### Genetic mapping of disease resistance genes Fhb1, Fhb2 and Lr34

in spring wheat (Triticum aestivum L.)

BY

PATRICIA ANNE CUTHBERT

A Thesis

Submitted to the Faculty of Graduate Studies

In Partial Fulfillment of the Requirements

For the Degree of

#### DOCTORATE OF PHILOSOPHY

Department of Plant Science

University of Manitoba

Winnipeg, Manitoba

© Copyright by Patricia Anne Cuthbert 2008

#### THE UNIVERSITY OF MANITOBA

#### FACULTY OF GRADUATE STUDIES \*\*\*\*\* COPYRIGHT PERMISSION

## Genetic mapping of disease resistance genes Fhb1, Fhb2 and Lr34 in spring wheat (*Triticum aestivum* L.)

#### BY

#### PATRICIA ANNE CUTHBERT

A Thesis/Practicum submitted to the Faculty of Graduate Studies of The University of

Manitoba in partial fulfillment of the requirement of the degree

#### **DOCTOR OF PHILOSOPHY**

#### PATRICIA ANNE CUTHBERT © 2008

Permission has been granted to the University of Manitoba Libraries to lend a copy of this thesis/practicum, to Library and Archives Canada (LAC) to lend a copy of this thesis/practicum, and to LAC's agent (UMI/ProQuest) to microfilm, sell copies and to publish an abstract of this thesis/practicum.

This reproduction or copy of this thesis has been made available by authority of the copyright owner solely for the purpose of private study and research, and may only be reproduced and copied as permitted by copyright laws or with express written authorization from the copyright owner.

#### ACKNOWLEDGMENTS

I wish to express my sincere appreciation to the following people for their contribution to this study:

Co-advisors, Drs. Daryl Somers and Anita Brule-Babel for their guidance and advice throughout the course of this study.

Drs. Dilantha Fernando, Gavin Humphreys, and Brent McCallum for serving on my advisory committee. Dr. Francois Belzile for serving as my external examiner.

National Science and Engineering Research Council for the financial support in the form of a scholarship.

Agriculture and Agri-Food Canada – Matching Initiative, Agriculture and Agri-Food Canada – Crop Genomics Initiative, and Western Grains Research Foundation for financial support.

Denis Green and the Agriculture and Agri-Food Canada wheat breeding field crew for their assistance seeding my rust field trials.

Roger Larios and University of Manitoba wheat breeding summer students for their assistance with seeding and field inoculations in Carman, Manitoba

Ron Kaethler, Kevin Morgan and Kirsten Slusarenko for all their assistance with FHB inoculum preparation for indoor and field inoculations.

Denis Green, Otto Gruenke, Leslie Betze, Allison Brown-Hoeppner, Monika Eng, Debbie Miranda, Mira Popovic, and Yanfen Zhang for assistance with hand harvesting the Fusarium head blight nurseries.

Dr. Jim Menzies for the development of population BW278/AC Foremost used in my experiments.

Pat Seto-Goh for assistance with leaf rust inoculum preparation and inoculations in the field and greenhouse.

Fellow Graduate Students for their friendship and help throughout the course of my project.

My husband Kelvin, Norm and Esther for all the love and support as I completed my thesis.

Mom, Dad, Janice, and Richard for the continuous love and encouragement. Thank you for your support.

## TABLE OF CONTENTS

.

ACKNOWLEDGMENTS	ii
TABLE OF CONTENTS	iii
LIST OF TABLES	vi
LIST OF FIGURES	. vii
LIST OF APPENDICES	viii
ABSTRACT	ix
1.0 GENERAL INTRODUCTION	1
2.0 LITERATURE REVIEW	6
2.1 Spring wheat	6
2.1.1 Importance of spring wheat and production statistics	6
2.1.2 Spring wheat origin and genetics	6
2.1.3 Molecular breeding of wheat	7
2.2 Molecular markers and mapping	9
2.2.1 Restriction Fragment Length Polymorphism – RFLP	. 10
2.2.2 Amplification-based markers	. 11
2.2.2.1 Random Amplified Polymorphic DNA – RAPDs	. 11
2.2.2.2 Amplified Fragment Length Polymorphisms – AFLPs	. 12
2.2.2.3 Microsatellites	. 13
2.2.3 Mapping populations	. 14
2.2.4 Genetic maps	. 15
2.2.5 Marker assisted selection (MAS)	. 17
2.3 Fusarium head blight	. 18
2.3.1 Symptoms	. 19
2.3.2 Disease cycle	. 20
2.3.3 Control	. 22
2.3.3.1 Cultural control	. 22
2.3.3.2 Chemical control	. 22
2.3.3.3 Biological control	. 23
2.3.3.4 Genetic control	. 23
2.4 Wheat leaf rust (brown rust)	. 26
2.4.1 Host-pathogen interaction	. 28
2.4.2 Symptoms	. 29
2.4.3 Disease cycle	. 30
2.4.4 Control	. 31
2.4.4.1 <i>Lr34</i>	. 32
2.4.5 Mapping leaf rust resistance genes	. 34
3.0 FINE MAPPING FHRI A MAJOR GENE CONTROLLING FUSARILIM HEAL	ר
BLIGHT RESISTANCE IN BREAD WHEAT (TRITICUM AESTIVUMI)	38
31 Abstract	38
3.2 Introduction	30
J.2 IIII.04401011	

