — barc121

0.0 — wPt-9824_4B
6.6 — wmc633

18.8 — wPt-0639

35.2 — cfa2040_7A
35.3 — wmc525

QHdg.jic-7A

56

Five QTL for plant height on chromosomes 1A, 1B, 2B, and 6A were identified in the disease field trial environments, while only two QTL for plant height were identified in the yield field trial environments on chromosomes 1A and 6A (Table 3.5). The plant height QTL identified on chromosomes 1A and 6A are found in the same genomic regions when compared between field trial types (Figure 3.2). The QTL with the highest LOD score in both the disease and yield field trials was found on chromosome 6A (Table 3.3). The positive allele for the 1A Ht QTL was from Torfrida while Pastiche provided the positive allele for the 6A Ht QTL.

Two QTL for thousand kernel weight were detected on chromosomes 1A and 6A with LOD scores of 5.1 and 5.5, respectively. The positive alleles for *QTkw.jic-1A* and *QTkw.jic-6A* were from Torfrida and Pastiche, respectively. The additive effect of each Tkw QTL was 1.3 g when compared to the population mean of 48.8 g, equating to an effect of about 2.7 %. Both QTL for Tkw to co-segregated with the plant height QTL identified from the disease and yield field trial measurements on chromosomes 1A and 6A.

3.5 Discussion

This study identified the QTL responsible for partial Stb resistance, grain yield and yield components in the Pastiche/Torfrida winter wheat cross. A recent study has determined that producers tend to use wheat cultivars which are high yielding but susceptible to septoria tritici blotch (Arraiano 2008). The effort by wheat breeders to incorporate genetic resistance to *M. graminicola* may have resulted in a cost to grain production. While many studies have identified QTL in hexaploid wheat for either resistance to economically important pathogens (Jahoor et al. 2004) or grain yield (Cuthbert et al. 2008; Kuchel et al. 2007), there are no reports of molecular mapping of both disease resistance, grain yield and yield components simultaneously in disease field trials and yield field trials.

A genetic map was constructed for the Pastiche/Torfrida winter wheat cross using 85 doubled haploid lines genotyped with microsatellite and DArT markers. The genetic

map was comprised of 32 linkage groups with a total length was 1283.2 cM and an average marker interval of 4.2 cM. The genetic map length of the Pastiche/Torfrida DH cross is shorter than other genetic maps developed for hexaploid wheat crosses (Chalmers et al. 2001), which can be mostly attributed to the shorter linkage groups representing chromosomes 3A, 3D, 4A, 4B, 4D, 5D, and 6D. Also the smaller population size of 85 individuals impacted the length of the genetic map. Clustering of marker loci was evident in certain genomic regions (1A, 2A, 3B, 6B, and 7B). The clustering of markers was probably due to reduced recombination (Tanksley et al. 1992).

QTL for partial Stb resistance have been identified on chromosomes 3A, 3B, 6B, and 7B by Eriksen et al. (2003). More recently Simon et al. (2004a) identified QTL for seedling or adult stage Stb resistance on chromosomes 1D, 2D, 3D, 6B, and 7B. To date, major resistance or partial resistance have yet to be reported for Stb resistance on chromosome 2A. The current study identified two QTL, designated *QStb.jic-2A* and *QYld.jic-2A*, on the short arm of chromosome 2A. These QTL co-localized and conferred an increase in grain yield of about 2.0% as well as a 31.2% decrease in flag leaf pycnidia density compared to the population mean when the Pastiche allele was present (Figure 3.1; Table 3.5). The complete genetic map of Pastiche/Torfrida (Appendix 8.4) has a high level of marker clustering in this interval with a total of twenty molecular markers, including 15 DArT and 6 SSR. The large amount of clustering of marker loci in the interval on 2A may indicate low levels of recombination and also a large physical distance (Tanksley et al. 1992). The SSR markers linked to these QTL for grain yield and Stb resistance could be directly useful for wheat breeding programs and MAS.

It is unknown if the relationship between the Stb resistance and grain yield increase found on chromosome 2A of Pastiche is linkage or pleiotrophy. While these QTL are identified in a relatively small genetic distance of less than 10 cM, it will be difficult to determine the underlying gene or genes responsible for these traits since it is very probable that this interval contains hundreds of genes. The physical to genetic distance ratio in wheat has been reported to vary from 0.02 to 16.7 megabase pairs per centiMorgan (Mb cM^{-1}) (Akhunov et al. 2003; Spielmeyer et al. 2000). Determining the

candidate genes from such a large number is not feasible; therefore, a smaller QTL interval and a smaller number of genes would need to be identified through fine mapping. This process involves mapping in a large population (>1000 individuals) created to localize the QTL to an interval, often less than one centiMorgan, saturated with marker loci (Chicaiza et al. 2006; Price 2006). The 2A interval identified in this study contains 15 DArT loci, which could be useful in a fine-mapping population since the sequences of these loci have been recently released to the public (http://www.diversityarrays.com/sequences.html). The DArT probes would need to be converted to an assay ideal for screening on a large number of recombinants for fine mapping. Fine mapping has been used in wheat for a number of QTL such as grain protein content (Olmos et al. 2003), grain weight (Röder et al. 2008), preharvest sprouting (Torada et al. 2008), leaf and stem rust resistance (Spielmeyer et al. 2008), and fusarium head blight resistance (Cuthbert et al. 2006). Fine mapping a QTL is generally an expensive process and should be used for a QTL with a large effect since it is difficult to assess the phenotypic differences of a small-effect QTL (Price 2006). Fine mapping the grain yield QTL and Stb resistance QTL identified on chromosome 2A of Pastiche is possible due to the large effect of these QTL (19.8% and 29.8% of the phenotypic variance, respectively).

Since grain yield is very important to producers for economic reasons, one of the major goals in wheat breeding programs will be maintaining or increasing grain yield of wheat cultivars. Due to increasing production costs of fossil fuels and chemical inputs, developing wheat cultivars that resist infection by economically important pathogens such as *M. graminicola* will help to reduce production risks to producers.

4.0 Molecular mapping of *StbSm3* in wheat with resistance to multiple isolates of *Mycosphaerella gramincola*

4.1 Abstract

Septoria tritici blotch (Stb), caused by the fungal pathogen *Mycosphaerella graminicola*, is a devastating foliar disease of wheat in regions with humid, temperate climates. While fungicides have been successfully used to control grain yield losses caused by this disease, the use of cultivars with genetic resistance is the most economical and environmentally sound method of sustainably reducing grain yield losses. Genetic resistance to Stb has been reported in numerous hexaploid wheat strains/cultivars. Salamouni, a hexaploid landrace from Lebanon, was previously studied and reported to contain three incompletely dominant resistance genes for septoria tritici blotch. Two of these genes confer resistance to *M. graminicola* isolate MG96-36 while all three genes confer resistance to *M. graminicola* isolate MG2. The chromosomal locations of the isolate-specific resistance gene and one of the multiple-isolate resistance genes were identified and named in a previous study *Stb14* and *Stb13,* respectively. The objective of the current study was to determine the chromosomal location of the third gene, which confers resistance to multiple *M. graminicola* isolates. Doubled haploid (DH) lines developed from a cross between the highly Stb resistant Salamouni and the highly Stb-susceptible hexaploid cultivar Katepwa were screened with both *M. graminicola* isolates. Resistant DH lines were screened with microsatellite markers on chromosome 7BL to find lines with homozygous Katepwa linked to *Stb13*, a Stb-resistance gene which confers resistance to *M. graminicola* isolates MG96-36 and MG2. The DH line 98S05B*13 was found to be resistant to both isolates and also contained Katepwa alleles at the markers linked to *Stb13*. This line was crossed to the susceptible parent and a segregating F_2 population was produced. DH line 98S05B*13 was found to contain the isolate-specific Stb resistance gene *Stb14* on chromosome 3BS. Marker-assisted selection was used on the F_2 population to select individual plants for presence (Salamouni allele at cfd79 and wmc623) and absence of *Stb14* (Katepwa allele at cfd79

and wmc623). The selected F_2 families were self pollinated to produce 62 $F_{2:3}$ families with *Stb14* and 56 without *Stb14*. Both *Stb14(+)* $F_{2:3}$ families and *Stb14*(-) $F_{2:3}$ families were screened for resistance with isolate MG96-36. The $F_{2:3}$ families without *Stb14* were more intermediate in their reaction; therefore, only families with *Stb14* were used for mapping purposes. The $F_{2:3}$ families were classified as homozygous resistant, segregating, or homozygous susceptible to *M. graminicola* isolate MG96-36. Bulked segregant analysis (BSA) identified a putative linkage to the gene of interest on chromosome 3A. A selection of polymorphic microsatellite markers on 3AS were used to construct a linkage group including the multiple-isolate Stb resistance gene. This chromosomal location has not been previously linked to any Stb resistance genes, therefore, this gene is new and will be designated *StbSm3*. The microsatellite markers barc321 and barc12 were found to be linked to and flank *StbSm3* at genetic distances of 1.9 and 2.5 cM, respectively.

4.2 Introduction

Septoria tritici blotch (Stb) is a devastating foliar disease of wheat caused by the fungal pathogen *Mycosphaerella graminicola* (anamorph *Septoria tritici*). Stb was first reported as a severe wheat disease in Europe in the 1970s and has since become widespread in regions with humid, temperate climates (Hardwick et al. 2001). The presence of the sexual state, *M. graminicola,* in western Canada in 2001 was reported by Horne et al. (2002). Grain yield losses have been observed to range from 25 to 50% during severe epidemics (Eyal et al. 1985; King et al. 1983). Chemical control has been used extensively to reduce losses of Stb; however, the fungus has adapted to the strobilurin group of fungicides which were routinely applied to crops for Stb control (Brunner et al. 2008; Cools and Fraaije 2008). Resistant cultivars can provide an effective and economical method of reducing crop losses. Until recently, wheat breeders have relied on unknown resistance loci to increase the genetic resistance in cultivars.

Resistance to *M. graminicola* has been reported to be both partial (quantitative) and specific (qualitative). Specific resistance may be oligogenic, near-complete and

follows a gene-for-gene relationship (Brading et al. 2002; McCartney et al. 2002). Partial resistance is more complex as it is incomplete and not always isolate-specific (Chartrain et al. 2004b; Jlibene et al. 1994; Simon and Cordo 1998).

To date, the chromosomal locations of 15 septoria tritici blotch resistance genes have been reported in hexaploid wheat (Table 2.2) (Adhikari et al. 2003; Adhikari et al. 2004b; Adhikari et al. 2004c; Adhikari et al. 2004d; Arraiano et al. 2007; Brading et al. 2002; Chartrain et al. 2005a; Chartrain et al. 2005c; Chartrain et al. 2009; Cowling 2006; Goodwin 2007; McCartney et al. 2003). These resistance genes have been designated as as *Stb1-Stb15*. Brading et al. (2002) was the first to report that *Stb6*, a Stb resistance gene mapped to chromosome 3AS, followed the gene-for-gene relationship. *Stb6* has also been identified as the most prevalent source of Stb resistance in wheat germplasm (Chartrain et al. 2005b).

Developing wheat cultivars for producers with durable resistance to devastating pathogens is an important objective for wheat breeders worldwide. It is believed that pyramiding multiple resistance genes in a single cultivar will help to delay a pathogen from overcoming single-gene resistance. Pyramiding Stb resistance genes, which are thought to follow gene-for-gene relationships, could provide durable resistance to *M. graminicola*. Screening segregating populations for which there are either specific virulence genes or molecular markers linked to the genes is required to develop resistance pyramids. This technique is very useful since selective races are not always available, and screening the number of lines required to successfully pyramid more than a few genes can be extremely laborious and resource intensive. Also, DNA-based markers can be used to select lines in the absence of disease epidemics.

Previous studies on the inheritance of genetic resistance in the hexaploid landrace Salamouni by McCartney et al. (2002) determined that three incompletely dominant and independent genes control resistance to two races of *M. graminicola* found in western Canada. The three genes confer resistance to *M. graminicola* isolate MG96-36 (representative of race 1) while only two of the three confer resistance to isolate MG2 (representative of race 2). Cowling (2006) was later able to map the chromosomal

locations of two Stb resistance genes, which have been designated *Stb13* and *Stb14*. *Stb13* conferred resistance to both *M. graminicola* races while *Stb14* was found to confer resistance to only race 2. The objective of the current study was to determine the chromosomal location of the third Stb resistance gene which confers resistance to two Canadian isolates (MG96-36 and MG2) in the landrace Salamouni using microsatellite markers and bulked segregant analysis.

4.3 Materials and Methods

4.3.1 Doubled haploid line selection

The highly Stb susceptible Canadian bread wheat cultivar Katepwa (Neepawa*6/RL-2938/3/Neepawa*6//CI-8154/2*Frocor) was used in reciprocal crosses with Salamouni. The crosses were completed in 2004 and doubled haploid (DH) lines were produced from F_1 plants using the wheat-maize pollination method followed by embryo rescue and chromosome doubling with colchicine (Laurie and Bennett 1988). A total of 67 DH lines was produced. The DH lines were previously screened with *M. graminicola* isolates MG96-36 (race 1) and MG2 (race 2) (Cowling 2006). DH lines that were resistant to both isolates were screened with the microsatellite marker wmc396, which is linked to the Stb resistance gene *Stb13*. The DH line 98S05B*13 was selected for further population development since it was resistant to both *M. graminicola* isolates (MG96-36 and MG2) and also contained the susceptible Katepwa allele at the marker wmc396 on chromosome 7A.

4.3.2 Mapping population development

The DH line 98S05B*13 was crossed back to the susceptible parent Katepwa to generate about 200 F_1 seeds (Cowling 2006). Ten F_1 plants were grown for inoculation and disease assessment. Five of the ten F_1 plants were inoculated with *M. graminicola* isolate MG96-36, while the other five were inoculated with isolate MG2. The F_1 plants were self pollinated by placing glassine bags on the spikes prior to anthesis to produce the F_2 population. Two groups of 100 plants from the F_2 population were inoculated with

either isolate MG96-36 or MG2. Both groups of F_2 plants were observed to segregate in a 3 resistant : 1 susceptible ratio, which is typical of a single gene.

Further microsatellite screening of DH line 98S05B*13 determined that it contained the Salamouni allele at microsatellite loci cfd79 and wmc623, both of which have been reported to flank the isolate-specific (MG2) Stb resistance gene *Stb14* (Cowling 2006). To control the effect of *Stb14*, marker-assisted selection was used to select F_2 individuals homozygous for either the resistant parental alleles (*Stb14* +) or the susceptible parental alleles (*Stb14* -) at microsatellite markers cfd79 and wmc623. These F_2 individuals were self-pollinated by placing glycine bags on spikes prior to anthesis. A summary of the development of the 98S05B*13/Katepwa mapping populations is depicted in Figure 4.1. A total of 62 $F_{2:3}$ families was produced and inoculated with isolate MG96-36. It was expected that a single gene was segregating in the families inoculated, therefore, a total of 20 plants per family was screened to ensure the homozygous susceptible reaction type was observed in the segregating families at least once 99% of the time.

Prior to planting, all seeds were germinated in the dark (2 days at 4°C followed by 2 days at room temperature) to ensure uniform development. One or two plants were grown in a four-litre pot filled with a 2 : 2 : 1 soil mix (soil/sand/peat). Plants were grown in controlled environments with temperatures of 18/14°C (day/night) with a 16-h photoperiod ($250\mu E\ m^{-2}s^{-1}$). All plants were fertilized with 20-20-20 fertilizer (3.75 mg/L) weekly and watered as required.

P: **98S05B*13 / Katepwa**

P1   P2

F_1:   5 F_1 plants

$\otimes$

F_2:
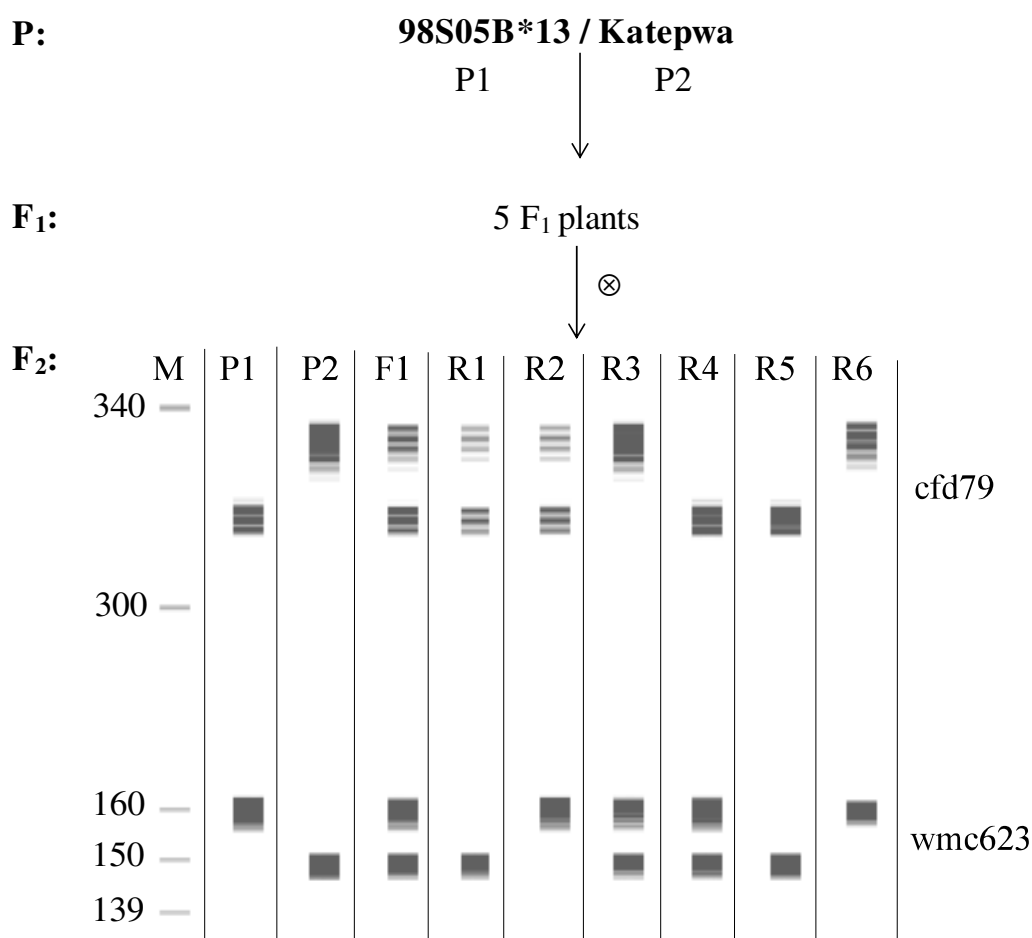
384 F_2 individuals from the 98S05B*13/Katepwa cross were screened with the microsatellite markers linked to *Stb14* (cfd79 and wmc623). F_2 individuals were selected based on presence of the Salamouni allele at both loci (*Stb14 +*) or for the Katepwa allele at both loci *(Stb14 -)*. These individuals were selfed to produce $F_{2:3}$ families.

$\otimes$
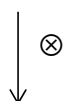
$F_{2:3}$:   62 *Stb14(+)* $F_{2:3}$ families phenotyped with MG96-36 (Race 1). Families were classified as either homozygous resistant (HR), segregating (SEG), or homozygous susceptible (HS).

Figure 4.1: Development of the 98S05B*13/Katepwa mapping population for molecular mapping of the third isolate-specific resistance gene in the hexaploid landrace Salamouni.

4.3.3 DNA isolation and microsatellite analysis

Leaf tissue was collected from the youngest non-inoculated leaf of individual F_2 plants. The harvested leaf tissue was lyophilized for 72h and then stored at -20°C. Lyophilized tissue from each plant and 200 µl of glass beads were placed in a 1.2 ml microtube. The microtubes were arranged in a 96-well plate. The plant tissue was ground to a fine powder by shaking the 96-well plate in a TissueLyzer II (Qiagen). DNA was extracted using the Patricia Warner DNA extraction method (Appendix 8.6). DNA was quantified using Hoechst 33258 stain and florescence was measured with a spectrophotometer. DNA samples were diluted to a final concentration of 10 ng/µl.

Polymerase chain reaction (PCR) was performed in 10-µl reaction volumes containing: 50 ng of template DNA, 0.5 U of Taq DNA polymerase (Promega), 1X PCR buffer (Applied Biosystems, Foster, CA, USA), 1.5 mM $MgCl_2$, 0.1 mM of each dNTP, 0.2 pmol of forward primer, 2.0 pmol of reverse primer, and 1.8 pmol of 6-FAM/HEX/NED-labelled M13 primer (5'-CACGACGTTGTAAAACGAC-3'; Applied Biosystems, Foster, CA, USA). Thermal cycling programs used were: 1 cycle of [94°C for 2 min], 30 cycles of [95°C for 1 min, (-0.5°C/s ramp to 61/55/51°C), 61/55/51°C for 50 sec, (+0.5°C/s ramp to 72°C), 72°C for 1 min], 1 cycle of [72°C for 5 min]. PCR fragments were resolved by capillary electrophoresis using an ABI PRISM 3130xL Genetic Analyzer (Applied Biosystems, Foster, CA, USA), and GeneScan 500 ROX as the internal molecular weight standard. Data generated (FSA files) by the ABI Genetic Analyzer was analyzed with GeneScan Analysis Software (Applied Biosystems, Foster, CA, USA) to standardize the M13 labelled PCR fragments size to the GeneScan-500 ROX size standard. After the genetic analyzer data was analyzed with GeneScan, a gel-like image was created using the Genographer version 1.6.0 available at http://hordeum.oscs.montana.edu/genographer/.

4.3.4 Mapping population inoculation

Single-spore isolates MG96-36 and MG2, representative of race 1 and race 2, respectively, were used to phenotype the mapping population. The single-spore isolates

were derived from a single sporulating pycnidium obtained from infected leaf tissue found in Manitoba (Grieger et al. 2005). The single spore was transferred to a yeast-malt agar plate containing 0.25% chloramphenicol (YMA[+]): 4g of Difco yeast extract (Difco Laboratories Inc., Detroit, MI), 4g of Difco malt extract, 4g of sucrose (Fisher Scientific, Pittsburgh, PA), 15 g of Difco agar, 250 mg of chloramphenicol (Sigma-Aldrich Corp., St. Louis, MO) and 1000 ml of distilled water (Eyal et al. 1987).

Cultures were maintained under fluorescent lights for seven days at room temperature. Plates were flooded with sterile distilled water and spores were dislodged with a wire loop to suspend the spores. The conidia spore suspension was filtered through sterile cheesecloth and quantified using a hemacytometer. The suspension was diluted to 1×10^7 spores per ml and a drop of Tween 20 (polyoxyethylene sorbitan monolaurate) was added per 50 ml of inoculum to reduce surface tension. Wheat seedlings at the three-leaf stage were sprayed with a DeVilbis-type sprayer until inoculum run-off. Inoculated plants were allowed to dry in the dark for about 1 hour and transferred to a humidity chamber for 67 to 72 h to maintain leaf wetness. Two ultrasonic humidifiers were used to maintain leaf wetness. The humidity chamber was located in a controlled environment set to a temperature of 21/19°C (day/night) and a photoperiod of 16h ($250\mu E\ m^{-2}s^{-1}$). Ultrasonic humidifiers provided sufficient moisture to maintain leaf wetness. After 72 h, seedlings were moved to a growth chamber set at 21°C day and 19°C night temperature with 16-h photoperiod ($390\ \mu E\ m^{-2}\ s^{-1}$) and a relative humidity between 70 to 80%.

Five to ten plants of the resistant DH line, 98S05B*13, and the susceptible parent line, Katepwa, as well as F_1 individuals were included in all inoculations to aid in comparing progeny reactions. Five to ten plants of ST6 were included to confirm the *M. graminicola* isolate in all inoculations since this line displays a differential reaction to race 1 (susceptible) and race 2 (resistant). Also, five to ten plants of Salamouni (resistant) and Erik (susceptible) were included in all inoculations to serve as additional controls. Due to growth space limitation, a total of 20 families consisting of 20 plants could be inoculated at one time. All individuals of a family were inoculated together.

4.3.5 Disease assessment

Plant reaction to *M. graminicola* was rated 17 days after inoculation (dai). The qualitative septoria tritici blotch disease scale developed by Rosielle (1972) and modified by McCartney et al. (2002) was used to rate all inoculated plants (Figure 4.2; Appendix 8.5). This scale includes consideration of expanded chlorotic lesions of heterozygous individuals better than the original scale. The rating scale was as follows: 0 = immune, characterized by an absence of pycnidial formation and occasional hypersensitive fleck, or no visible symptoms; 1 = highly resistant with hypersensitive flecking; 2 = resistant with small chlorotic or necrotic lesions, typically no pycnidial formation; 3 = intermediate characterized by coalescenece of chlorotic or necrotic lesions normally evident towards the leaf tips and to a lesser extent elsewhere on the leaf blade, very light pycnidial formation; 4 = susceptible with moderate pycnidial formation, coalesced necrotic lesions; 5 = very susceptible with large abundant pycnidia, necrotic lesions extensively coalesced. Plant ratings of 0 to 3 were considered resistant while plant ratings of 4 and 5 were considered susceptible. The intermediate rating 3 was considered resistant because growth and sporulation of the pathogen was severely restricted (McCartney et al. 2002). Plants with a rating of 3 have little necrosis and low pycnidia formation, both of which are important parameters in identifying susceptibility.

$F_{2:3}$ family ratings were classified as homozygous resistant (HR), segregating (SEG) or homozygous susceptible (HS) based on the reaction and rating of 20 individual seedlings per family. A goodness-of-fit chi-square (χ^2) was calculated on the phenotypic segregation data for each $F_{2:3}$ family.
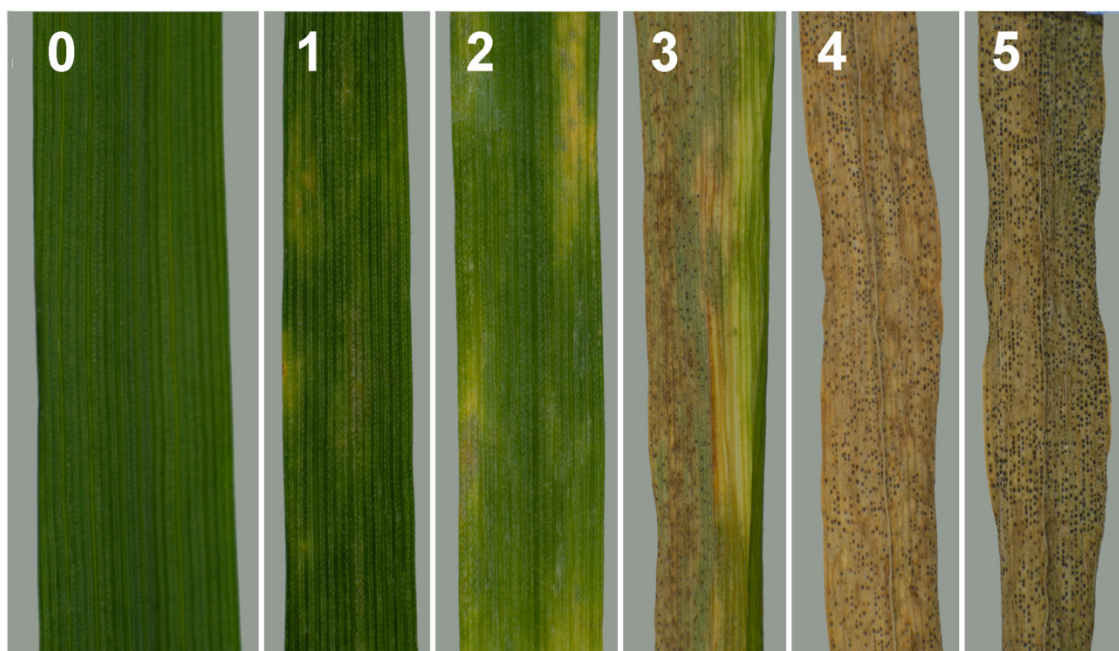
Figure 4.2: Modified Rosielle rating scale. Image source used with permission: Cowling (2006). **0** = immune, characterized by an absence of pycnidial formation, an occasional hypersensitive fleck, or no visible symptoms; **1** = highly resistance with hypersensitive flecking; **2** = resistant with small chlorotic or necrotic lesions, typically no pycnidial formation; **3** = intermediate, characterized by coalescence of chlorotic or necrotic lesions normally evident towards the leaf tips and to a lesser extent elsewhere on the leaf blade, very light pycnidial formation; **4** = susceptible with moderate pycnidial formation, coalesced necrotic lesions; **5** = very susceptible with large abundant pycidia, necrotic lesions, extensively coalesced.

4.3.6 Bulked segregant analysis

The bulked segregant analysis (BSA) method (Michelmore et al. 1991) was used to identify microsatellite markers putatively linked to the third Stb resistance gene contained in the 98S05B*13 population. The resistant DNA bulk was made by combining equal quantities of diluted F_2 DNA (10 ng/µl) from ten homozygous resistant $F_{2:3}$ families. The susceptible DNA bulk was made by combining equal quantities of diluted F_2 DNA (10 ng/µl) from ten homozygous susceptible $F_{2:3}$ families. Diluted DNA from the resistant parent (DH line 98S05B*13) and the susceptible parent (Katepwa) was used as the parental material for BSA. Microsatellite marker primers were selected for

BSA by using the wheat microsatellite consensus map (Somers et al. 2004), as well as the Komugi composite wheat map (National Bioresource Project 2009). Thirty-two primer pairs were selected which have been reported to be linked to the Stb resistance genes *Stb1* to *Stb12*, *Stb15*, and *TmStb1* to conduct targeted screening. Linkage was not observed for these primer pairs, therefore, a wide genome scan was initiated to screen the remainder of the genome. Primer pairs were selected at a distance of 10-15 cM to achieve sufficient genome coverage. In total, 378 primer pairs were screened on the parents and the two bulks.

4.3.7 Linkage analysis

The microsatellite markers identified during BSA to be putatively linked to the resistance gene were screened on the entire 98S05B*13/Katepwa mapping population (62 individuals) to confirm linkage. The chromosomal locations of these microsatellite markers were previously reported in other mapping populations (National Bioresource Project 2009; Röder et al. 1998; Somers et al. 2004; Song et al. 2005). These genetic maps were used to select additional microsatellite markers to help saturate the linkage group. The primer sequences and annealing temperatures for the microsatellite markers used in linkage analysis are listed in Table 4.1.

The software package JoinMap 4.0 (van Ooijen 2006) was used to calculate the linkage group and produce a genetic map of chromosome 3A. Genetic distances were calculated using Kosambi centiMorgans. The LOD threshold for the generated linkage group was 3.0.

Table 4.1: Primer sequences and annealing temperatures for the microsatellite loci that map to chromosome 3A

Marker	Forward primer (5' – 3')[A]	Reverse primer (5' – 3')	Annealing Temperature (°C)
barc321[B]	<u>CACGACGTTGTAAAACGAC</u>TGCACTTCCCACAACACATC	TTGCCACGTAGGTGATTTATGA	51
barc12[B]	<u>CACGACGTTGTAAAACGAC</u>CGACAGAGTGATCACCCAAATATAA	CATCGGTCTAATTGTCAATGTA	51
wmc11	<u>CACGACGTTGTAAAACGAC</u>TTGTGATCCTGGTTGTGTTGTGA	CACCCAGCCGTTATATATGTTGA	61
wmc532	<u>CACGACGTTGTAAAACGAC</u>GATACATCAAGATCGTGCCAAA	GGGAGAAATCATTAACGAAGGG	61
gwm369[C]	<u>CACGACGTTGTAAAACGAC</u>CTGCAGGCCATGATGATG	ACCGTGGGTGTTGTGAGC	61

[A] M13 tail sequence underlined in forward primer

[B] *Source:* Song et al. 2005.

[C] *Source:* Röder et al. 1998.

4.3.8 *M. graminicola* isolate IPO323 testing

Since the gene of interest in the current study was located on the short arm of chromosome 3A and the gene *Stb6* was previously reported to also map in this area, the Canadian differential wheat line set was screened with the *M. graminicola* isolate IPO323. The isolate IPO323 was used to determine the presence or absence of resistance gene *Stb6*. Resistance to this isolate was previously reported by Brading et al. (2002) to be conferred by a single resistance gene on chromosome 3A. The Canadian lines tested included Salamouni (6X), Katepwa (6X), Coulter (4X), Erik (6X), ST6 (6X) and the DH lines developed from the Salamouni/Katepwa cross: 98S08A*09 (*Stb14*), 98S08C*03 (*Stb13*), 98S05B*13. Flame (resistant) and Riband (susceptible) were included as known controls. IPO323 isolate screening was conducted at the John Innes Centre (Norfolk, UK) using the detached-leaf screening method previously developed by Arraiano et al. (2001a). Four replicates were used for detached-leaf testing.

4.4 Results

4.4.1 Mapping population disease assessment

Based on previous work conducted by McCartney et al. (2002) and Cowling (2006), the line 98S05B*13 was chosen because it was identified to be homozygous for Katepwa alleles at the markers linked to *Stb13* on chromosome 7B and was also resistant to both isolates with a chlorotic phenotype with no necrosis or pynidia (Figure 4.3). The reactions of the F_1 plants (98S05B*13/Katepwa) were similar for both isolates (Figure 4.3), with some chlorosis and necrotic lesions bearing pycnidia. Generally, the necrotic lesions were isolated, with minimal coalescence. This phenotypic reaction is typical of a 3 rating type according to the Modified Rosielle scale (Figure 4.2). An F_2 population was derived from the 98S05B*13/Katepwa cross. The F_2 groups segregated in a 3 resistant : 1 susceptible ratio when challenged with either *M. graminicola* isolate MG96-36 or MG2. The F_2 results were characteristic of a single gene segregating for resistance (P-values of 0.31 and 0.69) (Table 4.2; Table 4.3; Figure 4.4).

Figure 4.3: Phenotypic reactions of the doubled haploid line 98S05B*13 (**a, d**), Katepwa (**c, f**), and the F₁ from the cross 98S05B*13/Katepwa (**b, e**) to isolates MG96-36 (race 1) (**a-c**) and MG2 (race 2) **(d-f)** of *M. graminicola*.

Table 4.2: Phenotypic segregation ratios for the F_2 and $F_{2:3}$ generations produced from the cross 98S05B*13/Katepwa for reaction to *Mycosphaerella graminicola* isolate MG96-36 (race 1).

Cross	Observed F_2 (R : S)	Ratio tested in F_2 (R : S)	F_2 χ^2 (P)[A]	Observed $F_{2:3}$ (HR : SEG : HS)	Ratio tested in $F_{2:3}$	$F_{2:3}$ χ^2 (P)
98S05B*13/Katepwa	76 :19	3 : 1	1.01 (0.31)	-	-	-
Stb14 (+)[A]	-	-	-	21 : 28 : 13	1 : 2 : 1	2.65 (0.10)
Stb14 (-)[B]	-	-	-	2 : 12 : 6	1 : 2 : 1	2.40 (0.12)

[A] Chi-square value (χ^2) calculated using Yates correction factor where appropriate.
[B] The *Stb14*(+) $F_{2:3}$ families were homozygous for Salamouni alleles at microsatellite loci cfd79 and wmc623 on chromosome 3B.
[C] The *Stb14*(-) $F_{2:3}$ families were homozygous for Katepwa alleles at microsatellite loci cfd79 and wmc623 on chromosome 3B.

Table 4.3: Phenotypic segregation ratios for the F_2 generation produced from the cross 98S05B*13/Katepwa for reaction to *Mycosphaerella graminicola* isolate MG2 (race 2).

Cross	Observed F_2 (R : S)	Ratio tested in F_2 (R : S)	F_2 χ^2 (P)[A]
98S05B*13/Katepwa	72 :27	3 : 1	0.16 (0.69)

[A] Chi-square value (χ^2) calculated using Yates correction factor where appropriate.
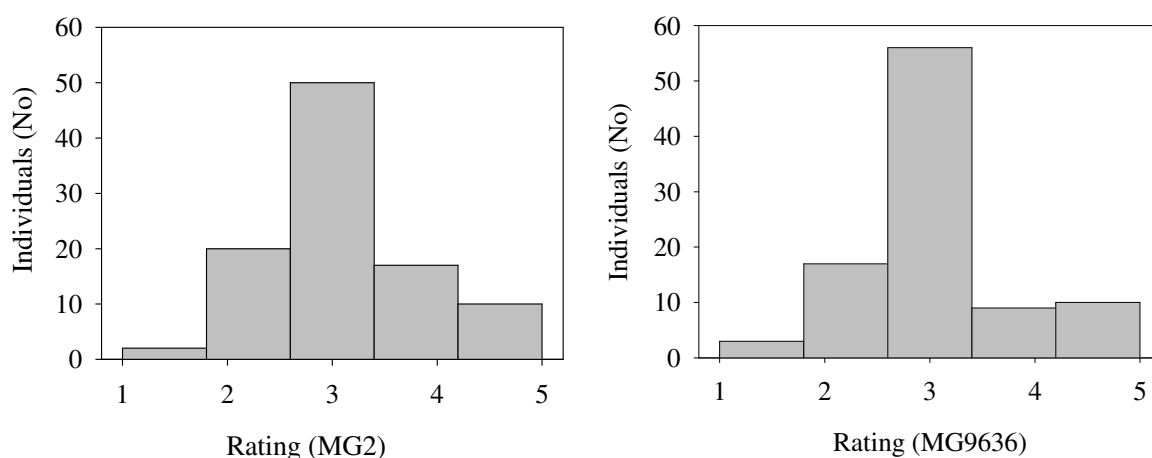
Figure 4.4: Histogram of phenotypic segregation ratios for the F_2 generation produced from the cross 98S05B*13/Katepwa for reaction to *Mycosphaerella graminicola* isolates MG2 (race 2) and MG96-36 (race 1).

The DH line 98S05B*13 was determined to contain the Salamouni allele for the microsatellite loci cfd79 and wmc623, which were previously reported to be linked to the resistance gene *Stb14* on chromosome 3B (Cowling 2006). Based on the previous reports, it was expected that the presence of *Stb14* along with the third multiple-isolate resistance gene would have resulted in a phenotypic segregation ratio of 15 resistant : 1 susceptible for the F_2 generation when inoculated with the MG2 isolate. Since this was not observed and the effect of *Stb14* on the third resistance gene was not known, MAS was used to select F_2 plants that contained the resistant or susceptible parent allele at both cfd79 and wmc623 to derive $F_{2:3}$ families. Out of a total of 384 F_2 plants screened, 62 were homozygous for Salamouni alleles and 56 were homozygous for the susceptible Katepwa alleles at these loci. The remainder of the F_2 plants were discarded since they were either heterozygous or recombinants. The F_2-derived F_3 families were categorized as either *Stb14*(+) or *Stb14*(-) respectively.

Initially, 20 *Stb14*(-) $F_{2:3}$ families were inoculated with the MG96-36 isolate. Ratings of these families were difficult because necrotic lesion coalescence was slightly

higher which made the distinction between reaction types 3 (resistant) or 4 (susceptible) difficult. The families thought to be positive for *Stb14* also were screened with the MG96-36 isolate. The reaction types of these families were easier to differentiate and the susceptible types were very similar to the susceptible parent Katepwa (Figure 4.3). From a total of 62 *Stb14*(+) families inoculated with the MG96-36 isolate, a segregation ratio of 21 homozygous resistant : 28 segregating : 13 homozygous susceptible was observed. The goodness-of-fit chi-square test for an expected segregation ratio of 1 homozygous resistant : 2 segregating: 1 homozygous susceptible was equal to 1.01 (P-value = 0.31) and therefore fits the hypothesis for segregation of a single resistance gene. F_2 individuals with a rating of 3 most often produced $F_{2:3}$ families segregating for Stb resistance, which indicates the resistance gene was heterozygous in the F_2 and confirms the intermediate rating of 3 as resistant.

4.4.2 Marker and linkage analysis

A total of 378 microsatellite markers (57% polymorphic) were used in BSA and identify two markers (barc321 and barc12) which had a banding pattern that showed clear linkage to the resistance gene in 98S05B*13. The banding pattern at locus barc321 is shown in Figure 4.5 (lanes 1-4 represent the parents and bulks). The Salamouni allele amplified only in the resistant bulk, while the Katepwa allele amplified only in the susceptible bulk. Figure 4.5 also shows the banding pattern of the 10 F_2 individuals which represent the homozygous resistant F_3 families (lanes 5 to 14) and 10 F_2 individuals which represent the homozygous susceptible F_3 families (lanes 15 to 24). This marker also demonstrates the linkage of barc321 to the resistance gene. The size difference of PCR fragments at the barc321 and barc12 loci was large which facilitated scoring of the Salamouni and Katepwa alleles. The Salamouni and Katepwa allele sizes for barc321 were 188 and 210 bp, respectively. The Salamouni and Katepwa allele sizes for barc12 were 174 and 194 bp, respectively. All allele sizes include the 19-bp M13 tail sequence.
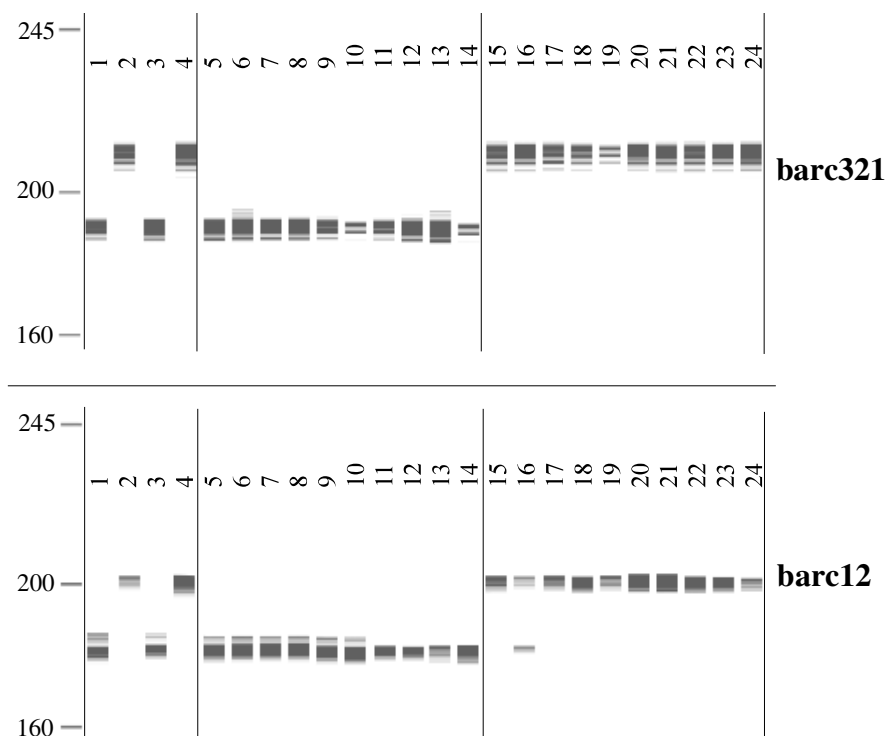
Figure 4.5: PCR fragments produced by amplification of microsatellite markers barc321and barc12 in the F$_2$ individuals selected from the 98S05B*13/Katepwa population. **Lane 1**, DH line 98S05B*13 (resistant). **Lane 2**, Katepwa (susceptible). **Lane 3**, resistant bulk. **Lane 4**, susceptible bulk. **Lanes 5-14**, homozygous resistant F$_{2:3}$ families included in the resistant bulk. **Lanes 15-24**, homozygous susceptible F$_{2:3}$ families included in the susceptible bulk. The size standard is identified to the left in base pairs (bp).

The barc321 and barc12 loci map to the short arm of chromosome 3A in the International Triticeae Mapping Initiative (ITMI) mapping population (W7984/Opata85) (Song et al. 2005). The wmc11, wmc532, and gwm369 loci were polymorphic between the parents and were also previously reported mapped to the short arm of chromosome 3A (Röder et al. 1998; Somers et al. 2004). These loci were also screened on the entire 98S05B*13/Katepwa *Stb14*(+) F$_2$ individuals which corresponded to the *Stb14*(+) F$_3$ families. A linkage group of about 25 cM was constructed using these microsatellite markers (Figure 4.6). The barc321 and barc12 loci were found to flank *StbSm3* at distances of 1.9 and 2.4 cM, respectively.