3.3 Materials and methods	
3.3.1 Mapping populations	
3.3.2 Genotyping and selection of recombinants	
3.3.3 Fusarium head blight phenotyping	
3.3.4 Construction of the genetic map	
3.4 Results	
3.4.1 Genotyping	47
3 4 2 Phenotyping	47
3 4 3 Genetic man	
3.5 Discussion	
5.5 Discussion	
4.0 MADDING OF FUD? ON CUDOMOSOME (DS) A GENE CONTR	DOLUNIC
ELISADIUM HEAD DI IGUT EIELD DESISTANCE IN DEAD WHEAT	
AESTIMINAL )	(IRIICOM
ALSTIVUML.)	
4.1 Abstract	
4.2 Introduction	
4.3 Materials and methods	
4.3.1 Population, genotyping and selection of recombinants	59
4.3.2 Fusarium head blight phenotyping – greenhouse	63
4.3.3 Fusarium head blight phenotyping - field trials	64
4.3.4 Construction of the genetic map	
4.3.5 Statistical analysis	
4.4 Results	66
4.4.1 Fusarium head blight phenotyping	
4.4.1.1 Greenhouse trials	
4.4.1.2 Field trials	
4.4.2 Genetic map	
4.5 Discussion	
5.0 MAPPING OF ADULT LEAF RUST RESISTANCE GENE $LR34$	AND THE
INTERACTION WITH FUSARIUM HEAD BLIGHT RESISTANCE GEN	JE $FHR2$ IN
SPRING WHEAT (TRITICUM AESTIVUM L.)	77
51 Abstract	
5.2 Introduction	
5.2 Materials and methods	
5.3 Materials and memory of $L_r^2 4$	
5.3.1 Genetic mapping of Lr54	80
5.3.1.1 Mapping population, genotyping and selection of lines	
5.3.1.2 Genotyping	
5.3.1.3 Leaf rust phenotyping – field	
5.3.1.4 Construction of the genetic map of 7DS	
5.3.1.5 Statistical analysis	83
5.3.2 Interaction between <i>Lr34</i> on <i>Fhb2</i>	83
5.3.2.1 Genetic population development	83
5.3.2.2 Fusarium head blight phenotyping	
5.3.2.3 Leaf rust phenotyping – growth cabinet	84
5.3.2.4 Genetic map	85

•

## - iv -

5.3.2.5 Statistical analysis	
5.4 Results	
5.4.1 Genetic mapping of <i>Lr34</i>	
5.4.1.1 Leaf rust field trials	88
5.4.1.2 Genetic map on 7DS	
5.4.2 Interaction between effects of <i>Lr34</i> on <i>Fhb2</i>	
5.4.2.1 Leaf rust growth cabinet test	
5.4.2.2 Fusarium head blight tests	
5.4.2.3 Genetic map	
5.4.2.4 Gene interaction	
5.5 Discussion	
6.0 GENERAL DISCUSSION	100
7.0 REFERENCES	107
	100
8.0 APPENDICES	

## LIST OF TABLES

Table		Page
3.1	Primer sequences, annealing temperatures, and allele sizes of markers used in the Thatcher/5*Sumai 3 (T/S) and HC374*3/98B69-L47 (HC/98) populations for fine mapping <i>Fhb1</i> in spring wheat	.50
4.1	Primer sequences, annealing temperatures, and allele sizes of markers on chromosome 6B used in the BW278/AC Foremost population for mapping <i>Fhb2</i> in spring wheat	.62
4.2	Analysis of variance for four phenotypic field variables (disease incidence, disease severity, visual rating index, and Fusarium-damaged kernels) collected over four site years (Carman 2003, Glenlea 2003, Carman 2004, Glenlea 2004)	.71
4.3:	Correlation coefficient (r) matrix calculated using averaged values for greenhouse disease severity and field data measurements (Visual Rating Index (VRI), Disease Incidence (DI), Disease Severity (DS), and Fusarium-damaged kernels (FDK)) collected in the greenhouse and two field locations in southern Manitoba (Glenlea and Carman) over two years (2003 and 2004). ( $\alpha$ =0.05)	.72
5.1	Primer sequences, annealing temperatures, and allele sizes of markers on chromosome 6B used in the BW278/AC Foremost population for mapping $Lr34$ in spring wheat	.87
5.2	Analysis of variance for leaf rust area under disease progress curve (AUDPC) collected in years (2003, 2004) in Glenlea, Manitoba	.89
5.3	Two-way analysis of variance to measure the effect of the interaction between Fusarium head blight (FHB) resistance gene $Fhb2$ and leaf rust resistance gene $Lr34$	.94

## LIST OF FIGURES

Figure		Page
3.1	Development of two large mapping populations for fine mapping <i>Fhb1</i> on the distal end of chromosome 3BS in spring wheat crosses	.43
3.2	Infection phenotype 21 days post-inoculation in 4 spikes from a susceptible plant and a resistant plant	.46
3.3	Phenotypic distribution of Type II resistance to FHB in fixed recombinant plants measured by single floret injection	.48
3.4	Fine map of the major FHB resistance gene, <i>Fhb1</i> , in two large bread wheat populations containing two sources of FHB resistance	.51
4.1	Phenotypic distribution of 95 RILs from the cross BW278/AC Foremost	.67
4.2	Genetic map position of <i>Fhb2</i> on chromosome 6BS in the cross BW278/AC Foremost	.69
5.1	Phenotypic distribution of leaf rust infection (%infection of flag leaf) collected in the field and growth cabinet for the 253 RILs (A) and 89 RILs (B), selected from the population BW278/AC Foremost	.90
5.2	Genetic map of the adult leaf rust resistance gene, <i>Lr34</i> on chromosome 7DS	.91
5.3	Distribution of the number of lines for 89 RILs from the cross BW278/AC Foremost carrying various combinations of resistance genes <i>Fhb2</i> and/or <i>Lr34</i> to demonstrate the effect of interaction of the two resistance genes	.98

## LIST OF APPENDICES

Appen	dix	Page
8.1	Dual floret inoculation conducted in the greenhouse when each wheat spike reached 50% anthesis. Each spike was inoculated by injecting a 10 $\mu$ l macroconidial suspension between the lemma and palea of a floret positioned at the inoculation point	22
8.2	Following point inoculation, a 10 x 5 cm clear, Bitran S Series (Fisher Scientific) liquid tight specimen bag was placed over the spike to increase humidity around the spike	22
8.3	Fusarium head blight field nursery at Carman, Manitoba 20031	23
8.4	A 50 gram sample from each field plot was visually assessed to determine the % of Fusarium damaged kernels in the harvested sample1	23

## ABSTRACT

# Cuthbert, Patricia Anne. PhD., The University of Manitoba, October 2007. Genetic Mapping of Disease Resistance Genes *Fhb1*, *Fhb2*, and *Lr34* in Spring Wheat (*Triticum aestivum* L.). Major Professors: Daryl J. Somers and Anita L. Brulé-Babel.

Fusarium head blight (FHB), caused primarily by Fusarium graminearum Schwabe (teleomorph Gibberella zeae (Schwein, Petch)) and leaf rust, caused by Puccinia triticina Eriks. (Anikster et al., 1997) (= P. recondita Rob. ex Desmaz, f. sp. tritici) are two of the most devastating fungal diseases of spring wheat which have resulted in multi-million dollar losses annually. Understanding the genetics and interaction of these diseases is key to facilitate the introgression of FHB and leaf rust resistance genes into adapted wheat cultivars. FHB resistance QTLs on chromosomes 3BS and 6BS were isolated and mapped as Mendelian factors in three large populations with fixed resistant and susceptible genetic backgrounds using microsatellite markers. FHB resistant parents in the crosses were Sumai 3, HC374= Nyubai, and BW278 = AC Domain\*2/Sumai 3. The QTL on 3BS (Offs.ndsu-3BS) was mapped in two genetic backgrounds, one fixed for resistance at other FHB resistance loci and one fixed for susceptibility at other FHB resistance loci: Thatcher/5\*Sumai 3 (T/S), and HC374/3\*98B69-L47 (HC/98) respectively. The QTL on 6BS was mapped in a population of BW278/AC Foremost that was fixed for resistance at other FHB loci. Phenotypic data were collected in the greenhouse using dual floret injections (DFI) to measure Type II resistance (disease severity (DS)) and macro conidial spray inoculation were conducted in field nurseries to evaluate field resistance (disease incidence (DI), disease severity (DS), visual rating index (VRI), and Fusarium damaged

kernels (FDK)). The major gene, *Fhb1* (syn. *Ofhs-ndsu-3BS*), was successfully mapped on chromosome 3BS in the same location of the two populations within a 1.27 centimorgan (cM) interval (T/S) and a 6.05 cM interval (HC/98). Fhb2 mapped all four field traits quantitatively to a coincident position on chromosome 6BS, flanked by GWM133 and GWM644. The greenhouse DS trait mapped 2 cM distal to Fhb2. The population BW 278/AC Foremost also segregates for two known leaf rust resistance genes Lr16, Lr34 and one unidentified resistance gene tentatively named LrF. Two groups of RILs were selected with varying combinations of disease resistance genes to leaf rust and FHB. The first group of RILs carried the susceptible allele for Lr16 and LrF and were recombinant between markers GWM1220 and GWM130 flanking Lr34 and segregated for Fhb2. These RILs were evaluated for leaf rust severity in the field during 2003 and 2004 to develop a high density map for Lr34. A second group of RILs from the same cross were genotypically selected to be fixed resistant or susceptible for Fhb2 and segregated for varying combinations of Lr16, LrF, and Lr34. This group of RILs was evaluated for leaf rust using growth cabinet inoculation and for Fusarium head blight in the field during 2003 and 2004. The phenotypic distribution for leaf rust was bimodal for both groups of RILs and Lr34 was mapped on 7D in both RIL populations. Leaf rust resistance gene Lr34 is known to enhance the level of disease resistance to many diseases in addition to its effects on leaf rust. However, there was no clear positive enhancement of FHB resistance when Lr34 and the un-linked gene, Fhb2 resistance alleles were present together. The successful mapping of resistance genes in wheat provides tightly linked markers that can reduce linkage drag associated with marker-assisted selection (MAS) and assist in the isolation, sequencing, and functional identification of the underlying resistance genes.