No previous Stb resistance genes have been linked to the microsatellite markers

barc312 and bac12, therefore this is probably a new Stb resistance gene. The Stb
resistance gene *Stb6* was previously reported to map to the short arm of chromosome 3A,
however, the linkage analysis of *Stb6* placed this resistance gene closer to microsatellite
locus gwm369.
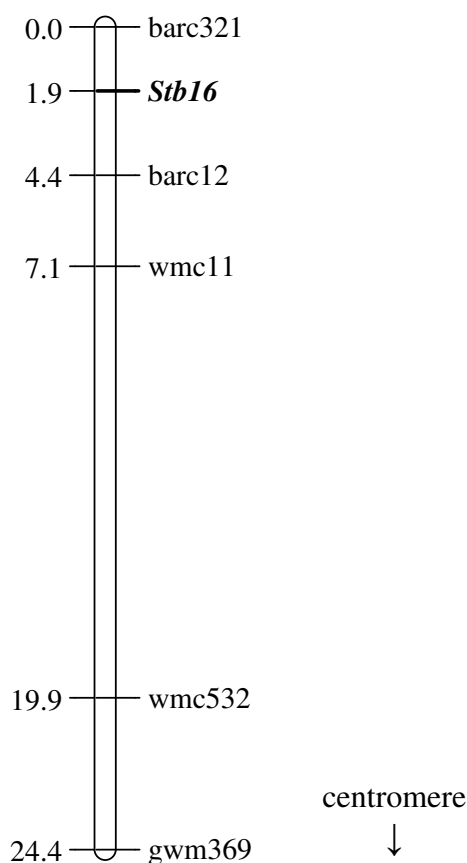
Figure 4.6: Linkage map of the short arm of chromosome 3A showing the septoria tritici
blotch resistance gene *StbSm3* and microsatellite loci. *StbSm3* confers resistance to
isolates MG96-36 and MG2 of *Mycosphaerella graminicola*. Linkage was calculated
using 62 $F_{2:3}$ families selected for presence of *Stb14* on chromosome 3B in a large F_2
population developed from a cross between resistant DH line 98S05B*13 and susceptible
cultivar Katepwa. Genetic distances in Kosambi centimorgans and indicated to the left of
linkage map.

4.4.3 *M. graminicola* isolate IPO323 testing

The detached-leaf testing of the Canadian differential Stb wheat set determined that all wheat lines, except for ST6, are resistant to the *M. graminicola* isolate IPO323. The known susceptible check cultivar Riband displayed a phenotype of extensive necrosis and pycnidial formation and the phenotype of ST6 progressed similarly to Riband so it was determined to be susceptible. The line ST6 was previously reported to contain the resistance gene *Stb7* on 4AL (McCartney et al. 2003). The phenotypes of the differential set are summarized in Figure 4.7. Since IPO323 has been reported to be avirulent to resistance gene *Stb6*, the hexaploid wheat lines Salamouni, Katepwa, 98S08A*09, 98S08C*03, 98S05B*13, and Erik as well as the tetraploid durum wheat Coulter which are resistant to IPO323 may contain the resistance gene *Stb6*.
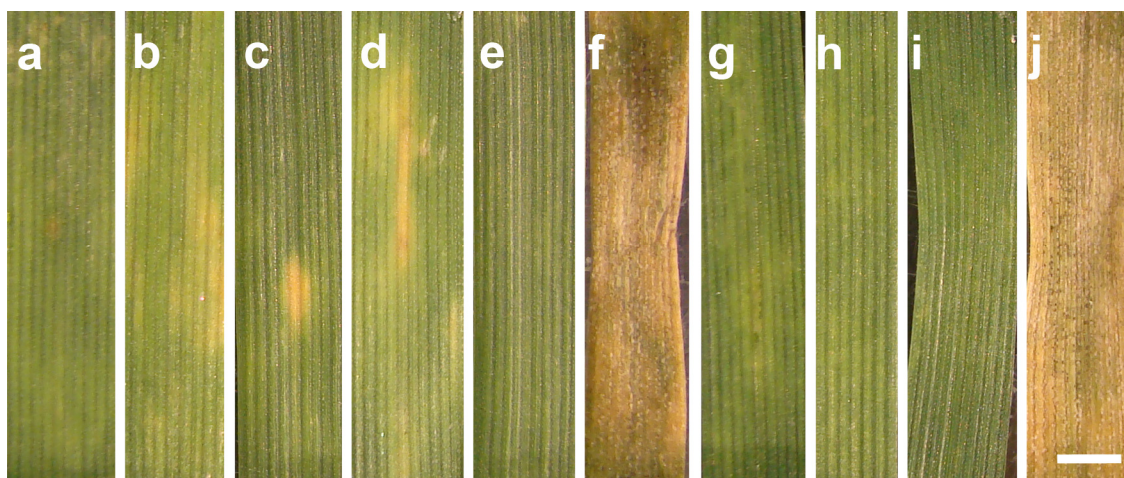
Figure 4.7: Reactions of Canadian differential lines to *Mycosphaerella graminicola* isolate IPO323 at 29 days after inoculation. **(a)**, Resistant reaction of Salamouni. **(b)**, Resistant reaction of Katepwa. **(c)**, **(d)**, and **(e)**, Resistant reaction types of double haploid lines produced from Salamouni/Katepwa cross (98S08A*09, 98S08C*03, and 98S05B*13 respectively). **(f)**, Susceptible reaction of ST6. **(g)**, Resistant reaction of Erik. **(h)**, Resistant reaction of Coulter. **(i)**, Resistant reaction of Flame. **(j)**, Susceptible reaction of Riband. Bar: 2.5 mm.

4.5 Discussion

This study determined the chromosomal location of the third resistance gene

previously identified in the highly resistant wheat landrace Salamouni. The third resistance gene will be designated *StbSm3*. The map location of *StbSm3* on 3AS is about 2 cM away from the microsatellite loci barc321 and barc12. *StbSm3* maps about 22.5 cM distal to the Stb resistance gene *Stb6*, which was reported to be linked within 2 cM of gwm369 (Brading et al. 2002; Chartrain et al. 2005b). *Stb6* is reported to be effective against *M. graminicola* isolate IPO323. To determine if the third resistance gene in Salamouni was different than *Stb6*, the Canadian Stb differential wheat lines were tested with *M. graminicola* isolate IPO323. It was observed that the wheat landrace Salamouni and wheat cultivar Katepwa were both highly resistant to IPO323 (Figure 4.7). Salamouni and Katepwa are the parents of the DH lines 98S08A*09 (*Stb14* mapping), 98S08C*03 (*Stb13* mapping), and 98S05B*13 (*StbSm3* mapping); therefore, it was expected and observed that these DH lines were also resistant to IPO323 (Figure 4.7). No other resistance loci besides *Stb6* have been reported to confer resistance to IPO323. It is probable that all material in the Canadian differential set, except for ST6, contains the resistance gene *Stb6*.

A quantitative trait locus (QTL) for partial resistance to STB on chromosome 3A has also been reported by Eriksen et al. (2003); however, this resistance locus was mapped closer to the centromere. The results of this study indicate that *StbSm3* most likely is a new resistance gene which has not been previously reported in the literature. A gene for resistance to tan spot, *tsn4*, has been previously mapped to chromosome 3A in the landrace Salamouni using monosomic lines (Tadesse et al. 2006). Since linkage analysis of *tsn4* has not been reported, it is not possible to determine if *StbSm3* and *tsn4* map to the same interval. It has been recently hypothesized that *M. graminicola* may produce toxins during the long latent infection period (Orton et al. 2011).

The resistance genes *Stb13* and *Stb14* were previously mapped by Cowling (2006) in Salamouni. The mapping of *StbSm3* to the short arm of chromosome 3A allowed the haplotyping at all three resistance genes of the 67 DH lines produced from reciprocal crosses of Salamouni/Katepwa. The reactions of 37 DH lines to both the MG96-36 and MG2 isolates were previously completed by Cowling (2006), and the phenotypes and

haplotypes for the DH lines are summarized in Appendix 8.7. Of the 37 DH lines phenotyped, four (98S05C*26, 98S05B*14, 98S05B*25, and 98S05B*07) were susceptible to both isolates. These four lines do not have *Stb13* or *StbSm3* based on the genotype at loci wmc396 and barc321, respectively; however, two of these lines probably contain the isolate-specific (MG2) resistance gene *Stb14*. The current study found that lack of *Stb14* in $F_{2:3}$ families of the 98S05B*13/Katepwa mapping population affected reactions and created difficulty in describing clear resistant/susceptible ratings. A possible explanation is that *Stb14* is epistatic to *StbSm3* and/or *Stb13*. Another possible explanation is that the resistance conferred by *Stb14* is reduced at higher levels of *M. graminicola* inoculum. Further studies will be necessary to determine the relationship and the extent of epistasis for the three resistance genes in Salamouni. Since all three resistance genes are linked to microsatellite loci these types of studies would be facilitated by the use of MAS. Populations with the specific combinations of the three resistance genes will need to be created. Flanking markers were reported for *Stb14*; however, no flanking marker was found for *Stb13* (Cowling 2006). Prior to developing these populations, fine mapping of the resistance genes *Stb13* and *Stb14* should be completed to increase the probability of selecting the desired resistance gene with MAS.

Plant breeders will need to manage the available genetic resistance for future cultivars since rapid breakdown of resistance has been reported (Ballantyne and Thomson 1995; Eyal et al. 1973). Deployment of a single Stb resistance gene (*Stb4*) in the cultivar Gene in Oregon, USA, resulted in the pathogen overcoming the resistance in only five years (Cowger et al. 2000). Breeding of wheat cultivars to be grown in western Canada should attempt to utilize all of the resistance genes identified in Salamouni. The high level of resistance conferred by the combination of the three loci could be a durable source of resistance as defined by Johnson (1984). Pyramiding these resistance genes into elite hexaploid germplasm will probably be the best method to use the specific resistance. DNA-based molecular markers will allow MAS to be very useful in developing cultivars quickly and without extensive phenotyping of segregating populations.

Assessing the disease reaction of wheat plants to *M. graminicola* remains a significant challenge. To date, a number of methods has been reported for Stb phenotyping and molecular mapping of Stb resistance. The most popular methods are the seedling test (Cowling 2006; Grieger et al. 2005; McCartney et al. 2002; McCartney et al. 2003), detached-leaf method (Arraiano et al. 2001a), first true leaf testing (Brading et al. 2002; Jing et al. 2008), and adult-plant testing (Arraiano et al. 2009). The first three methods have been used extensively in controlled environments for both qualitative and quantitative assessment, while adult-plant testing is often used in regions with high natural disease pressure for QTL mapping. Unfortunately, studies to compare the methods have not yet been conducted to determine if plant reactions are similar.

StbSm3 confers resistance to *M. graminicola* isolates MG96-36 and MG2 and was mapped to the short arm of chromosome 3A. The nearest molecular markers linked to *StbSm3* are barc321 and barc12 at distances of 1.9 and 2.5 cM, respectively. The interval *StbSm3* has been mapped to is relatively small compared to previous Stb resistance genes. This gene could be relatively easy to incorporate into breeding programs by using MAS. In addition, *StbSm3* may be an ideal resistance gene for fine mapping and possible map-based cloning. The small interval, and the current work on the physical mapping of chromosome 3AS (International Wheat Genome Sequencing Consortium; http://www.wheatgenome.org/) could facilitate fine mapping of this gene. Determining the underlying gene or genes responsible for the resistance to both races of *M. graminicola* in western Canada could be useful in developing a deployment strategy for genetic resistance in new wheat cultivars. Also, further investigation of the tan spot resistance gene *tsn4* may provide information on the possibility of a common locus for resistance to *M. graminicola* and *Pyrenophora tritici-repentis*.

5.0 Fine mapping *Stb14*, an isolate-specific septoria tritici blotch resistance gene in 'Salamouni'

5.1 Abstract

Septoria tritici blotch (Stb) is a devastating foliar disease of wheat caused by the ascomycete fungal pathogen *Mycosphaerella graminicola*. Genetic resistance has been identified and mapped to chromosomes in a number of wheat cultivars and lines, however, none of the underlying genes has been cloned and their function or structure is unknown. The resistance gene *Stb14* in the hexaploid landrace Salamouni was previously reported to confer resistance to *M. graminicola* isolate MG2 from western Canada and also was mapped to the short arm of chromosome 3B. The current study developed a large fine-mapping population with the intent to fine map the resistance gene *Stb14*. A total of 84 fixed recombinant F_4 families between the co-dominant microsatellite loci cfd79 and wmc623 was successfully produced from the cross 98S08A*09/Katepwa. Marker screening was completed to identify new microsatellite, sequence-tagged-site, and insertion-site-based polymorphism markers, however, only a few markers detected clear polymorphism between the parental lines. The new polymorphic markers were not observed to map within the *Stb14* interval. Further work to develop markers will need to be completed before the fine mapping of *Stb14* can be completed.

5.2 Introduction

Wheat (*Triticum aestivum* L.) is one of the most important food crops grown worldwide. In recent years, global wheat production has totalled over 683 million metric tonnes with an average value of over 87 billion dollars (FAOSTAT 2010). The demand for wheat continues to rise as the population increases and as new uses, such as biofuel, for wheat are discovered. To meet the projected global demand of one billion metric tonnes by the year 2020 (Rajaram 2001), wheat production will need to increase significantly. Currently, a number of production challenges impede world wheat

production including several biotic and abiotic stresses. Wheat breeders will need to incorporate genetic resistance to biotic stresses and tolerance to abiotic stresses that affect wheat crops.

An economically important biotic stress in wheat is the foliar disease septoria tritici blotch (Stb), caused by the pathogen *Mycosphaerella graminicola* (anamorph *Septoria tritici*). It is often the most important foliar disease in temperate and humid environments (Cowger et al. 2000; Hardwick et al. 2001). This disease has caused grain losses in the range of 25 to 50% during severe epidemics (Eyal et al. 1985; King et al. 1983).   Foliar fungicides have been used to reduce grain yield losses due to Stb, however, this type of control is expensive and not always effective. Strobilurin fungicides (Qo inhibitors) have been used extensively to control Stb from the late 1990s to the early 2000s, however, in 2002 isolates of *M. graminicola* were identified in Europe which were resistant to strobilurins (Fraaije et al. 2005; Torriani et al. 2009). More recently, isolates of *M. graminicola* resistant to triazole fungicides have been discovered (Brunner et al. 2008; Cools and Fraaije 2008; Cools et al. 2004). The most economical and environmentally safe method for controlling this devastating disease is to grow cultivars with durable genetic resistance.

Resistance to *M. graminicola* has been reported to be both partial (quantitative) and specific (qualitative). Specific resistance is oligogenic, near-complete and follows a gene-for-gene relationship (Brading et al. 2002; McCartney et al. 2002). Partial resistance is more complex as it is incomplete, polygenic, and not isolate-specific (Chartrain et al. 2004b; Jlibene et al. 1994; Simon and Cordo 1998).

Extensive effort has been invested by wheat researchers to identify major resistance loci effective against global *M. graminicola* isolates. To date, the chromosomal locations of 15 septoria tritici blotch resistance genes have been reported in hexaploid wheat (Table 2.2) (Adhikari et al. 2003; Adhikari et al. 2004b; Adhikari et al. 2004c; Adhikari et al. 2004d; Arraiano et al. 2007; Brading et al. 2002; Chartrain et al. 2005a; Chartrain et al. 2005c; Chartrain et al. 2009; Cowling 2006; Goodwin 2007; McCartney et al. 2003). These resistance genes have been designated *Stb1* to *Stb15*.

Although the chromosomal locations have been determined, generally the resistance genes have not been closely linked to flanking markers, often the linkage groups distances are large, and to date, none of the genes have been isolated (Table 2.2). Fine mapping these Stb resistance genes to identify diagnostic molecular markers will help wheat breeders rapidly develop elite wheat cultivars with durable resistance by using marker-assisted selection (MAS). Diagnostic markers should help to reduce introgression of undesirable genes which has been associated with MAS. Also, fine mapping will assist in future map-based cloning efforts and the study of the structure and function of the Stb resistance genes.

Fine mapping and map-based cloning of resistance genes in hexaploid wheat has lagged behind other food crops such as maize and rice largely due to the complexity of the wheat genome. Modern bread wheat is an allohexaploid consisting of three distinct, but related genomes (2n=6X=42), with a total haploid DNA content of about 1.6×10^{10} bp (Arumuganathan and Earle 1991) with over 50% repetitive sequences (Flavell and Smith 1976). Chromosome 3B was estimated to be the largest wheat chromosome at 9.95×10^8 bp, which is more than twice the size of the total rice genome (3.7×10^8 bp) (Paux et al. 2008). About 40 genes and quantitative trait loci (QTL) have been mapped to chromosome 3B (http://wheat.pw.usda.gov/GG2/maps.shtml) including the resistance genes *Stb2* (Adhikari et al. 2004c), *Sr2* (Spielmeyer et al. 2003), *Fhb1* (Cuthbert et al. 2006), and *Stb14* (Cowling 2006). The resistance gene *Stb14* confers resistance to the western Canadian *M. graminicola* isolate MG2, and was identified (McCartney et al. 2002) and mapped to the short arm of chromosome 3B (Cowling 2006) in the hexaploid landrace Salamouni.

The objective of the current study was to develop a fine-mapping population to isolate *Stb14*, a specific resistance gene contained on chromosome 3B in the hexaploid landrace Salamouni.

5.3 Materials and Methods

5.3.1 Mapping population development

Parental seed and F_1 individuals developed during the mapping of *Stb14* (Cowling 2006) were used to construct a large fine-mapping population (Figure 5.1). Twelve F_1 individual plants were selected from the cross of the resistant DH line 98S08A*09 and the susceptible cultivar Katepwa to be self pollinated. Each F_1 plant was grown in a 20-litre pot filled with a 2 : 2 : 1 soil mix (soil/sand/peat) and placed in a controlled environment with temperatures of 10/8°C (day/night) with a 16h photoperiod (250µE m^{-2}s^{-1}). A lower growing temperature was used to promote tiller production and increase seed production. The F_1 plants were fertilized with 20-20-20 fertilizer (3.75 mg/L) weekly and watered as required. A total of 2814 F_2 seeds was produced from the twelve F_1 plants (an average of about 234 seeds per plant). Seed produced per plant was lower than expected due to infection by powdery mildew (*Blumeria graminis* f.sp. *tritici*). The resistance gene *Stb14* was previously found to be flanked by the microsatellite (SSR) loci wmc500 and wmc623 at genetic distances of 2 and 5 cM, respectively, on chromosome 3B (Cowling 2006). Locus wmc500 was scored as a dominant marker and was difficult to score reliably due to a large number of clustered bands. Recombinant selection required that both parental alleles for the flanking loci be visualised, therefore, the co-dominant microsatellite locus cfd79 was selected for fine-mapping population development. The differences in PCR fragment size of alleles at loci cfd79 and wmc623 between the parents were found to be large and easy to score.

Parents: **98S08A*9 / Katepwa**

P1   P2

MG96-36:   Susceptible (4)   Susceptible (4-5)

MG2:   Resistant (3)   Susceptible (4-5)

F₁: 12 F₁ plants

F₂: 2367 F₂ seedlings genotyped with SSR markers linked to *Stb14* (cfd79 and wmc623).

Individuals with the following banding patterns were selected to be self pollinated.

F₃: Individuals from F₃ families derived from 192 F₂ individuals with

recombinant banding paterns R1 to R4 genotyped with SSR markers linked

to *Stb14* (cfd79 and wmc623) to identify fixed-recombinant F₃ individuals.

Fixed-recombinant F₃ individuals were self pollinated to produced fixed recombinant F₄ families.

Example of banding paterns of a R1 F₃ family:

Plant ID 1, 7, 15 or 16 would be selected to be self-pollinated in this example

F₄: 84 fixed-recombinant F₄ families developed for phenotypic assessment with Canadian isolate MG2.

Families were expected to be fixed present or absent for *Stb14* within this interval and the phenotype of each family was expected to be scored as either homozygous resistant (HR) or homozygous susceptible (HS).

Figure 5.1: Development of a large population for the fine mapping of *Stb14* on the short arm of chromosome 3B in the cross 98S08A*09/Katepwa.

About 2500 F_2 plants were grown and tissue was harvested for genotyping (Section 5.3.2) with SSR markers, cfd79 and wmc623, linked to *Stb14*. The recombinant F_2 plants (R1 to R6 banding patterns in Figure 5.1) were selected. Also, about 100 F_2 plants of each parental type (P1 and P2 in Figure 5.1) were selected to be used as checks during phenotyping. The remainder of the F_2 plants was discarded. A total of 715 single-recombinant F_2 plants (R1 to R4) were self-pollinated to produce F_3 families. Since the genetic distance between the markers cfd79 and wmc623 was found to be large (about 23 cM), a subset of 192 $F_{2:3}$ families was screened for fixed-recombinant plants. Each fixed-recombinant plant that was selected was homozygous for the resistant parent allele at one marker and homozygous for the susceptible parent allele at the other marker (Figure 5.1). The fixed recombinant F_3 plants were self-pollinated. A total of 84 unique fixed recombinant F_4 families were successfully produced. The F_2 or F_3 plants selected during genotyping were grown in four-litre pots following the methods previously described.

5.3.2 Genotyping

Leaf tissue was collected from the youngest leaf of F_1, F_2 and $F_{2:3}$ individual plants. The harvested leaf tissue was lyophilized for 72h and then stored at -20°C. Lyophilized tissue for each plant and 200 µl of glass beads were placed in a 1.2-ml microtube. The microtubes were arranged in a 96-well plate. The plant tissue was ground to a fine powder by shaking the 96-well plate in a TissueLyzer II (Qiagen). DNA was extracted using the Patricia Warner DNA extraction method (Appendix 8.6). DNA was quantified using Hoechst 33258 stain and diluted to a final concentration of 10 ng/µl.

Polymerase chain reaction (PCR) for microsatellite (SSR) and sequence-tagged site (STS) markers was performed in 10-µl reaction volumes containing: 50 ng of template DNA, 0.5 U of Taq DNA polymerase (Promega), 1X PCR buffer (Applied Biosystems, Foster, CA, USA), 1.5 mM MgCl$_2$, 0.1 mM each dNTP, 0.2 pmols forward primer, 2.0 pmols of reverse primer, and 1.8 pmols of 6-FAM/HEX/NED labelled M13 primer (5'-CACGACGTTGTAAAACGAC-3'; Applied Biosystems, Foster, CA, USA). Thermal cycling programs used were: 1 cycle of [94°C for 2 min], 30 cycles of [95°C for

1 min, (-0.5°C/s ramp to 61/55/51°C), 61/55/51°C for 50 sec, (+0.5°C/s ramp to 72°C), 72°C for 1 min], 1 cycle of [72°C for 5 min]. PCR fragments were resolved by capillary electrophoresis using an ABI PRISM 3130xL Genetic Analyzer (Applied Biosystems, Foster, CA, USA), and GeneScan 500 ROX as the internal molecular weight standard. Data generated (FSA files) by the ABI Genetic Analyzer was analyzed with GeneScan Analysis Software (Applied Biosystems, Foster, CA, USA) to standardize the M13-labelled PCR fragments size to the GeneScan-500 ROX size standard. After the genetic analyzer data was analyzed with GeneScan, a gel-like image was created using the Genographer version 1.6.0 available at http://hordeum.oscs.montana.edu/genographer/.