- X -

#### FOREWARD

This thesis follows the manuscript style outlined by the Department of Plant Science, University of Manitoba. 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 sections. A general review of the literature precedes the manuscripts, and a general discussion follows the manuscripts.

#### **1.0 GENERAL INTRODUCTION**

*Triticum aestivum* L. em. Thell (common or bread wheat) is one of the most important food sources for humans in the world, particularly in the temperate zone (Leonard and Martin 1963). In Canada, wheat production area has declined ten percent from 20.3 million hectares in 2006 to 17.1 million hectares in 2007 (Statistics Canada 2007). This decrease in wheat production area is primarily a result of low wheat commodity prices, as well as high disease pressure of Fusarium head blight and leaf rust.

Fusarium head blight (FHB), caused primarily by *Fusarium graminearum* Schwabe (teleomorph *Gibberella zeae* (Schwein. Petch)) and leaf rust, caused by *Puccinia triticina* Eriks. (Anikster et al., 1997) (= *P. recondita* Rob. ex Desmaz. f. sp. *tritici*), have resulted in multimillion dollar losses annually and losses are steadily increasing in Canada due to the spread of the disease and change in environmental conditions throughout the summer in Canada. Current agronomic practices and chemicals have met with limited success in controlling these diseases. The development of wheat cultivars with resistance to FHB and leaf rust is the most effective and economical approach for environmentally safe and sustainable long term control.

Some forms of plant disease resistance are genetically simple and have been analyzed extensively by traditional methods of plant pathology, plant breeding and genetics. Genetically complex forms of disease resistance, by contrast, are more poorly understood. In the past, classical quantitative genetics provided the tools for studying complex disease resistance. However, quantitative genetics is not suitable for dissecting

- 1 -

polygenic resistance characters into discrete genetic loci or defining the roles of individual genes in disease resistance. Therefore, an effective approach for studying complex and polygenic forms of disease resistance is known as Quantitative Trait Locus (QTL) mapping, which is based on linkage of DNA markers with the expression of the quantitative traits.

DNA-based molecular markers can be used to determine the chromosomal locations of genes of interest and to aid in the selection of desired genotypes, a process called marker assisted selection (MAS) (Mohan et al., 1997). Molecular markers can be used to select for multiple traits in segregating populations and decrease the number of backcrosses in a backcross program to restore the adapted genetic background. Because molecular markers provide information based on genotype they are independent of the environment making them very useful for improving traits that are polygenic and highly influenced by the environment. For some traits, molecular markers may be more cost effective than selection based on phenotype especially if there is the ability to select only the recombinant lines to decrease the number of lines that are screened.

Phenotypic scoring has a significant impact on QTL mapping. In studies with complex disease resistance genes, factors that can reduce the challenge associated with QTL mapping include: development of suitable mapping populations, use of appropriate inoculum, proper design and statistical analysis of controlled environment and field experiments, replication, and use of improved DNA marker systems such as microsatellite markers.

Microsatellite markers have been used to develop a wheat genetic consensus map of 1,235 microsatellite marker loci covering 2,569 cM over 21 chromosomes, giving an average marker interval of 2.2 cM (Somers et al. 2004). The microsatellite consensus map is a good estimation of the marker position from four genetic maps, assembled by consensus onto a single linkage map. The consensus map represents the highest-density public microsatellite map of wheat and is accompanied by an allele database showing the parent allele size for every marker mapped. The primary use of the consensus map is in molecular mapping of traits and plant breeding.

Molecular markers have been linked to QTLs associated with various types of FHB resistance, particularly in Sumai 3 (Bai et al., 1999, Anderson et al., 2001, Buerstmayr et al., 2002; Yang et al., 2003; Yang et al., 2005; Zhou et al., 2002). Other sources of FHB resistance include: Ning derivatives, Wangshuibai, Frontana, Wuhan and Nyubai (Zhou et al., 2002; Buerstmayr et al., 2002, Somers et al., 2003; Steiner et al., 2004; Han et al., 2005). The most prominent QTLs for FHB resistance have been associated with a specific type of resistance: Type II resistance to disease spread on chromosome 3BS (Waldron et al., 1999; Anderson et al., 2001; Bai et al., 1999; Ban 2000), and 6B (Anderson et al., 2001; Yang et al., 2003); Type 1 resistance to initial infection on 3A (Steiner et al., 2004), and 5A (Buerstmayr et al., 2002). A major QTL on chromosome 3BS, between microsatellite markers GWM493 and GWM533, is designated *Qfhs.ndsu-3BS*, was originally mapped by RFLP analysis (Waldron et al., 1999) and verified later by several research groups (Bai et al., 1999, Anderson et al., 2001; Zhou et al., 2002; Somers et al., 2003; Yang et al., 2003).

The adult leaf rust resistance gene, Lr34, expresses partial resistance in a quantitative manner (Drijepondt and Pretorious 1989; German and Kolmer 1992; Singh 1992) by increasing the latency period and a decreasing infection frequency and uredium size (Drijepondt and Pretorious 1989). Lr34 has been mapped to chromosme 7DS (Dyck 1987). In the study by Spielmeyer et al.. (2005), Lr34 and Yr18 were mapped to a single locus flanked by microsatellite markers GWM295 and GWM1220 on chromosome 7DS. The Lr34/Yr18 region has been associated with many traits and disease resistance in wheat including durable, adult plant resistance to leaf rust (Lr34) (Singh and Gupta 1991); durable adult plant resistance to stripe rust (Yr18) (McIntosh 1992; Singh 1992a); adult plant resistance to powdery mildew (Spielmeyer et al.. 2005); tolerance to barley yellow dwarf virus (Bdv1) (Singh 1993); enhanced expression of stem rust resistance (Dyck 1987, Vanegas et al.. 2007)); and leaf tip necrosis of flag leaves (Ltn) (Singh 1992b).

Although Lr34 is present in wheat worldwide, it is still not known whether some or all of the disease resistance traits are controlled by a single gene or by several tightly linked resistance genes. Fusarium head blight is one disease that has not been studied to determine if there is an enhancement in the level of FHB resistance when Lr34 is present. Sumai 3, an FHB resistant Chinese spring wheat variety possesses FHB resistance QTLs found on chromosomes 3BS, 5A and 6BS (Bai et al., 1999, Anderson et al., 2001; Zhou et al., 2002; Somers et al., 2003; Yang et al., 2003; Yang et al., 2005). Sumai 3 also contains the adult leaf rust resistant gene Lr34 (Brent McCallum, unpublished data). Although the QTLs are not linked with Lr34, it may be possible that there is a genetic interaction resulting in an increased level of FHB resistance when resistance alleles for Lr34 and FHB QTLs are present in the same lines. It would be beneficial for plant breeders to know if the presence of Lr34 in combination with the FHB resistance QTL on 6BS positively enhances FHB reactions.

Understanding the sources of FHB and leaf rust resistance aids in the genetic mapping of QTLs. The fine mapping of disease resistance gene(s) provides valuable information to plant breeders worldwide and helps to reduce linkage drag associated with marker assisted selection, assists in the cloning of functional resistance genes, and the development of wheat cultivars with multiple pest resistance.

The objectives of this study were: 1) To develop a fine map of the FHB resistance QTL *Qfhs.ndsu-3BS* on chromosome 3BS in two experimental populations with different FHB resistance sources, Sumai 3 and Nyubai; 2) To map the FHB resistance QTL on chromosome 6B by quantifying the phenotypic variation (disease incidence (DI), disease severity (DS), visual rating index (VRI), and Fusarium damaged kernels (FDK)) and classifying the resistance gene as a qualitative Mendelian factor; and 3) To isolate and map the *Lr34* locus using microsatellite markers in a fixed susceptible (AC Foremost) genetic background and in a population segregating for other leaf rust resistance; and to determine if any genetic interaction exists between *Lr34* and the un-linked Fusarium head blight resistance QTL on chromosome 6BS.

- 5 -

#### 2.1 Spring wheat

#### **2.1.1 Importance of spring wheat and production statistics**

*Triticum aestivum* L. em. Thell. (common or bread wheat) and *Triticum turgidum* (L.) Thell subsp. *durum* L. (durum wheat) are the two commercially important wheat species in Canada and are the hosts of Fusarium head blight (*Fusarium graminearum* Schw. (*Gibberella zeae* Schw. & Petch)) and leaf rust *Puccinia triticina* Eriks. (Anikster et al.. 1997) (= *P. recondita* Rob. ex Desmaz. f. sp. *tritici*). Wheat is a cool season crop grown in many parts of the world, primarily between the latitudes 30 and 60°N and 27 and 40°S (Percival 1921, Nuttonson 1955). It is one of the most important food sources for humans in the world, particularly in the temperate zone (Leonard and Martin 1963). Wheat is used to make leavened and unleavened breads, pasta, Asian noodles, confectionary products, industrial products, and animal feeds. In Canada, the spring wheat production area has averaged 18.5 million hectares over the last five year (Statistics Canada 2007).

#### 2.1.2 Spring wheat origin and genetics

Wheat was domesticated over 10,000 years ago in southwestern Asia (Poehlman and Sleper 1995). *T. aestivum* (2n-6x=42, AABBDD) and *T. turgidum* subsp. *durum* (2n=4x=28, AABB) are allopolyploids (Poehlman and Sleper 1995). *T. turgidum* subsp. durum was formed by the hybridization of two related species followed by chromosome doubling. Each species provided one genome, either the A or B genome, both containing

seven chromosome pairs. *T. monococcum* L. (2n=2x=14, AA) is thought to be the most closely related species to the A genome donor in existence today. The B genome donor is unknown. *T. aestivum* formed through hybridization of *T. turgidum* and a progenitor of *T. tauschii* (2n = 2x = 14, DD). The 21 chromosomes (gametic number) of hexaploid wheat have been assigned into seven homoeologous groups. Each homeologous group contains three homologous, or partially homologous chromosomes, one originating from each of the three genomes, A, B or D. Chromosomes are identified by a numbering system which includes the homoeologous group (1 to 7) and the genome (A, B or D). Spring wheat has a very complex genome, i.e., hexaploid, which can create problems with the use of molecular markers. The size of the wheat genome at  $1.6 \times 10^{10}$  bp compared to barley and maize at 5 x  $10^9$ , and rice at  $4 \times 10^8$  bp. For a detailed review, see Kimber and Sears (1987).