5.3.3 Marker screening

DNA-based molecular markers identified as linked within the *Stb14* interval were selected to be screened for polymorphism on each parent. Markers were selected from the bread wheat microsatellite consensus map (Somers et al. 2004), and the physical map developed for chromosome 3B (Paux et al. 2008). The majority of markers selected for screening were chosen from the physical map of chromosome 3B. Three types of markers were screened in this study, including microsatellites (SSR), sequence-tagged site (STS), and insertion site-based polymorphism (ISBP). Forward and reverse primers for each marker are identified in Table 5.1. Microsatellite loci with the prefix barc, cfa, cfd, gwm, and wmc as well as gpw3156, gpw3092, gpw3248, and gpw8100 were screened at Agriculture and Agri-Food Canada (AAFC). The remaining microsatellites with the gpw prefix loci and all wmm prefix were screened at INRA Clermont (France) by Dr. Pierre Sourdille. Microsatellite and STS markers were amplified and visualised using procedures outlined in Section 5.3.2.

Insertion-site-based polymorphism (ISBP) markers were screened using Real-Time PCR (RT-PCR) Melting Curve Analysis (MCA) (Appendix 8.8). Amplified PCR fragments from MCA were also visualised using 4% agarose gels to determine if dominant polymorphism was evident.

5.3.4 Genetic map construction

A genetic map of wheat chromosome 3BS for the cross 98S08A*9/Katepwa was constructed to determine genetic distances between molecular markers and also marker order. A total of 172 F_2 individuals were used along with the polymorphic SSR, STS, and ISBP markers from the 37 total screened markers. The software package JoinMap 4.0 (van Ooijen 2006) was used to calculate the linkage group and produce a genetic map of chromosome 3B. Genetic distances were calculated using Kosambi centiMorgans. The LOD threshold for the generated linkage group was 3.0.

5.4 Results

5.4.1 Genotyping

The number of selected F_2 single-recombinant plants totalled 715 in the 98S08A*09/Katepwa cross (R1 to R4; Figure 5.1). A total of 54 double recombinants were also identified in the F_2 plant screening (R5, and R6; Figure 5.1). The interval distance between loci cfd79 and wmc623 was calculated to be 22.5 cM. Since the interval distance was large, only 192 F_3 families were screened for fixed-recombinant individuals. From the 192 F_3 families, a total of 120 unique fixed recombinant F_3 plants were identified and self-pollinated.   Of the 120 fixed recombinant plants, 36 were lost due to pests in the greenhouse.

5.4.2 Marker screening and map construction

The reported linkage map for *Stb14* contained eight microsatellite loci and loci wmc500 and wmc623 were found to flank *Stb14* at distances of 2 and 5 cM, respectively (Cowling 2006). Molecular markers used in the initial mapping of *Stb14* relied solely on loci from the wheat consensus map (Somers et al. 2004), while this study used the microsatellite (SSR), sequence-tagged site (STS), and insertion-site-based polymorphism (ISBP) markers from the recent physical map of wheat chromosome 3B (Paux et al 2008). Diversity ARrays Technology (DArT) marker probes were not used since they were not publicly available at the time of genotyping.

The markers linked to *Stb14* were compared to the wheat physical map of chromosome 3B, and found to be contained within the deletion bin 3BS8-0.78-0.87. *Stb14* is probably also contained in this deletion bin; therefore, markers in this area were selected for screening. In the current study, all markers were screened against the resistant DH line 98S08A*9, susceptible parent Katepwa, at least one F_1 from the cross '98S08A*9/Katepawa', and four randomly selected F_2 plants.

A total of 10 STS markers were screened using annealing temperatures of either 51 or 61°C (Table 5.1). The markers STS15, STS17, and STS194 did not amplify while STS46, STS49, STS58, STS66, STS94, and STS142 were monomorphic. One STS locus, STS52, produced a banding pattern that was complex and not distinguishable.

Table 5.1: Primer sequences, visualisation method, and results for molecular markers screened on the parents and F_1 of the 98S08A*9/Katepwa recombinant population.

Marker	Type[A]	Forward primer (5' – 3')	Reverse primer (5' – 3')	Resolution method & result[B] MCA[C]	Agarose[C]	Sequencer[C]
cfp35	ISBP	CCGAAGGTTTCATTCCGTTTG	GTGCCCAACCCACTAGACAT	ND	ND	-
cfp45	ISBP	CCGGCTTTCTTTGTCCCTG	CTTTGGAGTTGCCCTTAAGC	ND	ND	-
cfp57	ISBP	GCATCCCCTCTTTCGTCTC	GCCGTTTCTCCCTTGAGC	ND	ND	-
cfp58	ISBP	CGGACACGGGATGAAGTC	GATGAAAAACCTACCCAAGCAC	Poly (88°C)	ND	ND
cfp59	ISBP	CGACACAAGGAGAGGCAAAG	AAATCAACCAGACCACCATTGC	Mono	Mono	-
cfp171	ISBP	TCGTCCGTAGTCAGATGATGCTC	CCAAACACACCCCAACACCC	Mono	Mono	-
cfp1738	ISBP	AAACAGCAACTAACACTGGGCAC	GGGGCGGCGAAGGTGAAG	Mono	Mono	-
cfp1836	ISBP	GCCTATTCACCCCCCCTCCC	AGACGGCATCCCACATGGAC	Mono	ND	-
cfp3102	ISBP	GTGTACGATTGAATCGGGGGC	TAGTAGAAACGGGGGCAATGGC	Mono	Mono	-
cfp3215	ISBP	TCACAAACCAAACAGCCGCC	CCAAAGCCTCTGCGTCACC	Poly (87°C)	ND	ND
cfp3410	ISBP	GCCAACTCAACAAACAACTTCGG	TCCAAATGATCACGCCAGGAAC	Mono	ND	-
cfp3530	ISBP	AAGAATCCCAGAAAACTAATTTGG	CCATTGAAAACACACATCACG	Mono	ND	-
cfd143	SSR	TTCTCCATGGGCAGCTACTT	ACTACTTGCGGACGGCTG	-	-	Mono
cfb3119	SSR	CONFIDENTIAL[D]	CONFIDENTIAL[D]	-	-	Mono
wmm242	SSR	CONFIDENTIAL[D]	CONFIDENTIAL[D]	-	-	NA
wmm626	SSR	CONFIDENTIAL[D]	CONFIDENTIAL[D]	-	-	NA
wmm1104	SSR	CONFIDENTIAL[D]	CONFIDENTIAL[D]	-	-	Poly
wmm1344	SSR	CONFIDENTIAL[D]	CONFIDENTIAL[D]	-	-	NA
wmm1648	SSR	CONFIDENTIAL[D]	CONFIDENTIAL[D]	-	-	Mono

wmm2176	SSR	CONFIDENTIAL[D]	CONFIDENTIAL[D]	-	-	Mono
gpw3092	SSR	GCTCGCAGGAGGTAGTGC	GCTCGCAGGAGGTAGTGC	-	-	Poly
gpw3156	SSR	CTGAGAGGACCCCGAACTC	CATGGGGATGATTCTGACAA	-	-	NA
gpw3248	SSR	CATGGACAGAGACGACAGGA	AAAAATGCACGTCCCAAAAT	-	-	Poly
gpw4146	SSR	CONFIDENTIAL[D]	CONFIDENTIAL[D]	-	-	Mono
gpw7031	SSR	CONFIDENTIAL[D]	CONFIDENTIAL[D]	-	-	Mono
gpw8100	SSR	GCCATTCTATCTGTGTTCTT	ATGCATGATTGGTTCTACTA	-	-	NA
barc75	SSR	AGGGTTACAGTTTGCTCTTTTAC	CCCGACGACCTATCTATACTTCTCTA	-	-	Poly
barc87	SSR	GCTCACCGGGCATTGGGATCA	GCGATGACGAGATAAAGGTGGAGAAC	-	-	Poly
cfa2226	SSR	GGAGAAAAGCAAACAGCGAC	CAGTAGCATCTTCCATGGCG	-	-	Poly
cfd28	SSR	TGCATCTTATTACTGGAGGCATT	CGCATGCCCTTATACCAACT	-	-	NA
cfd79	SSR	TCTGGTTCTTGGGAGGAAGA	CATCCAACAATTTGCCCAT	-	-	Poly
wmc54	SSR	TATTGTGCAATCGCAGCATCTC	TGCGACATTGGCAACCACTTCT	-	-	Mono
wmc500	SSR	ATAGCATGTTGGAACAGAGCAC	CTTAGATGCAACTCTATGCGGT	-	-	ND
wmc623	SSR	ACGATTGGCCACAGAGGAG	CAGTGACCAATAGTGGAGGTCA	-	-	Poly
wmc754	SSR	ATGGACTAGGTGGGCTGTGG	CAGGGTGTCTGGGTGTCTCA	-	-	Poly
wmc808	SSR	TGAACCATCATCGGAGCTTG	TTTTAGCCGAAGTCAAACATTGC	-	-	Mono
gwm389	SSR	ATCATGTCGATCTCCTTGACG	TGCCATGCACATTAGCAGAT	-	-	Poly
gwm533	SSR	AAGGCGAATCAAACGGAATA	GTTGCTTTAGGGGAAAAGCC	-	-	Poly
STS15	STS	CGGTCTGCCTCTACCAGAAG	ACGGGTTGTGGGATGTGTAT	-	-	NA
STS17	STS	GTTGTTTTCGGCCAGGTAGA	CCCTTGTGGTCCTACTGCAT	-	-	NA
STS46	STS	TTCCCGTGGGTCGTACTATG	CGCTGAGGGTACGAGTCTTC	-	-	Mono

STS49	STS	ATTGCGAAGAACACCCTCAC	ACGTCTTCAGCACCGAGTTC	-	-	Mono
STS52	STS	AGAGCGTGAAGGTGCTCAAG	TAGCTGCCTTTCTCGAGCTT	-	-	ND
STS58	STS	AAGTGCGACCAGGATGAGTC	ACGTTGAGCTCCTCGCTAAC	-	-	Mono
STS66	STS	AGTCAGGCGAAGAGCGATAA	AGCACTGCACAATGAGCATC	-	-	Mono
STS94	STS	TGCAGTCAAGCGATCTGAAC	ATCTGCAATGGTGGAGAGGT	-	-	Mono
STS142	STS	CGAGTACTACCTCGGCAAGC	CATAGAATGCCCCGAAACTG	-	-	Mono
STS194	STS	ACAGTCATCGGCAAGATTCC	ACCCGGAATATCAATCACCA	-	-	NA

[A] Type of molecular marker. ISBP = Insertion-Site Based Polymorphism. SSR = Microsatellite. STS = Sequence-Tagged Site.

[B] Visualisation methods. MCA = Melting curve analysis using Real-Time PCR. Agarose = PCR product from MCA was visualised in 4% agarose gel. Sequencer = PCR completed with M13 tailing on the forward primer and visualised with an ABI PRISM 3130xL Genetic Analyzer.

[C] ND = Not distinguishable. NA = No amplified product. Mono = Monomorphic. Poly = Polymorphic.

[D] Contact Pierre Sourdille of INRA Clermont (France) for further information on primer sequences of wmm markers.

Fifteen SSR loci were selected from the wheat physical map of chromosome 3B. Of these, four were polymorphic including cfa2226, wmm1104, gpw3092, and gpw3248 (Table 5.1). Loci cfa2226, gpw3092, and gpw3248 were polymorphic based on PCR fragment size, while wmm1104 was observed to be a dominant polymorphism. The remaining wmm loci were either monomorphic (Mono), not distinguishable (ND), or did not amplify (NA) (Table 5.1). All wmm loci screened in the current study as well as gpw7031 and gpw8100 were analysed by INRA Clermont (France) due to confidential primer sequences.

In addition, a selection of 12 ISBP loci were screened by INRA Clermont (France) using melting-curve analysis (MCA) and agarose (4%) visualization. The number of polymorphic loci was low, and only two loci, cfp58 and cfp3215, were polymorphic using MCA at temperatures of 88 and 87°C respectively (Table 5.1). The PCR products of eight ISBP markers (cfp35, cfp45, cfp57, cfp58, cfp59, cfp1836, cfp3215, cfp3410, and cfp3530) were not distinguishable when visualised using agarose gels due to a high number of bands. The remaining four ISBP loci (cfp59, cfp171, cfp1836, cfp3102) were monomorphic. Further attempts to use cfp58 or cfp3215 in the lab at Agriculture and Agri-Food Canada were unsuccessful as the PCR fragments were too numerous and the banding patterns were not distinguishable (Table 5.1).

5.4.3 Genetic map construction

The molecular markers previously used in mapping *Stb14* were included in the map constructed in this study, except for wmc500. Locus wmc500 was difficult to score reliably due to a complex banding pattern. In total, ten microsatellite loci on chromosome 3BS were screened on 172 F_2 individuals (Figure 5.2A). The locus order was consistent with the map reported in the physical mapping of chromosome 3B (Figure 5.2). No further mapping was completed since no new loci were found to link within the *Stb14* interval. The interval distance between cfd79 and wmc623 was about 7cM larger than reported previously (Cowling 2006).
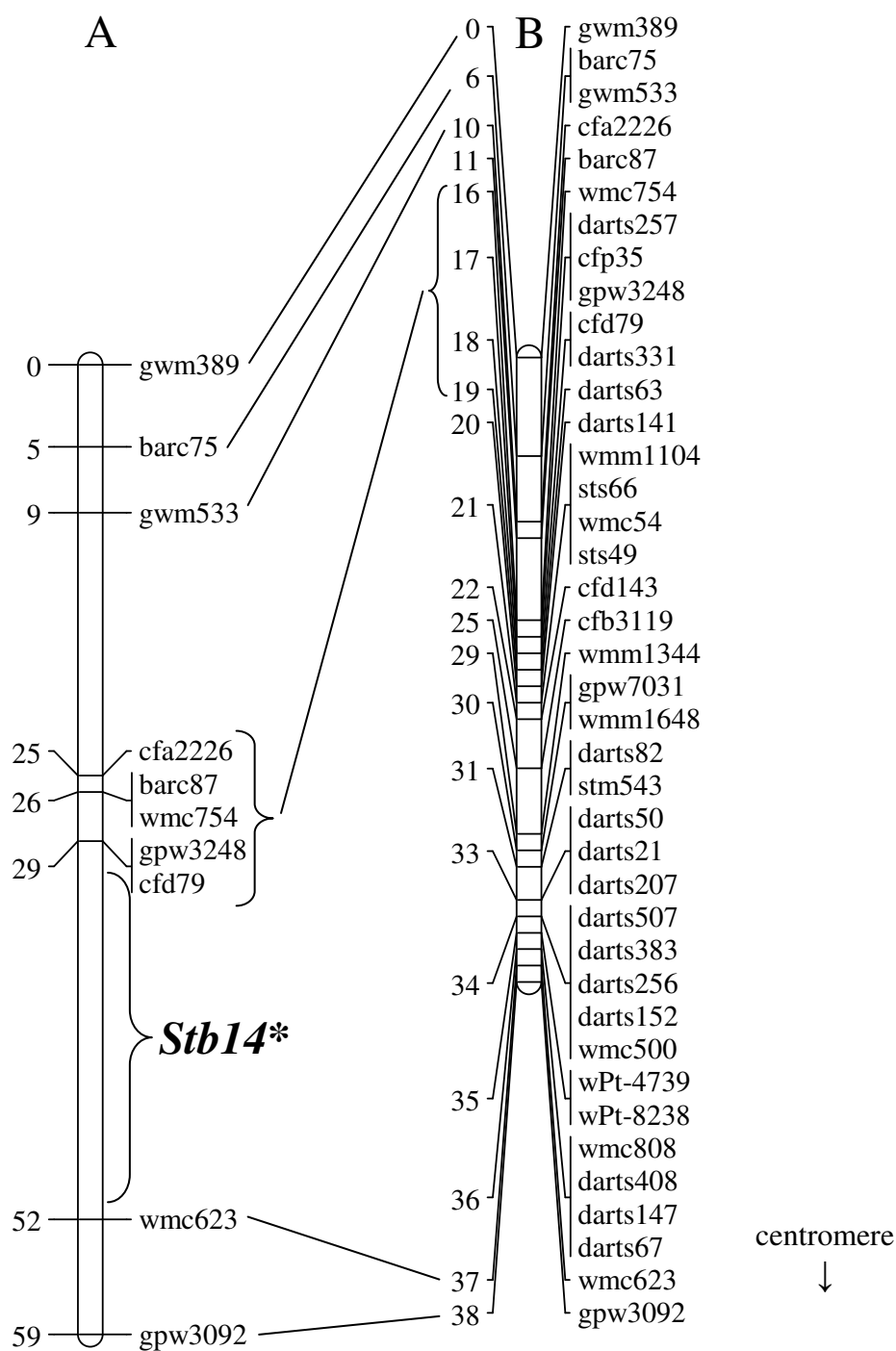
Figure 5.2: Comparison of the 98S08A*9/Katepwa 3BS genetic map and neighbour map of 3BS. **(A)** 98S08A*9/Katepwa F_2 3BS genetic map (172 F_2 individuals), **(B)** 3BS neighbour genetic map (13 populations) constructed by Paux et al. (2008). *Stb14 previously identified within this interval (Cowling 2006).

5.5 Discussion

The present study attempted to fine map the isolate-specific septoria tritici blotch resistance gene *Stb14* on chromosome 3B in the hexaploid wheat landrace Salamouni. A large population of fixed-recombinant lines was developed, which is suitable for phenotyping and genotyping with new molecular markers. The largest source of new molecular markers for this study was the physical mapping study of chromosome 3B (Paux et al. 2008). Three types of molecular markers were screened on the cross 98S08A*09/Katepwa to identify polymorphic markers within the *Stb14* interval. Microsatellite, sequence tagged site, and insertion site-based polymorphism markers were selected from the physical map of wheat chromosome 3B (Table 5.1).

The markers which were clearly polymorphic in the mapping population (cfa2226, gpw3248, and gpw3092) were not found to be linked within the cfd79 and wmc623 interval. Attempts to use the wmc500 marker did not result in a clear banding pattern and it was excluded from the genetic map since it could not be reliably scored. The wmm1104 locus was polymorphic; however, the primer sequences are confidential which would require sending the entire fine-mapping population to INRA Clermont (France) for screening in order to include it in the linkage analysis. The remaining microsatellite and sequence-tagged site markers were not easy to distinguish, did not amplify, or were monomorphic (Table 5.1).

Insertion site-based polymorphism (ISBP) markers are a new type of marker that exploits insertion sites of transposable elements (Paux et al. 2010; Paux et al. 2008) and are becoming useful in fine mapping genes in hexaploid wheat. Recently, the leaf rust resistance gene, *Lr34*, was fine mapped and a candidate gene was identified by using ISBP markers (Dakouri et al. 2010). The ISBP markers screened in this study did not show clear polymorphism and only two ISBP markers, cfp58 and cfp3215, were polymorphic at 88 and 87°C respectively using MCA. The PCR fragments generated by MCA were also visualised using a 4% agarose gel, however, many bands were observed and polymorphism was not clear. These loci were not screened on the fine-mapping population using MCA since the locations of these two ISBP loci on the physical map of

chromosome 3B were probably not within the *Stb14* interval.

The physical map of wheat chromosome 3B contained a large number of Diversity ARrays Technology (DArT) markers and, within the *Stb14* interval, 16 DArT markers were identified (Paux et al. 2008). This type of marker is array-based and is generally completed for wheat researchers by the company Triticarte (http://www.triticarte.com). The sequences of each DArT marker were previously confidential; however, in 2010, the DArT sequences were released to the public (Diversity ARrays Technology; http://www.diversityarrays.com/sequences.html). These DArT sequences will be very useful in creating PCR-based markers for further *Stb14* fine mapping. Determining single-nucleotide polymorphisms with these sequences could be completed either by using melting-curve analysis (Akey et al. 2001), or by developing cleaved amplified polymorphic sequence markers (Konieczny and Ausubel 1993). Due to time constraints of the current project, sequences were not exploited to develop new primers.

It is interesting to note that the genetic distance between the markers cfd79 and wmc623 is about 7 cM larger than observed previously by Cowling (2006). This could be due to a larger number of F_2 individuals that were used to construct the linkage group. The *Stb14* mapping study used only 68 reconstituted F_2 individuals while the current study used 172 F_2 individuals. Another reason that this interval is larger could be due to growing the F_1 plants in cooler temperatures. A recent study has observed that recombination frequencies between linked markers can increase substantially when plants are exposed to physical stress such as temperature (Wijnker and de Jong 2008).

In summary, the current study was able to develop a large population suitable for fine mapping the Stb resistance gene *Stb14*; however, there were few clearly polymorphic loci identified during marker screening. Future work on fine mapping of *Stb14* will be facilitated by the extensive population developed during this study. As new DNA-based molecular markers become available through annotation and assembly of the recently released hexaploid wheat genome sequence (International Wheat Genome Sequencing Consortium, http://www.wheatgenome.org), fine mapping and possibly map-based

cloning of *Stb14* could be completed quickly. The *Stb14* fine-mapping population also could be very useful for helping to assemble available sequence reads within the *Stb14* interval.

6.0 General Discussion and Conclusions

Septoria tritici blotch is a devastating foliar disease of wheat-growing regions worldwide and is caused by the pathogen *Mycosphaerella graminicola* (anamorph *Septoria tritici*). In some regions where Stb is a major disease, such as the United Kingdom (UK), producers successfully minimize grain yield losses by applying fungicides to the crop during the growing season. While this approach has worked, studies have reported numerous instances of *M. graminicola* isolates becoming resistant to these chemicals. The most economical and environmentally sound method for producers to minimize the risk of yield loss due to Stb is to use cultivars with durable genetic resistance. To date, few cultivars with adequate resistance have been available to producers. Previous research has shown that high-yielding wheat cultivars grown by producers are generally more susceptible to Stb, which indicates that a biological cost may be associated with Stb resistance (Dr. James K. M. Brown, John Innes Centre, pers. comm.).

The present study identified quantitative trait loci (QTL) for Stb resistance, Stb escape traits, grain yield and thousand-kernel weight by evaluating a doubled haploid (DH) population in separate disease field trials and yield field trials. The DH population was developed using the resistant winter wheat cultivar Pastiche and the susceptible winter wheat cultivar Torfrida. Pastiche has previously been reported to contain Stb resistance which was not isolate-specific and not accounted for by the disease-escape traits plant height, internode length, or heading date. A new QTL for decreased flag leaf pycnidial coverage was identified on the short arm of chromosome 2A which co-localized with a second new QTL for increased grain yield. A number of microsatellite and Diversity ARrays Technology (DArT) markers were closely associated with *QStb.jic-2A* and *QYld.jic-2A*. The microsatellite markers will be immediately useful for wheat breeders to incorporate this partial Stb resistance while increasing grain yield QTL. It is unknown if the relationship between the Stb resistance and grain yield increase found on chromosome 2A of Pastiche is linkage or pleiotropy. To determine this, further work

in fine mapping the interval would need to be completed. The fine mapping of this interval could be facilitated by use of DArT markers. This work may provide valuable insight into the function and structure of the underlying gene or genes.