#### 2.1.3 Molecular breeding of wheat

In the past, conventional wheat breeding has been used primarily to develop genetically uniform cultivars with good agronomic performance, disease resistance and quality characteristics. More recently, plant breeders have incorporated new technologies in their breeding programs. In particular, new technologies have contributed to the introgression of chromosome regions from wild relatives and the development of new selection strategies (Gupta and Varshney 2000).

Molecular marker technology offers a wide range of novel approaches to improve the efficiency of selection strategies. The DNA-based techniques detect sequence variation between varieties or accessions of wheat. Where sequence variation is situated in a region of the genome closely linked to a trait of interest, such as a disease resistance locus, the

- 7 -

variation can be used to predict the presence or absence of the resistance allele. The strength of prediction will depend upon the closeness of the genetic linkage between the sequence variant and the target locus. In wheat, some marker systems are more useful than others (Gupta and Varshney 2000). One of the challenges with wheat has been the general lack of significant polymorphism for some marker systems (Gupta and Varshney 2000).

Due to the complex genome of spring wheat, the application of several important techniques with molecular markers can be difficult. The presence of three related genomes of wheat (A, B, D) adds to the complexity of many marker assays, particularly Restriction Fragment Length Polymorphisms (RFLPs) analysis, since three sets of bands will usually appear one for each genome. In some cases this means that three loci can be mapped simultaneously; however, it is more common that only one genome will reveal polymorphisms with a given probe/enzyme combination. The three genomes also translate into 21 linkage groups in wheat, again adding to the complexity of the mapping work.

There is generally a low level of polymorphism in wheat relative to other cereal species and this means that a larger number of markers usually need to be screened than in the case of rice, maize, or barley (Chao et al., 1989; Liu et al., 1990). Further, the level of polymorphism is not consistent across genomes or crosses. Commonly, the D genome is more highly conserved between cultivars and is substantially more difficult to map. If random markers are being used in the mapping exercise, then the maps of the D genome tend to have the poorest coverage (Chalmers et al., 2001). The development of microsatellite markers specifically targeted to individual genomes (Pestova et al., 2000) should help address this problem.

The main application of molecular markers in cereals and other field crops can be divided into three categories: assessment of genetic variability and characterization of germplasm; identification and characterization of genomic regions controlling quantitative traits; and marker assisted selection following the identification of genomic regions (Dudley 2002; Ribaut et al., 2002).

#### 2.2 Molecular markers and mapping

Over the past twenty years there has been a massive effort to develop molecular marker linkage maps for many different crops (Somers 2004; Michelmore 1991; Roder 1998). It is the availability of these maps that has enabled the determination of the chromosomal location of genes of interest, construction of detailed genetic maps of quantitative and qualitative traits in segregating populations, marker-assisted selection and map-based cloning of genes. Molecular markers are genetic markers used in genomic analysis and provide the foundation for marker-assisted selection. A number of genetic marker systems have been developed for use in different plant species; however, some systems may not be suitable for all purposes. In general, the desirable characteristics of a marker system are to detect a high level of polymorphism, detect specific loci, provide clear and highly repeatable genetic information in a short period of time, and allow for automation (Liu et al., 1998). The marker systems available for any species will depend on the amount of pre-existing genome information.

There are two general approaches used to detect variation in a discrete region of DNA: hybridization-based and amplification-based methods (Gupta et al., 1999; Helentjaris et al., 1986; Weber and Helentjaris 1989; Chao et al., 1989; Liu and Tsunewaki

-9-

1991; Anderson et al. 1992; Devos et al. 1992; Devos and Gale 1993a,b; Xie et al. 1993). Restriction Fragment Length Polymorphisms (RFLPs) are a hybridization-based technology while Random Amplified Polymorphic DNAs (RAPDs), Amplified Fragment Length Polymorphisms (AFLPs) and Simple Sequence Repeats (SSRs or microsatellites) are amplification-based and use the polymerase chain reaction (PCR). Molecular markers can exhibit co-dominance or dominance. A co-dominant marker distinguishes between homozygous and heterozygous genotypes while a dominant marker is scored as present or absent and cannot identify heterozygous individuals (Somers 2004). The type of marker, co-dominant or dominant, determines the best generation to conduct MAS and also affects the value of the information and how it can be used. Dominant markers that are linked to the desired trait in coupling can still be used for MAS (Somers 2004).