The second part of this study was to map the location of the third multiple-isolate resistance gene in the resistant landrace Salamouni. A mapping population was constructed using the DH line 98S05B*13, which was observed to be resistant to both western Canadian *M. graminicola* isolates, MG96-36 (race 1) and MG2 (race 2). This DH line was created from a cross between Salamouni and the susceptible cultivar Katepwa and was found to lack *Stb13* based on haplotype. During the development of this mapping population, the DH line 98S05B*13 was found to be homozygous for Salamouni alleles at the microsatellite markers linked to *Stb14* on chromosome 3BS. The phenotypic segregation of the F_2 population when inoculated with either MG96-36 or MG2 fit a ratio of 3 resistant : 1 susceptible, characteristic of a single dominant resistance gene. It was expected that a phenotypic segregation ratio of 15 resistant : 1 susceptible would have been observed after inoculation with MG2, since 98S05B*13 probably contained *Stb14* and it was previously determined that *Stb14* confers resistance to *M. graminicola* isolate MG2 only. The F_2 population was screened with the markers linked to *Stb14* (cfd79 and wmc623) and individuals were selected for homozygous Salamouni alleles, *Stb14(+)*, or homozygous Katepwa alleles, *Stb14(-)*, at both microsatellite loci. The *Stb14(+)* $F_{2:3}$ families and *Stb14(-)* $F_{2:3}$ families were screened with isolate MG96-36. Ratings for *Stb14(-)* families were difficult since necrotic lesions were slightly more coalesced, and clear differentiation of resistant and susceptible types was complicated. Phenotypes of the *Stb14(+)* $F_{2:3}$ families were easier to rate since resistant and susceptible types were easy to classify. The third multiple-isolate resistance gene was mapped using phenotypes of the *Stb14(+)* $F_{2:3}$ families and bulked segregant analysis (Michelmore et al. 1991). This third resistance gene was flanked by the microsatellite markers barc321 and barc12, both of which map to the short arm of chromosome 3A. This is a new resistance gene and is tentatively designated *StbSm3*.

This study observed a possible epistatic relationship between the genes *Stb14* and

StbSm3, however, the extent of the interaction was not determined. Now that linked microsatellite markers are available for all three resistance genes (*Stb13*, *Stb14*, and *StbSm3*) specific crosses can be developed to produce a series of lines with each combination of the resistance genes. Since Katepwa is susceptible to tan spot (*Pyrenophora tritici-repensis*) these lines could be used to determine if the tan spot resistance gene (*tsn4*) in Salamouni maps near *StbSm3*. If these two genes are linked, it is possible this is a broad-spectrum leaf spotting resistance locus which, could be very useful for wheat breeding programs worldwide.

       The resistance gene identified as *StbSm3* in the current study maps close to the telomere of chromosome 3AS while *Stb2*, a previously mapped resistance gene (Adhikari et al. 2004c), maps close to the telomere of chromosome 3BS. It is quite possible that these resistance genes could have evolved from a common ancestor. Analyzing the structure and function of the underlying genetic resistance at these loci could help determine the possible evolution of these genes.

       To date, sixteen Stb resistance genes (designated *Stb1* to *StbSm3*) have been mapped and identified in various hexaploid wheat cultivars or lines. None of these resistance genes have been cloned and their structure and function remain unknown. Information on gene structure and function could be helpful to incorporate durable genetic resistance in new wheat cultivars and could be enhanced by determining the ideal combinations of genetic resistance.

       The final study of this project attempted to fine map the Stb resistance gene *Stb14*, previously reported to be on the short arm of chromosome 3B (Cowling 2006). Wheat chromosome 3B has been of great interest to researchers due to the high number of disease resistance genes that have been mapped to its short arm including *Stb2* (Adhikari et al. 2004c), *Sr2* (Spielmeyer et al. 2003), *Fhb1* (Cuthbert et al. 2006), and *Stb14* (Cowling 2006). This study developed a large fine mapping population of 84 fixed-recombinant F_4 families using the co-dominant flanking microsatellite markers cfd79 and wmc623. Marker screening was completed in an attempt to identify new microsatellite, sequence tagged-site, and insertion-site-based polymorphism markers,

however, only a few detected a clear polymorphism between the parental lines. Further work on fine mapping *Stb14* can be facilitated by use of the hexaploid wheat genome sequence to develop new reliable polymorphic markers. The physical mapping of chromosome 3B has determined that a number of DArT markers maps to the *Stb14* interval, however, the sequences of these DArT markers were not publicly available when the marker screening was completed in the current study. The use of melting-curve analysis could be a very useful approach to screen the DArT markers for single nucleotide polymorphisms (SNPs) quickly in the 98S08A*09/Katepwa fine-mapping population. The DArT makers could also be converted to cleaved amplified polymorphic sequence (CAPS) markers, however, this approach is currently more expensive due to DNA sequencing costs. The future of precise genetic research in wheat is dependent on reliable genomic sequence information. The International Wheat Genome Sequencing Consortium (IWGSC; http://www.wheatgenome.org/) is making significant progress in developing physical maps for a number of the 21 haploid chromosomes of hexaploid wheat. These physical maps will serve as a scaffold to help in assembling the sequence reads produced recently (Cerealsdb; http://www.cerealsdb.uk.net/). The fixed-recombinant F_4 families developed in this study could be used in the alignment of genomic sequence reads within the wheat 3B deletion bin 3BS8-0.78-0.87. The fine mapping and map-based cloning of *Stb14* should be completed within a few years if wheat genome sequence research continues to advance quickly.

Breeding for improved resistance and mapping resistance loci is highly dependent on the ability to accurately score phenotypic reactions. Research on septoria tritici blotch has used a number of phenotyping methods such as the seedling test, detached-leaf method, first true leaf testing, and adult plant testing. To date, no study has evaluated all phenotyping methods to determine if plant reaction is identical. A future study in this area would be useful to determine if wheat researchers worldwide are using optimal phenotyping protocols.

In conclusion, identifying and mapping genetic resistance effective against a range of *M. graminicola* isolates is a major step towards developing cultivars with

improved and durable genetic resistance. Reducing producer dependence on fungicides by developing resistant cultivars with improved grain yield and grain quality will be critical for cultivar adoption and ultimately wheat production. This study was able to map two new resistance loci that are effective against multiple isolates or natural populations of *M. graminicola*. These loci have been mapped to relatively small genetic intervals and are flanked with microsatellite markers. Wheat breeders should be able to incorporate these loci using MAS in either germplasm enhancement schemes or in direct cultivar development. The major benefit of MAS will be in reducing the number of progeny that will need to be screened phenotypically to pyramid multiple sources of resistance. A second benefit for using MAS is the alleviation of experiment contamination during phenotypic screening due to the long latent period of the disease; also field screening is often complicated by presence of other foliar diseases such as leaf rust, stagonospora nodorum blotch, tan spot, spot blotch, and septoria avenae blotch. These foliar diseases can create significant challenges when trying to complete reliable field screening.

The level of Stb resistance in Canadian wheat cultivars could be greatly improved using identified genetic resistance, however, breeders must be cautious to not introduce susceptibility to other important wheat pathogens. In the 1970s in the UK a decrease in stagonospora nodorum blotch (*Stagonospora nodorum*) was observed while Stb increased. Studies have suggested that this shift may be due to decreasing atmospheric SO_4 concentration. A more recent study has noted that the shift could be attributed to breeding priorities shifting to increase the level of resistance to stagonospora nodorum blotch, which, in turn could have increased wheat susceptibility to Stb (Arraiano et al. 2009). To effectively incorporate resistance to the leaf spot complex in wheat, it will be imperative that breeders maintain or increase the current level of resistance to all leaf spotting pathogens during germplasm enhancement and cultivar development.

7.0 Literature Cited

Ackermann MD, Stewart S, Ibanez W (1995) Pathogenic variability of *Septoria tritici* isolates from South America. In: Gilchrist SL, van Ginkel M, McNab A, Kema GHJ (eds) Proceedings of a *Septoria tritici* Workshop. CIMMYT, Mexico, DF, pp 41-51

Adhikari TB, Anderson JM, Goodwin SB (2003) Identification and molecular mapping of a gene in wheat conferring resistance to *Mycosphaerella graminicola*. Phytopathology 93:1158-1164

Adhikari TB, Balaji B, Breeden J, Anderson JM, Goodwin SB (2004a) Quantification of *Mycosphaerella graminicola* in wheat by real-time PCR. Phytopathology 94:S2-S2

Adhikari TB, Cavaletto JR, Dubcovsky J, Gieco JO, Schlatter AR, Goodwin SB (2004b) Molecular mapping of the *Stb4* gene for resistance to septoria tritici blotch in wheat. Phytopathology 94:1198-1206

Adhikari TB, Wallwork H, Goodwin SB (2004c) Microsatellite markers linked to the *Stb2* and *Stb3* genes for resistance to septoria tritici blotch in wheat. Crop Sci 44:1403-1411

Adhikari TB, Yang X, Cavaletto JR, Hu X, Buechley G, Ohm HW, Shaner G, Goodwin SB (2004d) Molecular mapping of *Stb1*, a potentially durable gene for resistance to septoria tritici blotch in wheat. Theor Appl Genet 109:944-953

Akbari M, Wenzl P, Caig V, Carling J, Xia L, Yang SY, Uszynski G, Mohler V, Lehmensiek A, Kuchel H, Hayden MJ, Howes N, Sharp P, Vaughan P, Rathmell B, Huttner E, Kilian A (2006) Diversity arrays technology (DArT) for high-throughput profiling of the hexaploid wheat genome. Theor Appl Genet 113:1409-1420

Akey JM, Sosnoski D, Parra E, Dios S, Hiester K, Su B, Bonilla C, Jin L, Shriver MD (2001) Melting curve analysis of SNPs (McSNP®): A gel-free and inexpensive approach for SNP genotyping. Biotechniques 30:358-367

Akhunov ED, Goodyear AW, Geng S, Qi LL, Echalier B, Gill BS, Miftahudin, Gustafson JP, Lazo G, Chao SM, Anderson OD, Linkiewicz AM, Dubcovsky J, La Rota M, Sorrells ME, Zhang DS, Nguyen HT, Kalavacharla V, Hossain K, Kianian SF, Peng JH, Lapitan NLV, Gonzalez-Hernandeiz JL, Anderson JA, Choi DW, Close TJ, Dilbirligi M, Gill KS, Walker-Simmons MK, Steber C, McGuire PE, Qualset CO, Dvorak J (2003) The organization and rate of evolution of wheat genomes are correlated with recombination rates along chromosome arms. Genome Res 13:753-763

Arraiano LS (2008) Genetics and breeding for durable resistance to Septoria tritici blotch. In: McDonald BA (ed) 7th International Mycosphaerella and Stagonospora Symposium,

Monte Veritá Conference Centre, Ascona, Switzerland

Arraiano LS, Balaam N, Fenwick PM, Chapman C, Feuerhelm D, Howell P, Smith SJ, Widdowson JP, Brown JKM (2009) Contributions of disease resistance and escape to the control of septoria tritici blotch of wheat. Plant Pathol 58:910-922

Arraiano LS, Brading PA, Brown JKM (2001a) A detached seedling leaf technique to study resistance to *Mycosphaerella graminicola* (anamorph *Septoria tritici*) in wheat. Plant Pathol 50:339-346

Arraiano LS, Brown JKM (2006) Identification of isolate-specific and partial resistance to septoria tritici blotch in 238 European wheat cultivars and breeding lines. Plant Pathol 55:726-738

Arraiano LS, Brown JKM (2007) Genetic analysis of septoria tritici blotch to improve resistance in European wheat breeding programmes. In: Buck HT, Nisi JE, Salomon N (eds) Wheat Production in Stressed Environments. Springer, Dordrecht, pp 109-112

Arraiano LS, Chartrain L, Bossolini E, Slatter HN, Keller B, Brown JKM (2007) A gene in European wheat cultivars for resistance to an African isolate of *Mycosphaerella graminicola*. Plant Pathol 56:73-78

Arraiano LS, Worland AJ, Ellerbrook C, Brown JKM (2001b) Chromosomal location of a gene for resistance to septoria tritici blotch (*Mycosphaerella graminicola*) in the hexaploid wheat 'Synthetic 6x'. Theor Appl Genet 103:758-764

Arumuganathan K, Earle E (1991) Nuclear DNA content of some important plant species. Plant Molecular Biology Reporter 9:208-218

Asins MJ (2002) Present and future of quantitative trait locus analysis in plant breeding. Plant Breed 121:281-291

Bahat A, Gelernter I, Brown MB, Eyal Z (1980) Factors affecting the vertical progression of septoria leaf blotch in short-statured wheats. Phytopathology 70:179-184

Bailey KL, Duczek LJ (1996) Managing cereal diseases under reduced tillage. Can J Plant Pathol 18:159-167

Ballantyne B (1989) Pathogenic variation in Australian cultures of *Mycosphaerella graminicola*. In: Fried PM (ed) Septoria disease of cereals: Proceeding of the 3rd international workshop, Zurich, Switzerland, pp 152-154

Ballantyne B, Thomson F (1995) Pathogenic variation in Australian isolates of *Mycosphaerella graminicola*. Aust J Agric Res 46:921-934

Baltazar BM, Scharen AL, Kronstad WE (1990) Association between dwarfing genes

Rht1 and *Rht2* and resistance to septoria tritici blotch in winter wheat (*Triticum aestivum* L. em Thell). Theor Appl Genet 79:422-426

Bannon FJ, Cooke BM (1998) Studies on dispersal of *Septoria tritici* pycnidiospores in wheat-clover intercrops. Plant Pathol 47:49-56

Bernatzky R, Tanksley SD (1986) Toward a saturated linkage map in tomato based on isozymes and random cDNA sequences. Genetics 112:887-898

Botstein D, White RL, Skolnick M, Davis RW (1980) Construction of a genetic linkage map in man using Restriction Fragment Length Polymorphisms. American Journal of Human Genetics 32:314-331

Brading PA, Verstappen ECP, Kema GHJ, Brown JKM (2002) A gene-for-gene relationship between wheat and *Mycosphaerella graminicola*, the septoria tritici blotch pathogen. Phytopathology 92:439-445

Brokenshire T (1975) Role of Graminaceous species in epidemiology of *Septoria tritici* on wheat. Plant Pathol 24:33-38

Brown AGP, Rosielle AA (1980) Prospects for control of Septoria. Western Australian Journal of Agriculture 21:8-11

Brown JKM, Kema GHJ, Forrer HR, Verstappen ECP, Arraiano LS, Brading PA, Foster EM, Fried PM, Jenny E (2001) Resistance of wheat cultivars and breeding lines to septoria tritici blotch caused by isolates of *Mycosphaerella graminicola* in field trials. Plant Pathol 50:325-338

Brown JS, Kellock AW, Paddick RG (1978) Distribution and dissemination of *Mycosphaerella graminicola* (Fuckel) Schröeter in relations to epidemiology of speckled leaf blotch of wheat. Aust J Agric Res 29:1139-1145

Brunner PC, Stefanato FL, McDonald BA (2008) Evolution of the *CYP51* gene in *Mycosphaerella graminicola*: evidence for intragenic recombination and selective replacement. Mol Plant Pathol 9:305-316

Buckler ES, Thornsberry JM (2002) Plant molecular diversity and applications to genomics. Curr Opin Plant Biol 5:107-111

Burr B, Burr FA (1991) Recombinant inbreds for molecular mapping in maize - Theoretical and practical considerations. Trends Genet 7:55-60

Canadian Grain Commission (2009) Canadian Grain Exports, Crop Year 2008-2009. p 3

Canadian Grain Commission (2010) Canadian wheat classes. http://www.grainscanada.gc.ca/wheat-ble/classes/classes-eng.htm. Accessed 20 June

2011

Chalmers KJ, Campbell AW, Kretschmer J, Karakousis A, Henschke PH, Pierens S, Harker N, Pallotta M, Cornish GB, Shariflou MR, Rampling LR, McLauchlan A, Daggard G, Sharp PJ, Holton TA, Sutherland MW, Appels R, Langridge P (2001) Construction of three linkage maps in bread wheat, *Triticum aestivum*. Aust J Agric Res 52:1089-1119

Chao S, Sharp PJ, Worland AJ, Warham EJ, Koebner RMD, Gale MD (1989) RFLP-based genetics maps of wheat homoeologous group-7 chromosomes. Theor Appl Genet 78:495-504

Chartrain L, Berry ST, Brown JKM (2005a) Resistance of wheat line Kavkaz-K4500 L.6.A.4 to Septoria tritici blotch controlled by isolate-specific resistance genes. Phytopathology 95:664-671

Chartrain L, Brading PA, Brown JKM (2005b) Presence of the *Stb6* gene for resistance to septoria tritici blotch (*Mycosphaerella graminicola*) in cultivars used in wheat-breeding programmes worldwide. Plant Pathol 54:134-143

Chartrain L, Brading PA, Makepeace JC, Brown JKM (2004a) Sources of resistance to septoria tritici blotch and implications for wheat breeding. Plant Pathol 53:454-460

Chartrain L, Brading PA, Widdowson JP, Brown JKM (2004b) Partial resistance to septoria tritici blotch (*Mycosphaerella graminicola*) in wheat cultivars Arina and Riband. Phytopathology 94:497-504

Chartrain L, Joaquim P, Berry ST, Arraiano LS, Azanza F, Brown JKM (2005c) Genetics of resistance to septoria tritici blotch in the Portuguese wheat breeding line TE 9111. Theor Appl Genet 110:1138-1144

Chartrain L, Sourdille P, Bernard M, Brown JKM (2009) Identification and location of *Stb9*, a gene for resistance to septoria tritici blotch in wheat cultivars Courtot and Tonic. Plant Pathol 58:547-555

Chicaiza O, Khan IA, Zhang X, Brevis JC, Jackson L, Chen X, Dubcovsky J (2006) Registration of five wheat isogenic lines for leaf rust and stripe rust resistance genes. Crop Sci 46:485-487

Chungu C, Gilbert J, Townley-Smith F (2001) Septoria tritici blotch development as affected by temperature, duration of leaf wetness, inoculum concentration, and host. Plant Dis 85:430-435

Churchill GA, Doerge RW (1994) Empirical threshold values for quantitative trait mapping. Genetics 138:963-971

Coakley SM, McDaniel LR, Shaner G (1985) Model for predicting severity of septoria tritici blotch on winter wheat. Phytopathology 75:1245-1251

Cohen L, Eyal Z (1993) The histology of processes associated with the infection of resistant and susceptible wheat cultivars with *Septoria tritici*. Plant Pathol 42:737-743

Cook RJ (1999) Management by chemicals. In: Lucas JA, Bowyer P, Anderson HM (eds) Septoria on cereals: a Study of Pathosystems. CAB International, Wallingford, UK

Cools HJ, Fraaije BA (2008) Are azole fungicides losing ground against Septoria wheat disease? Resistance mechanisms in *Mycosphaerella graminicola*. Pest Manag Sci 64:681-684

Cools HJ, Fraaije BA, Lucas JA (2004) Molecular mechanisms conferring reduced sensitivities to triazoles in UK isolates of Septoria tritici. Phytopathology 94:S20-S21

Cowger C, Hoffer ME, Mundt CC (2000) Specific adaptation by *Mycosphaerella graminicola* to a resistant wheat cultivar. Plant Pathol 49:445-451

Cowger C, Mundt CC (2002) Effects of wheat cultivar mixtures on epidemic progression of Septoria tritici blotch and pathogenicity of *Mycosphaerella graminicola*. Phytopathology 92:617-623

Cowling SG (2006) Identification and mapping of host resistance genes to septoria tritici blotch of wheat. MSc Thesis Department of Plant Science. University of Manitoba, Winnipeg, p 129

Cunfer BM, Ueng PP (1999) Taxonomy and identification of *Septoria* and *Stagonospora* species on small-grain cereals. Annu Rev Phytopathol 37:267-284

Cuthbert JL, Somers DJ, Brûlé-Babel AL, Brown PD, Crow GH (2008) Molecular mapping of quantitative trait loci for yield and yield components in spring wheat (*Triticum aestivum* L.). Theor Appl Genet 117:595-608

Cuthbert PA, Somers DJ, Thomas J, Cloutier S, Brûlé-Babel A (2006) Fine mapping *Fhb1*, a major gene controlling fusarium head blight resistance in bread wheat (*Triticum aestivum* L.). Theor Appl Genet 112:1465-1472

Dakouri A, McCallum BD, Walichnowski AZ, Cloutier S (2010) Fine-mapping of the leaf rust Lr34 locus in *Triticum aestivum* (L.) and characterization of large germplasm collections support the ABC transporter as essential for gene function. Theor Appl Genet 121:373-384

Danon T, Eyal Z (1990) Inheritance of resistance to two *Septoria tritici* isolates in spring and winter bread wheat cultivars. Euphytica 47:203-214

Danon T, Sacks JM, Eyal Z (1982) The relationships among plant stature, maturity class, and susceptibility to septoria leaf blotch of wheat. Phytopathology 72:1037-1042

Darvasi A, Weinreb A, Minke V, Weller JI, Soller M (1993) Detecting marker-QTL linkage and estimating QTL gene effect and map location using a saturated genetic-map. Genetics 134:943-951

Daud HM, Gustafson JP (1996) Molecular evidence for *Triticum speltoides* as a B-genome progenitor of wheat (*Triticum aestivum*). Genome 39:543-548

DePauw R, Hunt T (2001) Canadian Wheat Pool. In: Bonjean AP, Angus WJ (eds) The World Wheat Book: a History of wheat breeding. Lavoisier Publishing Inc., London, pp 479-515

Dreher K, Khairallah M, Ribaut JM, Morris M (2003) Money matters (I): costs of field and laboratory procedures associated with conventional and marker-assisted maize breeding at CIMMYT. Mol Breed 11:221-234

Dubin HJ, Rajaram S (1996) Breeding disease-resistant wheats for tropical highlands and lowlands. Annu Rev Phytopathol 34:503-526

Edwards MD, Stuber CW, Wendel JF (1987) Molecular-marker-facilitated investigations of quantitative-trait loci in maize. I. Numbers, genomic distribution and types of gene action Genetics 116:113-125

Endo TR, Gill BS (1996) The deletion stocks of common wheat. Journal of Heredity 87:295-307

Entz MH, Vandenberg CGJ, Lafond GP, Stobbe EH, Rossnagel BG, Austenson HM (1990) Effect of late-season fungicide application on grain-yield and seed size distribution in wheat and barley. Can J Plant Sci 70:699-706

Eriksen L, Borum F, Jahoor A (2003) Inheritance and localisation of resistance to *Mycosphaerella graminicola* causing septoria tritici blotch and plant height in the wheat (*Triticum aestivum* L.) genome with DNA markers. Theor Appl Genet 107:515-527

Eujayl I, Sorrells ME, Baum M, Wolters P, Powell W (2002) Isolation of EST-derived microsatellite markers for genotyping the A and B genomes of wheat. Theor Appl Genet 104:399-407

Eyal Z (1981) Intergrated control of septoria diseases of wheat. Plant Dis 65:763-768

Eyal Z (1999a) Breeding for resistance to *Septoria* and *Stagonospora* diseases of wheat. In: Lucas JA, Bowyer P, Anderson HM (eds) Septoria of cereals: a Study of Pathosystems. CABI Publisher, Wallingford, UK, pp 332-344

Eyal Z (1999b) The septoria tritici and stagonospora nodorum blotch diseases of wheat. Eur J Plant Pathol 105:629-641

Eyal Z, Amiri Z, Wahl I (1973) Physiologic specialization of *Septoria tritici*. Phytopathology 63:1087-1091

Eyal Z, Brown MB (1976) Quantitative method for estimating density of *Septoria tritici* pycnidia on wheat leaves. Phytopathology 66:11-14

Eyal Z, Scharen AL, Huffman MD, Prescott JM (1985) Global insights into virulence frequencies of *Mycosphaerella graminicola*. Phytopathology 75:1456-1462

Eyal Z, Scharen AL, Prescott JM, van Ginkel M (1987) The Septoria Disease of Wheat: Concepts and methods of disease management. CIMMYT, Mexico

FAOSTAT (2010) Food and Agricultural Commodities Production. http://faostat.fao.org/ Accessed November 14, 2010

Fernandez MR, Zentner RP, McConkey BG, Campbell CA (1998) Effects of crop rotations and fertilizer management on leaf spotting diseases of spring wheat in southwestern Saskatchewan. Can J Plant Sci 78:489-496

Fischer RAT, Edmeades GO (2010) Breeding and Cereal Yield Progress. Crop Sci 50:S85-S98

Flavell RB, Smith DB (1976) Nucleotide-sequence organization in the wheat genome. Heredity 37:231-252

Flor HH (1946) Genetics of pathogenicity in *Melampsora lini*. Jour Agric Res 73:335-357

Fraaije BA, Cools HJ, Fountaine J, Lovell DJ, Motteram J, West JS, Lucas JA (2005) Role of ascospores in further spread of QoI-resistant cytochrome b alleles (G143A) in field populations of *Mycosphaerella graminicola*. Phytopathology 95:933-941

Gale MD, Devos KM (1998) Plant comparative genetics after 10 years. Science 282:656-659

Garcia C, Marshall D (1992) Observations on the ascogenous stage of *Septoria tritici* in Texas. Mycological Research 96:65-70

Gilbert J, Woods SM (2001) Leaf spot diseases of spring wheat in southern Manitoba farm fields under conventional and conservation tillage. Can J Plant Sci 81:551-559

Gilbert J, Woods SM, Tekauz A (1998) Relationship between environmental variables and the prevalence and isolation frequency of leaf-spotting pathogens in spring wheat.

Can J Plant Pathol 20:158-164

Goodwin SB (2007) Back to basics and beyond: increasing the level of resistance to Septoria tritici blotch in wheat. Austral Plant Pathol 36:532-538

Goodwin SB, Cavaletto JR, Lowe I, Thompson I, Xu SX, Adhikari TB, Dubcovsky J (2008) Validation of a new map location for the *Stb3* gene for resistance to septoria tritici blotch in wheat. In: McDonald BA (ed) 7th International Mycosphaerella and Stagonospora Symposium, Monte Veritá Conference Centre, Ascona, Switzerland

Grieger A, Lamari L, Brûlé-Babel A (2005) Physiologic variation in *Mycosphaerella graminicola* from western Canada. Can J Plant Pathol 27:71-77

Gupta PK, Rustgi S, Sharma S, Singh R, Kumar N, Balyan HS (2003) Transferable EST-SSR markers for the study of polymorphism and genetic diversity in bread wheat. Molecular Genetics and Genomics 270:315-323

Gupta PK, Varshney RK, Sharma PC, Ramesh B (1999) Molecular markers and their applications in wheat breeding. Plant Breed 118:369-390

Hackett CA, Broadfoot LB (2003) Effects of genotyping errors, missing values and segregation distortion in molecular marker data on the construction of linkage maps. Heredity 90:33-38

Halama P (1996) The occurrence of *Mycosphaerella graminicola*, teleomorph of *Septoria tritici* in France. Plant Pathol 45:135-138

Hardwick NV, Jones DR, Slough JE (2001) Factors affecting diseases of winter wheat in England and Wales, 1989-98. Plant Pathol 50:453-462

Hayden MJ, Nguyen TM, Waterman A, McMichael GL, Chalmers KJ (2008) Application of multiplex-ready PCR for fluorescence-based SSR genotyping in barley and wheat. Mol Breed 21:271-281

Helentjaris T, Slocum M, Wright S, Schaefer A, Nienhuis J (1986) Construction of genetic linkage maps in maize and tomato using Restriction Fragment Length Polymorphisms. Theor Appl Genet 72:761-769

Hess DE, Shaner G (1987) Effect of moisture and temperature on development of septoria tritici blotch in wheat. Phytopathology 77:215-219

HGCA (2010) Fungicide performance tool. http://www.hgca.com/content.output/878/878/Resources/Tools/Fungicide%20performance%20tool.mspx. Accessed November 7, 2010

Hoorne C, Lamari L, Gilbert J, Ballance GM (2002) First report of *Mycosphaerella*

graminicola, the sexual state of *Septoria tritici*, in Manitoba, Canada. Can J Plant Pathol 24:445-449

Hulbert SH, Webb CA, Smith SM, Sun Q (2001) Resistance gene complexes: Evolution and utilization. Annu Rev Phytopathol 39:285-312

Hunter T, Coker RR, Royle DJ (1999) Studies on the sexual plase of leaf blotch in UK winter wheat. In: Lucas JA, Bowyer P, Anderson HM (eds) Septoria on Cereals: a Study of Pathosystems. CAB International, Wallingford, UK, pp 108-114

Jaccoud D, Peng K, Feinstein D, Kilian A (2001) Diversity arrays: a solid state technology for sequence information independent genotyping. Nucleic Acids Res 29:E25

Jahoor A, Eriksen L, Backes G (2004) QTLs and genes for disease resistance in barley and wheat. In: Gupta P, Varshney RK (eds) Cereal Genomics. Kluwer Academic Publishers, Netherlands, pp 199-251

James WC (1971) An illustrated series of assessment keys for plant diseases, their preparation and usage. Canadian Plant Disease Survey 51:39-65

Jansen RC (1993) Interval mapping of multiple quantitative trait loci. Genetics 135:205-211

Jing HC, Lovell D, Gutteridge R, Jenk D, Kornyukhin D, Mitrofanova OP, Kema GHJ, Hammond-Kosack KE (2008) Phenotypic and genetic analysis of the *Triticum monococcum-Mycosphaerella graminicola* interaction. New Phytol 179:1121-1132

Jlibene M, Gustafson JP, Rajaram S (1992) A field disease evaluation method for selecting wheats resistant to *Mycosphaerella graminicola*. Plant Breed 108:26-32

Jlibene M, Gustafson JP, Rajaram S (1994) Inheritance of resistance to *Mycosphaerella graminicola* in hexaploid wheat. Plant Breed 112:301-310

Jlibene M, Mazouz H, Farih A, Saadaoui EM (1995) Host-pathogen interaction of wheat (*Triticum aestivum*) and *Septoria tritici* in Morocco. In: Gilchrist SL, Van Ginkel M, McNab A, Kema GHJ (eds) Proceedings of a *Septoria tritici* Workshop. CIMMYT, Mexico, DF, pp 34-40

Johnson R (1984) A critical analysis of durable resistance. Annu Rev Phytopathol 22:309-330

Kema CHJ, Verstappen ECP, Waalwijk G (2000) Avirulence in the wheat septoria tritici leaf blotch fungus *Mycosphaerella graminicola* is controlled by a single locus. Molecular Plant-Microbe Interactions 13:1375-1379

Kema GHJ, Annone JG, Sayoud R, van Silfhout CH, van Ginkel M, deBree J (1996a)

Genetic variation for virulence and resistance in the wheat-*Mycosphaerella graminicola* pathosystem .1. Interactions between pathogen isolates and host cultivars. Phytopathology 86:200-212

Kema GHJ, Sayoud R, Annone JG, van Silfhout CH (1996b) Genetic variation for virulence and resistance in the wheat-*Mycosphaerella graminicola* pathosystem .2. Analysis of interactions between pathogen isolates and host cultivars. Phytopathology 86:213-220

Kema GHJ, van Silfhout CH (1997) Genetic variation for virulence and resistance in the wheat *Mycosphaerella graminicola* pathosystem .3. Comparative seedling and adult plant experiments. Phytopathology 87:266-272

Kema GHJ, van Silfhout CH, Annone JG, Sayoud R, van Ginkel M, de Bree J (1995) Genetic variation for virulence in *Septoria tritici*. In: Gilchrist SL, Van Ginkel M, McNab A, Kema GHJ (eds) Proceedings of a *Septoria tritici* Workshop. CIMMYT, Mexico, DF, pp 52-54

Kema GHJ, Verstappen ECP, Todorova M, Waalwijk C (1996c) Successful crosses and molecular tetrad and progeny analyses demonstrate heterothallism in *Mycosphaerella graminicola*. Current Genetics 30:251-258

Kema GHJ, Yu DZ, Rijkenberg FHJ, Shaw MW, Baayen RP (1996d) Histology of the pathogenesis of *Mycosphaerella graminicola* in wheat. Phytopathology 86:777-786

Kilian A, Huttner E, Wenzl P, Jaccoud D, Carling J, Caig V, Evers M, Heller-Uszynska K, Uszynski G, Cayla C, Patarapuwadol S, Xia L, Yang S, Thomson B (2005) The fast and the cheap: SNP and DArT-based whole genome profiling for crop improvement. In: Tuberosa R, Phillips RL, Gale M (eds) Proceedings of the international congress in the wake of the double helix: from the green revolution to the gene revolution. Avenue Media, Bologna, Italy, pp 443–461

King JE, Cook RJ, Melville SC (1983) A review of Septoria diseases of wheat and barley. Ann Appl Biol 103:345-373

Kolmer J, Chen X, Jin Y (2009) Diseases which challenge global wheat production: the wheat rusts. In: Carver BF (ed) Wheat: Science and Trade. Wiley-Blackwell, Ames, Iowa, USA, pp 89-124

Konieczny A, Ausubel FM (1993) A procedure for mapping *Arabidopsis* mutations using co-dominant ecotype-specific PCR-based markers. Plant J 4:403-410

Kosambi DD (1944) The estimation of map distances from recombination values. Ann Eugenics 12:172-175

Krupinsky J (1999) Influence of cultural practices on Septoria/Stagonospora diseases. In: van Ginkel M, McNab A, Krupinsky J (eds) Septoria and Stagonospora Diseases of Cereals: Compilation of Global Research. CIMMYT, Mexico, pp 105-110

Kuchel H, Williams KJ, Langridge P, Eagles HA, Jefferies SP (2007) Genetic dissection of grain yield in bread wheat. I. QTL analysis. Theor Appl Genet 115:1029-1041

Lagercrantz U, Ellegren H, Andersson L (1993) The abundance of various polymorphic microsatellite motifs differ between plants and vertebrates. Nucleic Acids Research 21:1111-1115

Lander ES, Botstein D (1989) Mapping mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics 121:185-199

Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newburg L (1987) MAPMAKER: An interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174-181

Langridge P, Lagudah ES, Holton TA, Appels R, Sharp PJ, Chalmers KJ (2001) Trends in genetic and genome analyses in wheat: a review. Aust J Agric Res 52:1043-1077

Laurie DA, Bennett MD (1988) The production of haploid wheat plants from wheat x maize crosses. Theor Appl Genet 76:393-397

Liu BH (1998) Statistical Genomics: Linkage, mapping and QTL analysis. CRC Press, New York, NY

Loughman R, Thomas GJ (1992) Fungicide and cultivar control of *Septoria* diseases of wheat. Crop Prot 11:349-354

Mackay TFC (2001) The genetic architecture of quantitative traits. Annu Rev Genet 35:303-339

Magboul AM, Geng S, Gilchrist DG, Jackson LF (1992) Environmental influence on the infection of wheat by *Mycosphaerella graminicola*. Phytopathology 82:1407-1413

Mann CE, Rajaram S, Villareal RL (1985) Progress in breeding for *Septoria tritici* resistance in semidwarf spring wheat at CIMMYT. In: Scharen AL (ed) Septoria of cereals: Proceedings of the Workshop, Montana State University, Bozeman, pp 22-26

Marshall D (1985) Geographic distribution and aggressiveness of *Septoria tritici* on wheat in the United States. Phytopathology 75:1319-1319

Marshall D (2009) Diseases which challenge global wheat production: Powdery mildew and leaf and head blights. In: Carver BF (ed) Wheat: Science and Trade. Wiley-Blackwell, Ames, Iowa, USA, pp 155-169

McCallum BD, DePauw RM (2008) A review of wheat cultivars grown in the Canadian prairies. Can J Plant Sci 88:649-677

McCartney CA, Brûlé-Babel AL, Lamari L (2002) Inheritance of race-specific resistance to *Mycosphaerella graminicola* in wheat. Phytopathology 92:138-144

McCartney CA, Brûlé-Babel AL, Lamari L, Somers DJ (2003) Chromosomal location of a race-specific resistance gene to *Mycosphaerella graminicola* in the spring wheat ST6. Theor Appl Genet 107:1181-1186

McCouch SR, Kochert G, Yu ZH, Wang ZY, Khush GS, Coffman WR, Tanksley SD (1988) Molecular mapping of rice chromosomes. Theor Appl Genet 76:815-829

McDonald BA, Zhan J, Yarden O, Hogan K, Garton J, Pettway RE (1999) The population genetics of *Mycosphaerella graminicola* and *Stagonospora nodorum*. In: Lucas JA, Bowyer P, Anderson HM (eds) Septoria on cereals: a Study of Pathosystems. CABI Publishing, Wallingford, UK, pp 44-69

Michelmore RW, Paran I, Kesseli RV (1991) Identification of markers linked to disease-resistance genes by bulked segregant analysis: A rapid method to detect markers in specific genomic regions by using segregating populations. Proc Natl Acad Sci U S A 88:9828-9832

Mohan M, Nair S, Bhagwat A, Krishna TG, Yano M, Bhatia CR, Sasaki T (1997) Genome mapping, molecular markers and marker-assisted selection in crop plants. Mol Breed 3:87-103

Morris M, Dreher K, Ribaut JM, Khairallah M (2003) Money matters (II): costs of maize inbred line conversion schemes at CIMMYT using conventional and marker-assisted selection. Mol Breed 11:235-247

Mundt CC, Brophy LS, Schmitt MS (1995) Choosing crop cultivars and cultivar mixtures under low versus high disease pressure: A case study with wheat. Crop Prot 14:509-515

Mundt CC, Hoffer ME, Ahmed HU, Coakley SM, DiLeone JA, Cowger C (1999) Population genetics and host resistance. In: Lucas JA, Bowyer P, Anderson HM (eds) Septoria on cereals: a Study of Pathosystems. CABI Publishing, Wallingford, UK, pp 115-130

Narvaez I, Caldwell RM (1957) Inheritance of resistance to leaf blotch of wheat caused by *Septoria tritici*. Phytopathology 47:529-530

National Bioresource Project (2009) Komugi Integrated Wheat Science Database. Composite Wheat Map. http://shigen.lab.nig.ac.jp/wheat/komugi/maps/markerMap.jsp Accessed 10 November 2010

Olmos S, Distelfeld A, Chicaiza O, Schlatter AR, Fahima T, Echenique V, Dubcovsky J (2003) Precise mapping of a locus affecting grain protein content in durum wheat. Theor Appl Genet 107:1243-1251

Orton ES, Deller S, Brown JKM (2011) Mycosphaerella graminicola: From genomics to disease control. Mol Plant Pathol 12:413-424

Palmer CL, Skinner W (2002) *Mycosphaerella graminicola*: latent infection, crop devastation and genomics. Mol Plant Pathol 3:63-70

Parlevliet JE (1979) Components of resistance that reduce the rate of epidemic development. Annu Rev Phytopathol 17:203-222

Paterson AH, Bowers JE, Burow MD, Draye X, Elsik CG, Jiang CX, Katsar CS, Lan TH, Lin YR, Ming RG, Wright RJ (2000) Comparative genomics of plant chromosomes. Plant Cell 12:1523-1539

Patterson FL, Roberts JJ, Finney RE, Shaner GE, Gallun RL, Ohm HW (1975) Registration of Oasis wheat. Crop Sci 15:736-737

Patterson FL, Shaner GE, Huber DM, Ohm HW, Finney RE, Gallun RL, Roberts JJ (1979) Registration of Sullivan wheat. Crop Sci 19:297

Paux E, Faure S, Choulet F, Roger D, Gauthier V, Martinant JP, Sourdille P, Balfourier F, Le Paslier MC, Chauveau A, Cakir M, Gandon B, Feuillet C (2010) Insertion site-based polymorphism markers open new perspectives for genome saturation and marker-assisted selection in wheat. Plant Biotechnol J 8:196-210

Paux E, Sourdille P, Salse J, Saintenac C, Choulet F, Leroy P, Korol A, Michalak M, Kianian S, Spielmeyer W, Lagudah E, Somers D, Kilian A, Alaux M, Vautrin S, Berges H, Eversole K, Appels R, Safar J, Simkova H, Dolezel J, Bernard M, Feuillet C (2008) A physical map of the 1-gigabase bread wheat chromosome 3B. Science 322:101-104

Pedersen EA, Hughes GR (1992) The effect of crop rotation on development of the septoria disease complex on spring wheat in Saskatchewan. Can J Plant Pathol 14:152-158

Pedersen EA, Hughes GR (1993) Relative occurrence of *Phaeosphaeria nodorum* and *Mycosphaerella graminicola* on spring wheat in Saskatchewan. Can J Plant Pathol 15:113-118

Pestsova E, Ganal MW, Röder MS (2000) Isolation and mapping of microsatellite markers specific for the D genome of bread wheat. Genome 43:689-697

Prairie Grain Development Committee (2010) Prairie Recommending Committee for Wheat, Rye and Triticale - Operating procedures

Price AH (2006) Believe it or not, QTLs are accurate! Trends in Plant Science 11:213-216

Prosser J (1993) Detecting single-base mutations. Trends in Biotechnology 11:238-246

Qi LL, Echalier B, Chao S, Lazo GR, Butler GE, Anderson OD, Akhunov ED, Dvorak J, Linkiewicz AM, Ratnasiri A, Dubcovsky J, Bermudez-Kandianis CE, Greene RA, Kantety R, La Rota CM, Munkvold JD, Sorrells SF, Sorrells ME, Dilbirligi M, Sidhu D, Erayman M, Randhawa HS, Sandhu D, Bondareva SN, Gill KS, Mahmoud AA, Ma XF, Miftahudin, Gustafson JP, Conley EJ, Nduati V, Gonzalez-Hernandez JL, Anderson JA, Peng JH, Lapitan NLV, Hossain KG, Kalavacharla V, Kianian SF, Pathan MS, Zhang DS, Nguyen HT, Choi DW, Fenton RD, Close TJ, McGuire PE, Qualset CO, Gill BS (2004) A chromosome bin map of 16,000 expressed sequence tag loci and distribution of genes among the three genomes of polyploid wheat. Genetics 168:701-712

Qiagen (2005) HotStarTaq PCR Handbook.