#### 2.2.1 Restriction Fragment Length Polymorphism – RFLP

Restriction fragment length polymorphisms are highly reproducible, co-dominant markers that can identify unique loci (Mohan et al., 1997; Gupta et al., 1999; Liu 1998). RFLPs are typically detected by digesting genomic DNA with a restriction endonuclease, separating DNA fragments by electrophoresis, and hybridizing a Southern blot with a labeled DNA probe. A polymorphism is the result of the presence or absence of a restriction site or an insertion or deletion of DNA between restriction sites. Only a small fraction of RFLP probes detect polymorphism (Gupta et al., 1999). These markers have been successfully used for mapping plant genomes, including wheat (Chao et al., 1989, Helentjaris et al., 1986). The use of RFLPs is limited because the procedures involved are very labor intensive, require a relatively high level of technical skill, and are expensive for

the quantity of information obtained (Gupta et al., 1999; D'Ovidio et al., 1990). This method also requires a large amount of high quality DNA and is therefore not suitable when a limited amount of plant material is available. The greatest barrier to the use of RFLPs in MAS is the low level of polymorphism in a number of important crops including wheat (He et al., 1992). This low frequency is sometimes attributed to the polyploid nature of these crops, the high proportion of repetitive DNA and large genome size (Gupta et al., 1999).

#### 2.2.2 Amplification-based markers

Polymerase chain reaction (PCR) has facilitated the rapid development of many DNA amplication strategies that are all fundamentally similar. PCR based DNA markers rely on sequence variation in annealing sites or DNA length differences between amplified products. Techniques are robust and amenable to automation therefore can be widely applied to large scale marker development or implementation (Gupta et al., 1999).

#### **2.2.2.1 Random Amplified Polymorphic DNA – RAPDs**

Random amplified polymorphic DNA markers are simple, dominant, PCR-based markers (D'Ovidio et al.. 1990; Weining and Langridge 1991; Devos and Gale 1992; Somers 2004). The PCR reaction uses a single DNA primer of arbitrary sequence to amplify random segments of genomic DNA and the PCR amplicons are separated by electrophoresis to visualize polymorphisms (Williams et al.. 1990). The primers used in the RAPD system are a short arbitrary DNA sequence, often only ten base pairs, which can amplify multiple regions in the genome. The bands produced may or may not represent

some degree of sequence homology. To amplify specific loci, longer primers can be used. RAPD technology has proven useful in wheat studies; however, they have not been widely used due to low levels of detectable polymorphism and lack of reproducible results (Gupta et al., 1999). Because the primers are short relative to other marker systems, template DNA quality and quantity along with amplification conditions (concentration of MgCl<sub>2</sub>, Taq polymerase, and annealing/denaturing temperature) must be carefully controlled to ensure reproducible results especially among different labs (Devos and Gale 1992; D'Ovidio et al., 1990).

RAPD technology has been used widely in cereals to measure and characterize genetic diversity, to create linkage maps and to tag genes controlling important traits. The most prevalent difficulty associated with the RAPD technique is the lack of reproducibility and lack of locus specificity, particularly in polyploid species such as wheat. However, RAPD markers are simpler, more cost effective and less labour intensive than than RFLPs (Gupta et al., 1999). Very small amounts of DNA are required with no prior template DNA sequence information. Genetic variation at many loci from different regions of the genome can be examined quickly with RAPD markers.

#### 2.2.2.2 Amplified Fragment Length Polymorphisms – AFLPs

Amplified fragment length polymorphisms (AFLPs) are a relatively new type of marker system based on the arbitrary selective PCR amplification of DNA fragments generated by restriction digestion of genomic DNA. Specific DNA adapters are ligated to restriction digested DNA. AFLPs can be generated for any organism without prior knowledge of the DNA sequence and requires only a small amount of DNA. AFLPs are more reproducible than RAPDs and provide greater genome coverage. AFLPs have been limited in use for MAS because they are a dominant marker and are more labor intensive than RAPDs but have a higher multiplex ratio (Vos et al., 1995). A high multiplex ratio means they provide genetic information about many loci in a single PCR reaction. Studies have observed that AFLP is the most efficient mapping technique to detect polymorphism when compared to RFLPs and RAPDs (Powell et al., 1996; Lin et al., 1996).

#### 2.2.2.3 Microsatellites

Microsatellites or simple sequence repeats (SSR) are based on the number of contiguous direct repeat units at a particular locus in the genome (Gupta et al., 1999; Somers 2004; Devos et al. 1995; Roder et al. 1995). PCR primers complementary to flanking DNA sequences amplify the repeat region. PCR amplicons are then separated using electrophoresis. SSR markers are co-dominant, highly polymorphic relative to RFLPs and RAPDs, and many amplify only a single locus in common wheat (Roder et al., 1998; Gupta et al., 1999). SSRs can be isolated by searching sequence databases (such as GenBank and EMBL) and screening genomic or cDNA libraries for repeat sequences. SSR primers identified in one species can also be used for other related species (Roder et al., 1998; Pestsova et al., 2000).