Quarrie SA, Lazic-Jancic V, Kovacevic D, Steed A, Pekic S (1999) Bulk segregant analysis with molecular markers and its use for improving drought resistance in maize. Journal of Experimental Botany 50:1299-1306

Rajaram S (2001) Prospects and promise of wheat breeding in the 21st century. Euphytica 119:3-15

Ribaut JM, Hoisington D (1998) Marker-assisted selection: new tools and strategies. Trends in Plant Science 3:236-239

Ribaut JM, Jiang C, Hoisington D (2002) Simulation experiments on efficiencies of gene introgression by backcrossing. Crop Sci 42:557-565

Rillo AO, Caldwell RM (1966) Inheritance of resistance to *Septoria tritici* in *Triticum aestivum* subsp. *vulgare* Bulgaria 88. Phytopathology 56:897-&

Robinson RA (1969) Disease resistance terminology. Review of Applied Mycology 48:593-606

Röder MS, Huang X-Q, Borner A (2008) Fine mapping of the region on wheat chromosome 7D controlling grain weight. Funct Integr Genomics 8:79-86

Röder MS, Korzun V, Wendehake K, Plaschke J, Tixier MH, Leroy P, Ganal MW (1998) A microsatellite map of wheat. Genetics 149:2007-2023

Rosielle AA (1972) Sources of resistance in wheat to speckled leaf blotch caused by *Septoria tritici*. Euphytica 21:152-&

Rosielle AA, Brown AGP (1979) Inheritance, heritability and breeding behavior of three

sources of resistance to *Septoria tritici* in wheat. Euphytica 28:385-392

Royle DJ (1994) Understanding and predicting epidemics: a Commentary based on selected pathosystems. Plant Pathol 43:777-789

Saadaoui EM (1987) Physiological specialization of *Septoria tritici* in Morocco. Plant Dis 71:153-155

Saari EE, Prescott JM (1975) Scale for appraising foliar intensity of wheat diseases. Plant Disease Reporter 59:377-380

Sachidanandam R, Weissman D, Schmidt SC, Kakol JM, Stein LD, Marth G, Sherry S, Mullikin JC, Mortimore BJ, Willey DL, Hunt SE, Cole CG, Coggill PC, Rice CM, Ning ZM, Rogers J, Bentley DR, Kwok PY, Mardis ER, Yeh RT, Schultz B, Cook L, Davenport R, Dante M, Fulton L, Hillier L, Waterston RH, McPherson JD, Gilman B, Schaffner S, Van Etten WJ, Reich D, Higgins J, Daly MJ, Blumenstiel B, Baldwin J, Stange-Thomann NS, Zody MC, Linton L, Lander ES, Altshuler D, Int SNP, Map, Working, Group, (2001) A map of human genome sequence variation containing 1.42 million single nucleotide polymorphisms. Nature 409:928-933

Saiki RK, Gelfand DH, Stoffel S, Scharf SJ, Higuchi R, Horn GT, Mullis KB, Erlich HA (1988) Primer directed enzymatic amplification of DNA with a thermostable DNA-polymerase. Science 239:487-491

Saintenac C, Falque M, Martin OC, Paux E, Feuillet C, Sourdille P (2009) Detailed recombination studies along chromosome 3B provide new insights on crossover distribution in wheat (*Triticum aestivum* L.). Genetics 181:393-403

Sanderson FR (1972) A *Mycosphaerella* species as the ascogenous state of *Septoria tritici*. New Zealand Journal of Botany 10:707-710

Sanderson FR, Gaunt RE (1980) Commercial control of speckled leaf blotch (*Mycosphaerella graminicola*, imperfect state *Septoria tritici*) on wheat using fungicides. Proceeding of 3rd International Wheat Conference, Madrid, Spain, pp 554-559

Sanger F, Nicklen S, Coulson AR (1977) DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci U S A 74:5463-5467

Sax K (1923) The association of size differences with seed coat pattern and pigmentation in *Phaseolus vulgaris*. Genetics 8:552-560

Schuelke M (2000) An economic method for the fluorescent labeling of PCR fragments. Nature Biotechnology 18:233-234

Scott PR, Sanderson FR, Benedikz PW (1988) Occurrence of *Mycosphaerella graminicola*, teleomorph of *Septoria tritici*, on wheat debris in the UK. Plant Pathol

37:285-290

Shaner G (1981) Effect of environment on fungal leaf blights of small grains. Annu Rev Phytopathol 19:273-296

Shaner G, Buechley G (1995) Epidemiology of leaf blotch of soft red winter wheat caused by *Septoria tritici* and *Stagonospora nodorum*. Plant Dis 79:928-938

Shaner G, Finney RE (1976) Weather and epidemics of Septoria leaf blotch of wheat. Phytopathology 66:781-785

Shaw MW (1991) Interacting effects of interrupted humid periods and light on infection of wheat leaves by *Mycosphaerella graminicola* (*Septoria tritici*). Plant Pathol 40:595-607

Shaw MW, Royle DJ (1993) Factors determining the severity of epidemics of *Mycosphaerella graminicola* (*Septoria tritici*) on winter wheat in the UK. Plant Pathol 42:882-899

Shearer BL, Wilcoxson RD (1978) Variation in size of macropycnidiospores and pycnidia of *Septoria tritici* on wheat. Canadian Journal of Botany 56:742-746

Shipton WA, Boyd SRJ, Rosielle AA, Shearer BI (1971) Common Septoria Diseases of Wheat. Bot Rev 37:231-262

Simon MR, Ayala FM, Cordo CA, Roder MS, Borner A (2004a) Molecular mapping of quantitative trait loci determining resistance to septoria tritici blotch caused by *Mycosphaerella graminicola* in wheat. Euphytica 138:41-48

Simon MR, Cordo CA (1998) Diallel analysis of four resistance components to *Septoria tritici* in six crosses of wheat (*Triticum aestivum*). Plant Breed 117:123-126

Simon MR, Worland AJ, Struik PC (2004b) Influence of plant height and heading date on the expression of the resistance to Septoria tritici blotch in near isogenic lines of wheat. Crop Sci 44:2078-2085

Sleper DA, Poehlman JM (2006) Breeding Field Crops, Fifth edn. Blackwell Publishing Ames, Iowa

Somasco OA, Qualset CO, Gilchrist DG (1996) Single-gene resistance to Septoria tritici blotch in the spring wheat cultivar 'Tadinia'. Plant Breed 115:261-267

Somers D (2004) Molecular marker systems and their evaluation for cereal genetics. In: Gupta P, Varshney RK (eds) Cereal Genomics. Kluwer Academic Publishers, Netherlands, pp 35-82

Somers DJ, Isaac P, Edwards K (2004) A high-density microsatellite consensus map for bread wheat (*Triticum aestivum* L.). Theor Appl Genet 109:1105-1114

Somers DJ, Thomas J, DePauw R, Fox S, Humphreys G, Fedak G (2005) Assembling complex genotypes to resist Fusarium in wheat (*Triticum aestivum* L.). Theor Appl Genet 111:1623-1631

Song QJ, Shi JR, Singh S, Fickus EW, Costa JM, Lewis J, Gill BS, Ward R, Cregan PB (2005) Development and mapping of microsatellite (SSR) markers in wheat. Theor Appl Genet 110:550-560

Southern EM (1975) Detection of specific sequences among DNA fragments separated by gel electrophoresis. Journal of Molecular Biology 98:503-&

Spielmeyer W, Moullet O, Laroche A, Lagudah ES (2000) Highly recombinogenic regions at seed storage protein loci on chromosome IDS of *Aegilops tauschii*, the D-genome donor of wheat. Genetics 155:361-367

Spielmeyer W, Sharp PJ, Lagudah ES (2003) Identification and validation of markers linked to broad-spectrum stem rust resistance gene Sr2 in wheat (*Triticum aestivum* L.). Crop Sci 43:333-336

Spielmeyer W, Singh RP, McFadden H, Wellings CR, Huerta-Espino J, Kong X, Appels R, Lagudah ES (2008) Fine scale genetic and physical mapping using interstitial deletion mutants of *Lr34/Yr18*: a disease resistance locus effective against multiple pathogens in wheat. Theor Appl Genet 116:481-490

Sprague R (1950) Diseases of cereals and grasses in North America. Ronald Press Co.

Statistics Canada (2010) July 31 Estimate of Production of Principal Field Crops. Field Crop Reporting Series - Catalogue no 22-002-X, vol 89, no 5, p 19

Stephenson P, Bryan G, Kirby J, Collins A, Devos K, Busso C, Gale M (1998) Fifty new microsatellite loci for the wheat genetic map. Theor Appl Genet 97:946-949

Sutton JC, Vyn TJ (1990) Crop sequences and tillage practices in relation to diseases of winter wheat in Ontario. Can J Plant Pathol 12:358-368

Tadesse W, Hsam SLK, Zeller FJ (2006) Evaluation of common wheat cultivars for tan spot resistance and chromosomal location of a resistance gene in the cultivar 'Salamouni'. Plant Breed 125:318-322

Tanksley SD (1993) Mapping polygenes. Annu Rev Genet 27:205-233

Tanksley SD, Ganal MW, Prince JP, Devicente MC, Bonierbale MW, Broun P, Fulton TM, Giovannoni JJ, Grandillo S, Martin GB, Messeguer R, Miller JC, Miller L, Paterson

AH, Pineda O, Roder MS, Wing RA, Wu W, Young ND (1992) High-density molecular linkage maps of the tomato and potato genomes. Genetics 132:1141-1160

Tanksley SD, Young ND, Paterson AH, Bonierbale MW (1989) RFLP mapping in plant breeding - new tools for an old science. Bio-Technology 7:257-264

Tautz D, Renz M (1984) Simple sequences are ubiquitous repetitive components of eukaryotic genomes. Nucleic Acids Research 12:4127-4138

Thiel T, Michalek W, Varshney RK, Graner A (2003) Exploiting EST databases for the development and characterization of gene-derived SSR-markers in barley (Hordeum vulgare L.). Theor Appl Genet 106:411-422

Tompkins DK, Fowler DB, Wright AT (1993) Influence of agronomic practices on canopy microclimate and septoria development in no-till winter wheat produced in the Parkland region of Saskatchewan. Can J Plant Sci 73:331-344

Torada A, Koike M, Ikeguchi S, Tsutsui I (2008) Mapping of a major locus controlling seed dormancy using backcrossed progenies in wheat (*Triticum aestivum* L.). Genome 51:426-432

Torriani SFF, Brunner PC, McDonald BA, Sierotzki H (2009) QoI resistance emerged independently at least 4 times in European populations of *Mycosphaerella graminicola*. Pest Manag Sci 65:155-162

van Ginkel M, Rajaram S (1995) Breeding for Resistance to *Septoria tritici* a CIMMYT. In: Gilchrist SL, van Ginkel M, McNab A, Kema GHJ (eds) Proceedings of a *Septoria tritici* Workshop. CIMMYT, Mexico, DF, pp 55-59

van Ginkel M, Rajaram S (1999) Breeding for resistance to the Septoria/Stagonospora blights of wheat. In: van Ginkel M, McNab A, Krupinsky J (eds) Septoria and Stagonospora Diseases of Cereals: a Compilation of Global Research. CIMMYT, Mexico, DF, pp 117-126

van Ginkel M, Scharen AL (1988a) Diallel analysis of resistance to *Septoria tritici* isolates in durum wheat. Euphytica 38:31-38

van Ginkel M, Scharen AL (1988b) Host pathogen relationships of wheat and *Septoria tritici*. Phytopathology 78:762-766

van Ooijen JW (2004) MapQTL ® 5, Software for the mapping of quantitative trait loci in experimental populations. Kyazma B.V., Wageningen, Netherlands

van Ooijen JW (2006) JoinMap ® 4, Software for the calculation of genetic linkage maps in experimental populations. Kyazma B.V., Wageningen, Netherlands

Varshney RK, Korzun V, Börner A (2004) Molecular maps in cereals: Methodology and progess. In: Gupta P, Varshney RK (eds) Cereal Genomics. Kluwer Academic Publishers, Netherlands, pp 35-82

Visscher PM, Haley CS, Thompson R (1996) Marker-assisted introgression in backcross breeding programs. Genetics 144:1923-1932

Voorrips RE (2002) MapChart: Software for the graphical presentation of linkage maps and QTLs. Journal of Heredity 93:77-78

Vos P, Hogers R, Bleeker M, Reijans M, Vandelee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M, Zabeau M (1995) AFLP: A new technique for DNA fingerprinting. Nucleic Acids Research 23:4407-4414

Wainshilbaum SJ, Lipps PE (1991) Effect of temperature and growth stage of wheat on development of leaf and glume blotch caused by *Septoria tritici* and *Stagonospora nodorum*. Plant Dis 75:993-998

Wang J, Basten CJ, Zeng ZB (2010) Windows QTL Cartographer 2.5_006. http://statgen.ncsu.edu/qtlcart/WQTLCart.htm (1 December 2010). Department of Statistics, North Carolina State University, Raleigh, NC

Wenzl P, Carling J, Kudrna D, Jaccoud D, Huttner E, Kleinhofs A, Kilian A (2004) Diversity Arrays Technology (DArT) for whole-genome profiling of barley. Proc Natl Acad Sci U S A 101:9915-9920

Wiese MV (1987) Compendium of wheat diseases. APS Press, St. Paul, MN

Wijnker E, de Jong H (2008) Managing meiotic recombination in plant breeding. Trends in Plant Science 13:640-646

Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Research 18:6531-6535

Wilson RE (1979) Resistance to *Septoria tritici* in two wheat cultivars, determined by independent, single dominant genes:16-18

Young ND (1994) Constructing a plant genetic linkage map with DNA markers. In: Vasil IK, Phillips RL (eds) DNA-based markers in plants. Kluwer, Dordrecht/Boston/London, pp 39-57

Young ND, Zamir D, Ganal MW, Tanksley SD (1988) Use of isogenic lines and simultaneous probing to identify DNA markers tightly linked to the *Tm-2α* gene in tomato. Genetics 120:579-585

Zadoks JC, Chang TT, Konzak CF (1974) Decimal code for growth stages of cereals. Weed Res 14:415-421

Zeng ZB (1994) Precision mapping of quantitative trait loci. Genetics 136:1457-1468

Zhang X, Haley SD, Jin Y (2001) Inheritance of Septoria tritici blotch resistance in winter wheat. Crop Sci 41:323-326

Ziv O, Eyal Z (1978) Assessment of yield component losses caused in plants of spring wheat cultivars by selected isolates of *Septoria tritici*. Phytopathology 68:791-796

8.0 Appendices

Appendix 8.1: Summary of typical inputs used on Pastiche/Torfrida field trials conducted in north-western Europe.

Crop growth stage[A]	Product name	Product Company	Type	Rate
Pre-emerg	Stomp 400 +	BASF	Herbicide	3.0 L ha^{-1}
	Lexus SX	DuPont	Herbicide	20.0 g ha^{-1}
12	Luxan Metaldehyde	Luxan	Mollusicide	6.00 kg ha^{-1}
23	IPU (Arelon 500)	Nufarm UK Ltd.	Herbicide	2.00 L ha^{-1}
	Foundation	Headland Agrochemicals Ltd.	Herbicide	1.00 L ha^{-1}
25	Extran (33.5% N)	Yara UK Ltd.	Fertiliser	53.00 kg ha^{-1}
	Opus	BASF	Fungicide[C]	0.50 L ha^{-1}
	Bravo 500	Syngenta	Fungicide[C]	1.00 L ha^{-1}
	Flexity	BASF	Fungicide[C]	0.50 L ha^{-1}
30	New 5 C Cycocel	BASF	PGR[B]	1.75.0 L ha^{-1}
31-32	Proline	Bayer CropScience	Fungicide[C]	0.80 L ha^{-1}
	Bravo 500	Syngenta	Fungicide[C]	1.00 L ha^{-1}
	Talius	DuPont	Fungicide[C]	0.15 L ha^{-1}
31-32	Moddus	Syngenta	PGR[B]	0.40 L ha^{-1}
	New 5C Cycocel	BASF	PGR[B]	0.75 L ha^{-1}
32	Extran (33.5% N)	Yara UK Ltd.	Fertiliser	40.00 kg ha^{-1}
39	Tomahawk	Makhteshim-Agan	Herbicide	1.50 L ha^{-1}
39-43	Opus	BASF	Fungicide[C]	0.75 L ha^{-1}
	Comet 200	Bayer CropScience	Fungicide[C]	0.50 L ha^{-1}
	Talius	DuPont	Fungicide[C]	0.15 L ha^{-1}
49-51	Dursban	Dow AgroSciences	Insecticide	0.60 kg ha^{-1}
59-61	Fandango	Bayer CropScience	Fungicide[C]	1.25 L ha^{-1}
	Phantom	BASF	Insecticide	280.0 g ha^{-1}

[A]- Indicated as Zadoks scale (Zadoks et al. 1974).
[B]- PGR = Plant growth regulator.
[C]- Fungicides only applied to yield field trials

Appendix 8.2: Histograms of the least square means for traits measured in **(A)** disease field trials, and **(B)** yield field trials.

A: Disease field trials
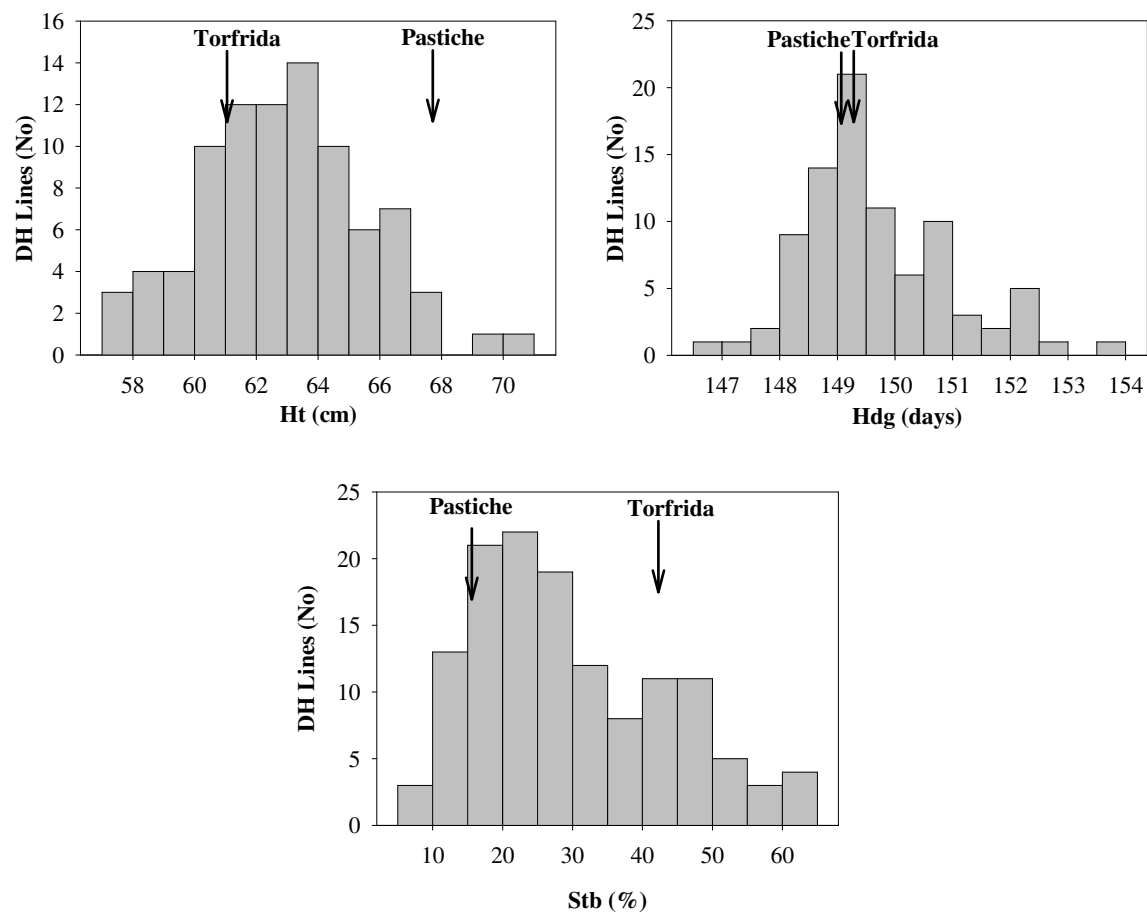
B: Yield field trials
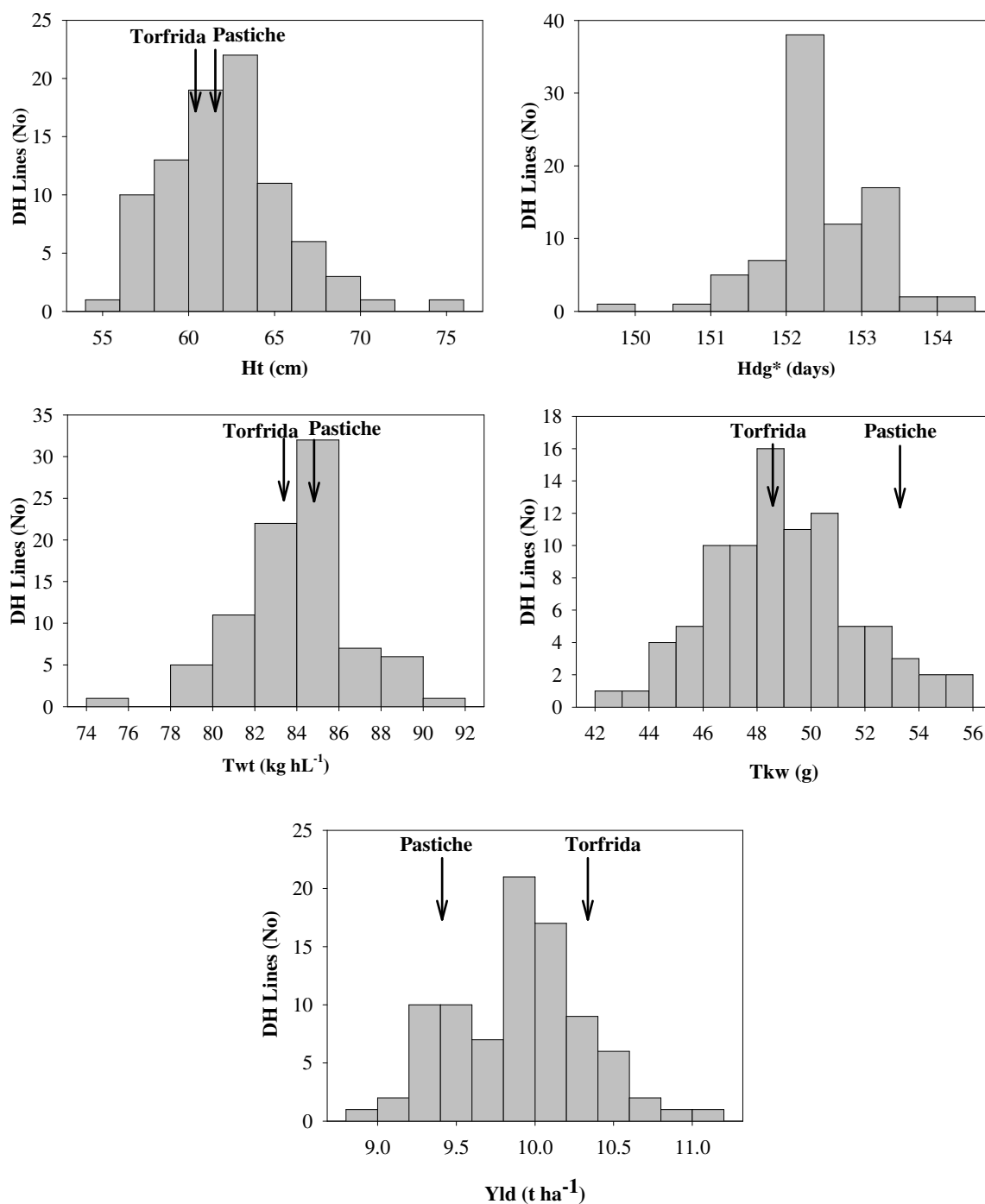
* The parent cultivars Pastiche and Torfrida were not included in the yield field trial
Sej09, therefore, heading date for the cultivars Pastiche and Torfrida is not indicated.

Appendix 8.3: Analysis of Variance (ANOVA) for combined trait data measured in field trials conducted in north-western Europe between 2006 and 2009.

Source of Variation	DF	Type III SS	Mean Square	Expected Mean Squares	Error Term	Error DF	F-Value	P-Value
Septoria tritici blotch (Stb)[A]								
Entry	131	514.9	3.9	σ^2(Residual) + 1.9999 σ^2(Env*Entry) + Q(Entry)	1 MS(Env*Entry) + 222x10^{-9} MS(Residual)	393.0	5.53	<0.0001
Env	3	156.8	52.3	σ^2(Residual) + 2 σ^2(Env*Entry) + 132 σ^2(Rep(Env)) + 264 σ^2(Env)	1.0038 MS(Rep(Env)) + 1 MS(Env*Entry) - 1.0038 MS(Residual)	6.4	21.34	0.0010
Rep(Env)	4	8.0	2.0	σ^2(Residual) + 131.5 σ^2(Rep(Env))	MS(Residual)	524	9.64	<0.0001
Env *Entry	393	279.5	0.7	σ^2(Residual) + 1.9999 σ^2(Env*Entry)	MS(Residual)	524	3.55	<0.0001
Residual	524	105.1	0.2	σ^2(Residual)				
Plant height to flag leaf (Ht)[B]								
Entry	131	6286.3	48.0	σ^2(Residual) + 2 σ^2(Env*Entry) + Q(Entry)	MS(Env*Entry)	2.2	2.24	<0.0001
Env	1	1218.5	1218.5	σ^2(Residual) + 2 σ^2(Env*Entry) + 130σ^2 (Rep(Env)) + 260 σ^2(Env)	0.9924 MS(Rep(Env)) + MS(Env*Entry) - 0.9924 MS(Residual)	16.3	16.32	0.0229
Rep(Env)	2	117.2	58.6	σ^2(Residual) + 131 σ^2 (Rep(Env))	MS(Residual)	11.8	11.83	<0.0001
Env *Entry	129	2763.5	21.4	σ^2(Residual) + 2 σ^2 (Env*Entry)	MS(Residual)	4.3	4.33	<0.0001
Residual	256	1287.8	5.0	σ^2(Residual)				
Days to heading (Hdg)[B]								
Entry	129	154.1	1.2	σ^2(Residual) + Q(Entry)	MS(Residual)	129	7.76	<0.0001
Rep	1	0.1	0.1	σ^2(Residual) + 130 σ^2(Rep)	MS(Residual)	129	0.90	0.3447
Residual	129	19.9	0.2	σ^2(Residual)				
Test weight (Twt)[B]								
Entry	131	3251.6	24.8	σ^2(Residual) + 1.9796 σ^2(Env*Entry) + Q(Entry)	1.0002 MS(Env*Entry) - 0.0002 MS(Residual)	129.0	1.00	0.5024

	df	SS	MS	Expected Mean Square	Error Term		F	Pr > F
Env	1	35305.0	35305.0	$\sigma^2(\text{Residual}) + 1.9692\ \sigma^2(\text{Env*Entry}) + 128\ \sigma^2(\text{Rep(Env)}) + 256\ \sigma^2(\text{Env})$	0.9922 MS(Rep(Env)) + 0.9949 MS(Env*Entry) - 0.9871 MS(Residual)	214.7	241.67	0.0024
Rep(Env)	2	276.7	138.4	$\sigma^2(\text{Residual}) + 129\ \sigma^2(\text{Rep(Env)})$	MS(Residual)	8.6	8.58	0.0002
Env *Entry	129	3204.9	24.8	$\sigma^2(\text{Residual}) + 1.9793\ \sigma^2(\text{Env*Entry})$	MS(Residual)	1.5	1.54	0.0018
Residual	256	4127.0	16.1	$\sigma^2(\text{Residual})$				

Thousand kernel weight (Tkw)[B]

	df	SS	MS	Expected Mean Square	Error Term		F	Pr > F
Entry	131	7555.6	57.7	$\sigma^2(\text{Residual}) + 1.9908\ \sigma^2(\text{Env*Entry}) + Q(\text{Entry})$	0.9987 MS(Env*Entry) + 0.0013 MS(Residual)	9.4	9.41	<0.0001
Env	3	26339.0	8779.8	$\sigma^2(\text{Residual}) + 1.9884\ \sigma^2(\text{Env*Entry}) + 130.58\ \sigma^2(\text{Rep(Env)}) + 261.16\ \sigma^2(\text{Env})$	0.9987 MS(Rep(Env)) + 0.9975 MS(Env*Entry) - 0.9962 MS(Residual)	181.3	181.28	<0.0001
Rep(Env)	4	180.9	45.2	$\sigma^2(\text{Residual}) + 130.75\ \sigma^2(\text{Rep(Env)})$	MS(Residual)	15.8	15.79	<0.0001
Env *Entry	391	2399.2	6.1	$\sigma^2(\text{Residual}) + 1.9934\ \sigma^2(\text{Env*Entry})$	MS(Residual)	2.1	2.14	<0.0001
Residual	519	1486.0	2.9	$\sigma^2(\text{Residual})$				

Grain yield (Yld)[B]

	df	SS	MS	Expected Mean Square	Error Term		F	Pr > F
Entry	131	190.1	1.5	$\sigma^2(\text{Residual}) + 1.9939\ \sigma^2(\text{Env*Entry}) + Q(\text{Entry})$	0.9991 MS(Env*Entry) + 0.0009 MS(Residual)	391.4	1.82	<0.0001
Env	3	1518.2	506.1	$\sigma^2(\text{Residual}) + 1.9923\ \sigma^2(\text{Env*Entry}) + 130.83\ \sigma^2(\text{Rep(Env)}) + 261.66\ \sigma^2(\text{Env})$	0.9987 MS(Rep(Env)) + 0.9983 MS(Env*Entry) - 0.997 MS(Residual)	6.6	353.45	<0.0001
Rep(Env)	4	4.4	1.1	$\sigma^2(\text{Residual}) + 131\ \sigma^2(\text{Rep(Env)})$	MS(Residual)	520	2.33	0.0548
Env *Entry	391	312.5	0.8	$\sigma^2(\text{Residual}) + 1.9956\ \sigma^2(\text{Env*Entry})$	MS(Residual)	520	1.68	<0.0001
Residual	520	247.3	0.5	$\sigma^2(\text{Residual})$				

[A]- Trait measured in disease field trials.

[B]- Trait measured in yield field trials.

Appendix 8.4: Complete set of 32 linkage groups assigned to the 21 chromosomes of the Pastiche/Torfrida winter wheat cross. Molecular markers are indicated to the right of the chromosome and genetic distances are indicated to the left of the chromosome in Kosambi centiMorgans. The letter "C" indicates the approximate location of the centromere.

1A
0.0 wPt-1011_1A
2.3 wPt-8412_3B|6B
wPt-2847_1A
2.5 wPt-0128_1A
3.1 wPt-4658_1A
5.3 wPt-0164_1A
5.4 wPt-6754_1A
9.1 gwm497_1A
13.9 dupw038
32.1 wPt-8455_1A
33.4 wPt-4029_1A
33.5 wPt-6358_1A|3B
35.1 gwm136
42.4 wPt-9757_1A
42.7 wmc093
46.4 wPt-3870_1A
48.0 wPt-6122_1A
C → 59.8 psp3151
64.4 barc083
67.8 gwm135
72.0 wmc024
74.0 wPt-3698_1A
77.8 wPt-2872_1A
82.3 wPt-6654
83.0 gwm164
83.1 psp3027
wPt-1426

1B
0.0 wPt-2575
2.7 wPt-5801_1B
C → 22.9 wPt-8971
30.3 gwm011
wPt-0260_1B
31.8 wPt-2786_6B
gwm413
40.8 wPt-3566_1B
wPt-0705_1B
46.4 wPt-2315_1B
59.6 gwm268
59.7 wPt-1403_1B
61.6 wPt-9809_1B
74.8 wPt-2526_1B
98.8 wmc044
115.1 wmc728
116.3 wPt-4721_1B
116.4 wPt-1973

1D
0.0 gwm232
7.1 gdm111

0.0 wPt-3738_1D
C → 7.4 gwm106
10.2 wPt-3707_1D
30.1 gwm458
46.6 wPt-3743_1D
gwm642
53.4 wPt-3358

2A
0.0 gwm497_2A
2.3 wPt-8049
5.0 barc212
6.6 barc124_2A
6.9 Dart4
wPt-1224
7.6 wPt-9672
gwm636
wPt-3565
Dart2
7.7 wPt-1112
wPt-6158
wPt-8407
wPt-5738_2B
wPt-9839
7.8 wPt-8132
8.5 wPt-9961
9.3 wPt-8573
10.6 wPt-8242
11.4 cfd036_2A
26.5 wPt-3896_2A

0.0 gwm095
psp3088
6.8 gwm425
C → 24.0 wPt-2882
wPt-8569_1A|2B
24.1 wPt-0094_2B
25.4 wPt-2544_2D
35.6 wPt-7024_2A|3B
40.2 wPt-3281_2A
42.1 wPt-1499_2A
46.2 wPt-7739
49.6 wPt-5887_2A
53.7 wPt-6775
55.9 wPt-6233

2B
0.0 wPt-8398
2.8 wPt-8326_2A|2B
6.4 wmc154
25.8 wPt-6311_2B
39.0 wPt-1266_7B
43.3 gwm068_2B
44.2 wPt-0335_2B
54.4 wPt-5298
57.0 wPt-0981
62.5 wPt-3132_2B
C → 63.3 wPt-5242
wPt-0697
63.7 wPt-0473
wPt-9257
64.1 wPt-1127
66.7 wPt-0523
69.1 wPt-9856
75.3 wPt-2397_2B
75.7 gwm526
85.6 wmc149
98.9 wPt-3378_2B
101.3 wPt-4917_2B

2D
0.0 barc124_2D
11.9 gdm035
15.4 cfd036_2D

0.0 gwm484
2.3 barc168
C → 21.3 wmc018
43.7 gwm539
63.3 wPt-1554_2D
wPt-2781_2D
64.3 wPt-5865_2A
64.6 wPt-2160
67.5 wPt-3728_2D
70.5 wPt-0638_2D

3A

0.0 — wPt-2967_3A
0.6 — wPt-3697_3A
0.9 — wPt-2698_3A
2.4 — gwm155

3B

0.0 — wPt-7225_3B
0.3 — gwm389
4.1 — gwm533
5.3 — wPt-3921_3B
18.5 — barc087
22.1 — gwm493
22.3 — wPt-0302
22.4 — wPt-3536
22.8 — wPt-2757_3B
26.7 — wPt-3038
32.7 — wPt-5064
34.9 — barc102
36.6 — wPt-7212_3B
52.2 — wmc054
53.5 — wPt-5318
53.8 — wPt-5836
56.2 — wPt-0773
58.9 — wPt-1484_3B
59.8 — wPt-6216_3B
61.8 — wPt-6239
63.6 — wPt-0682
64.1 — wPt-2377_3B
— wPt-4209_3B
64.7 — gwm274
— gwm261
64.8 — gwm285
65.0 — wPt-6973_3B
71.7 — wPt-9310_3B
71.8 — wPt-1625_3B
C → 79.0 — barc164
83.5 — wPt-2458

0.0 — wPt-2416_3B
4.8 — wPt-7595
12.6 — wPt-5072_3B
12.8 — wPt-0324_3B
13.0 — wPt-7614_3B
14.3 — psp3001
15.3 — wPt-7526
24.6 — gwm114
31.5 — wPt-8021_3B

49.0 — wPt-5704_3B
54.1 — wPt-3005_3B
61.8 — wPt-5358

3D

0.0 — wPt-5313_1B|3D
1.3 — wPt-6909_3D

C → 0.0 — barc125
— gwm456
4.8 — gdm072

4A

0.0 — dupw004
3.6 — wPt-2788_4A

4B

0.0 — wPt-0246_4B
1.4 — wPt-5559_4B

4D

0.0 — wmc285

25.8 — wPt-8836_4D

5A

0.0	wPt-1954_5A
2.8	barc056
2.9	gwm293_5A
	gwm304
4.7	gwm129
5.8	barc180
C →	
25.7	barc141

5B

0.0	wPt-3350
2.9	wPt-4602_6D
4.1	wPt-3127
C →8.6	barc021

0.0	barc140
	wPt-8094_5B
1.2	wPt-9598_5B

0.0	gwm497_5B
8.4	wPt-7238_5B
12.4	wPt-3922_5B
13.2	wPt-3569_5B
14.7	wPt-0484_5B

5D

C → 0.0	gwm293_5D
	gwm159
26.1	gwm583

6A

0.0	Dart1
	wPt-4016_6A
2.5	gwm334
3.7	wPt-6904_6A
7.4	wPt-8266_6A

0.0	wPt-7599_6A|6B
21.7	psp3152
24.4	barc146
	barc171
C →24.6	psp3071
25.4	barc003
27.2	barc107
46.3	gwm570

0.0	wPt-4229_6A
4.1	wPt-0404
13.7	dupw167
13.8	wPt-7204_6A

6B

0.0	wPt-6127
2.0	wPt-8894
2.1	wPt-3116_6B
	wPt-3774_6B
2.4	dupw217
2.5	wPt-5188_2B|6B
2.8	wPt-5234_6B
6.4	wPt-0245
13.9	wPt-5256_6B
16.1	wPt-7745_6B
16.7	wPt-0259_6A|6B
16.9	wPt-7906
47.9	barc198
	wmc397
52.5	gwm626
67.4	gwm219
C →	
98.5	barc134
100.8	wPt-9930_6B
	wPt-6023
109.5	wPt-1325_6B

6D

0.0	barc173
14.2	wPt-7394
21.4	wPt-6500
C →	

7A

0.0	wPt-1510_7A
1.6	wPt-6034_7A
25.3	wPt-4880_7A
26.6	wPt-6447_7A
	wPt-1179_5B
46.0	wPt-3702
60.0	gwm060
64.4	barc127
68.0	wPt-1928_7A
81.2	wPt-7188_1B
89.5	wPt-9857_1D
101.4	gwm068_7A
102.6	wPt-2266_7A
102.7	barc108
104.0	wmc247
114.6	Dart3
	barc029
114.8	barc121

C →

0.0	wPt-9824_4B
6.6	wmc633
18.8	wPt-0639
35.2	cfa2040_7A
35.3	wmc525

7B

0.0	gwm046
6.3	wPt-7653_7B
10.2	wPt-4771
34.7	wPt-7318_7B

C →

0.0	wPt-6156_7B
1.6	wPt-2356_7B
4.1	wPt-8938
9.2	wPt-5216
9.6	gwm577
11.5	wPt-5138_7B
12.8	wPt-8007_7B
	wPt-7413_7B
13.0	wPt-8561_7B
	wPt-0600_7B
13.1	wPt-3190_7B
	wPt-7720_7B
13.3	wPt-3776
13.4	wPt-1093
14.2	barc032
14.3	wPt-8084_7D
17.3	wPt-4038_7B
20.6	wPt-7108_7B
22.6	wPt-5816_7B
22.7	wPt-6673
22.9	wPt-1209_7B
23.0	wPt-0504_7B
24.2	wPt-1085_7B
24.5	wPt-0465_7B
24.7	wPt-1533_7B
27.6	wPt-0530_7B
32.8	wPt-5069_7B

7D

0.0	gwm295

C →

31.5	barc111
43.8	wPt-1859_7D
54.8	wPt-7114_5B
66.7	cfa2040_7D
76.4	barc076

Appendix 8.5: Disease rating scale developed by Rosielle (1972).

0 = **immune** – no pycnidial formation, no visible symptoms or an occasional hypersensitive fleck.

1 = **highly resistant** – no, or only an occasional isolated pycnidium formed, particularly in older tissue, hypersensitive flecking in younger leaf tissue.

2 = **resistant** – very light pycnidial formation, some coalescement of lesions, mainly towards the leaf tip and in older tissue.

3 = **intermediate** – light pycnidial formation, coalescement of lesions normally evident towards the leaf tips and elsewhere on the leaf blade.

4 = **susceptible** – moderate pycnidial formation, lesions much coalesced.

5 = **very susceptible** – large, abundant pycnidia, lesions extensively coalesced.

An 'X' following the Arabic numeral rating indicated extensive leaf necrosis. The Arabic numeral rating for such host genotypes corresponded to pycnidial formation as above.

Appendix 8.6: Wheat and Barley DNA extraction protocol (Patricia Warner)

1. Tissue is harvested, frozen and lyophilized. The tissue (2 x 2.5cm sections) is then placed in 1.2 mL collection tubes with about 200 µl glass beads (2mm) and shaken on the paint shaker for 10-15 mins to grind the tissue to a fine powder.

2. Prepare Extraction Buffer (0.1M Tris-HCl pH 7.5, 0.05 EDTA pH 8.0, 1.25% SDS).

For 1 litre:	100 mL 1.0 M Tris-HCl pH 7.5
	100 mL 0.5M EDTA pH 8.0
	125 mL 10% SDS
	675 mL ddH$_2$O

3. Preheat extraction buffer to 65°C and also allow the plates containing the tissue to warm up to room temperature if they have been stored at -20°C.

4. Add 500 µl of extraction buffer to each tube, seal the plates with caps and shake thoroughly. Incubate the plate at 65°C for 30 minutes.

5. Place the plates in the fridge (or freezer) to cool them down to room temperature (about 15 minutes) before adding 250 µl 6M ammonium acetate, which is stored at 4°C. Shake vigorously to mix in the ammonium acetate and then leave to stand for 15 minutes in the fridge.

6. Centrifuge the plate for 15 minutes at 5000 rpm (Sigma 4-15 centrifuge) to collect the precipitated proteins and plant tissue.

7. Recover 600 µl of the supernatant into new collection microtubes containing 360µl of iso-propanol in each well. Mix thoroughly and allow the DNA to precipitate for 5 minutes.

8. Centrifuge the samples for 15 minutes at 5000 rpm in order to pellet the DNA and then tip off the supernatant. Allow the remaining fluid to drain off the DNA pellet by inverting the tubes onto a piece of paper towel. ONLY INVERT THE TUBES FOR LESS THAN 1 MINUTE OTHERWISE YOU WILL LOSE THE DNA PELLETS.

9. Wash the pellet in 500 µl of 70% ethanol.

10. Centrifuge the plate for 15 minutes at 5000 rpm and again discard the supernatant.

11. Allow DNA pellets to dry completely.

12. Resuspend the pellet in 250 µl of 0.1X TE. Leave the DNA to dissolve overnight at 4°C in the fridge. Try to dislodge the pellet.

13. Spin down the un-dissolved cellular debris by centrifuging the plate for 20minutes at 5000 rpm.

14. Transfer approximately 200-220 µl supernatant into a 96 well microtitre plate. Avoid pipetting any debris at the bottom of the well.

Appendix 8.7: Haplotypes of septoria tritici blotch resistance genes *Stb13*, *Stb14*, and *StbSm3* as well as phenotypic reactions to *Mycosphaerella graminicola* isolates MG96-36 and MG2 for selected doubled haploid lines produced from the Salamouni/Katepwa cross. Alleles are identified as SAL (Salamouni, resistant parent) and KAT (Katepwa, susceptible parent). The chromosome location of the three Stb resistance genes are identified.

DH Line	*Stb13*[A] 7BL	*Stb14*[B] 3BS	*StbSm3*[C] 3AS	MG96-36[D] Race 1	MG2[D] Race 2
Parents					
Salamouni	SAL	SAL	SAL	0-1 (R)	0 (R)
Katepwa	KAT	KAT	KAT	4-5 (S)	4-5 (S)
Stb14 mapping[E]					
98S08A*09	KAT	SAL	KAT	4 (S)	3 (R)
Stb13 mapping[E]					
98S08C*03	SAL	KAT	KAT	3 (R)	3 (R)
98S05B*01	SAL	KAT	KAT	2-3 (R)	2 (R)
StbSm3 mapping					
98S05B*13	KAT	SAL	SAL	2 (R)	2 (R)
Not previously mapped					
98S08B*04	SAL	SAL	SAL	2 (R)	3 (R)
98S08D*03	SAL	SAL	SAL	2 (R)	2-3 (R)
98S05C*17	SAL	SAL	SAL	2 (R)	2 (R)
98S05B*31	SAL	SAL	KAT	0 (R)	1 (R)
98S08A*20	SAL	SAL	KAT	2 (R)	3 (R)
98S08C*05	SAL	SAL	KAT	1 (R)	2 (R)
98S08D*17	SAL	SAL	KAT	1-2 (R)	2 (R)
98S05B*03	SAL	SAL	KAT	1-2 (R)	1-2 (R)
98S05C*21	SAL	SAL	KAT	2 (R)	2 (R)
98S05C*31	SAL	SAL	KAT	2 (R)	2 (R)
98S05C*13	SAL	SAL	KAT	1-2 (R)	3 (R)
98S05B*09	KAT	SAL	SAL	2 (R)	2 (R)
98S05B*11	KAT	SAL	SAL	2 (R)	2-3 (R)
98S05A*23	KAT	SAL	SAL	2 (R)	2 (R)
98S08A*17	KAT	SAL	SAL	2 (R)	3 (R)
98S05C*22	KAT	SAL	SAL	2 (R)	2 (R)
98S08D*20	KAT	SAL	SAL	2 (R)	3 (R)
98S05A*24	SAL	KAT	SAL	2 (R)	2 (R)
98S08C*02	SAL	KAT	SAL	2 (R)	3 (R)
98S05B*04	SAL	KAT	SAL	3-4 (S)	2 (R)
98S08C*15	SAL	KAT	KAT	2-3 (R)	3 (R)
98S05A*07	SAL	KAT	KAT	2-3 (R)	2-3 (R)
98S08C*08	KAT	KAT	SAL	2-3 (R)	3 (R)

98S08A*10	KAT	KAT	SAL	3-4 (S)	3 (R)
98S05C*05	KAT	SAL	KAT	2 (R)	3 (R)
98S05C*15	KAT	SAL	KAT	2-3 (R)	2 (R)
98S05C*26	KAT	SAL	KAT	3-4 (S)	3-4 (S)
98S05B*14	KAT	SAL	KAT	3-4 (S)	3-4 (S)
98S05B*25	KAT	KAT	KAT	3-4 (S)	3-4 (S)
98S05B*07	KAT	KAT	KAT	4 (S)	5 (S)

[A] *Stb13* reported to be linked to microsatellite locus wmc396 at a distance of 9 cM (Cowling 2006).

[B] *Stb14* reported to flanked by the microsatellite loci cfd79 and wmc623 at distances of 11 and 5 cM, respectively (Cowling 2006). Allele based on marker wmc623.

[C] *StbSm3* flanked by the microsatellite loci barc321 and barc12 at distances of 1.9 and 2.5 cM, respectively. Allele based on marker barc321.

[D] DH line phenotypic rating measured with Modified Rosielle Scale by Cowling (2006).

[E] DH lines listed were used in the mapping of septoria tritici blotch resistance genes *Stb13* or *Stb14* (Cowling 2006).

Appendix 8.8: Protocol used by INRA Clermont for insertion-site based polymorphism (ISBP) marker screening

Products	1 reaction
Template DNA at 10 ng/μL	2.50 μL
PCR Buffer 10X (contains 31mM MgCl$_2$)	1.00 μL
Betaine 5M	2.00 μL
dNTP10mM	0.40 μL
Primer F (10μm)	0.25 μL
Primer R (10μm)	0.25 μL
DNA Taq polymerase	0.25 μL
SYBR Green 1.6X	1.00 μL
H$_2$O sterile 7.5 μL	2.35 μL
Total Volume	**10 μL**

Thermal cycling programs for ISBP markers: 1 cycle of [95°C – 5 min], 7 cycles of [95°C – 30 sec, 62°C (-1°C per cycle) – 30 sec, 72°C – 30 sec], 31 cycles of [95°C – 30 sec, 55°C – 30 sec, 72°C – 30 sec], 11 cycles of [95°C – 30 sec, 56°C – 30 sec, 72°C – 30 sec], 1 cycle of [72°C – 5 min], 15°C forever.