Microsatellites are genome specific and will amplify a specific locus containing the microsatellite in the A, B, or D genome. In some cases, however, the primer pairs will amplify fragments located in two different genomes and the loci map to apparently homoeologous sites on chromosomes. The use of deletion lines allows researchers to assign the markers to defined chromosome regions (Roder et al., 1998). Microsatellites

detect a higher level of polymorphism and informativeness in hexaploid wheat than any other marker system because they target highly variable regions of the genome (Roder et al., 1995; Somers 2004). However due to the large genome size, the development of microsatellite markers in wheat is extremely time consuming and expensive. Only 30% of all primer pairs developed from microsatellite sequences is functional and suitable for genetic analysis (Roder et al., 1995 and Bryan et al., 1997). Nevertheless, SSRs are an ideal type of marker for MAS because they are co-dominant and easy to score.

Microsatellite markers have been widely used because of their abundance, locus specificity, and high polymorphism to construct genetic maps, tag resistance genes (Peng et al., 1999), enable marker assisted selection in wheat (Huang et al., 2000), and assess genetic diversity in closely related collections of bread wheat accessions (Plascheke et al., 1995; Huang et al., 2002).

#### 2.2.3 Mapping populations

A number of different types of populations can be used to perform genetic mapping; however the most commonly used include:  $F_2$ , backcross (BC<sub>1</sub>F<sub>1</sub> and BC<sub>2</sub>F<sub>1</sub>), doubled haploid (DH) lines, and recombinant inbred lines (RILs) (Mohan et al., 1997; Michelmore et al., 1991; Young 1996; Paterson 1991). The mapping population selected may be dependent upon the use of a dominant or co-dominant marker type (Mohan et al., 1997; Gupta et al., 1996).

Doubled haploid (DH) lines and recombinant inbred lines (RILs) are homozygous at all or the majority of all gene loci (Burr et al., 1988). Self pollination of these lines will generate offspring that are genetically identical or nearly identical to the parent. The homozygosity of the DH lines and RILs means dominant and co-dominant markers provide the same amount of genetic information in these populations (Poehlman and Sleper 1995). These lines can be tested for an unlimited number of traits in an unlimited number of environments. Also, a more accurate assessment of the genetic component of variance can be made for quantitative traits because a genotype is represented by a line instead of a single individual (Burr et al., 1988). DH lines can be developed quickly but are labour intensive to produce (Poehlman and Sleper 1995). The development of RILs is not as labour intensive but requires multiple cycles of self pollination. RILs have more recombination events than DH lines because of the additional meioses in RIL development (Burr et al., 1988). Therefore, RILs produce genetic maps of higher resolution than a similar sized DH population. In general, DH populations are favored in species where efficient protocols for DH line development are available because of their rapid advance to homozygosity.

#### 2.2.4 Genetic maps

Molecular markers have permitted a number of genetic maps to be developed for economically important plants. The value of genetic maps has steadily increased since they were first introduced in the 1980s. Wheat molecular genetic maps first comprised RFLP markers (Chao et al., 1989; Devos and Gale 1993; Devos and Gale 1997) and over time, PCR-based markers became the dominant marker type for genetic map construction, including RAPDs (Williams et al., 1990), AFLPs (Vos et al., 1995), and microsatellites (SSRs) (Röder et al., 1998; Pestsova et al., 2000; Gupta et al., 2002). The primary reason to shift toward PCR-based markers and particularly SSR marker maps is the potential to use the maps in plant breeding (Gupta and Varshney 2000). Conventional plant breeding requires the analysis of thousands of plants in a short time period at low cost. Microsatellite markers and high-throughput capillary electrophoresis are good platforms upon which to implement marker-assisted selection (MAS) in breeding programs (Paterson 1991; Gupta et al., 1999; Mohan et al., 1997).

Molecular breeding is more effective if the molecular map is densely populated with markers (Röder et al., 1998). This provides molecular breeding strategies with more choice in the quality of markers and a higher probability of identifying polymorphic markers in an important chromosome interval. The first microsatellite map in wheat possessed 279 microsatellites (Röder et al., 1998). This marker density is useful for QTL and gene mapping, but is limiting for the precise transfer of QTLs between different genetic backgrounds. Specifically, the limitation comes from the lack of polymorphic markers immediately flanking QTLs. Somers et al.. (2004) developed a wheat genetic consensus map of 1,235 microsatellite marker loci covering 2,569 cM of 21 chromosomes, giving an average interval distance of 2.2 cM (Somers et al., 2004). The microsatellite consensus map is a good estimation of the marker position from four genetic maps, assembled by consensus onto a single linkage map. The consensus map represents the highest-density public microsatellite map of wheat and is accompanied by an allele database showing the parent allele sizes for every marker mapped. The primary use of the consensus map is in molecular mapping of traits and plant breeding.

Wheat genomics research is increasing the use of genetic maps, particularly in mapbased gene cloning efforts. Map-based cloning requires an accurate, fine genetic map to correctly position a gene of interest between close flanking markers (Peters et al., 2003).

- 16 -

Beginning with a robust, high-density map, the efforts to add more markers for fine mapping are greatly improved and narrows the number of possible bacterial artificial clones (BACs) harbouring the gene.

#### 2.2.5 Marker assisted selection (MAS)

Marker assisted selection (MAS) is the use of markers linked to genes of interest to indirectly select for these genes in segregating populations (Mohan et al., 1997; Gupta et al.. 1999; Somers 2004). MAS requires markers tightly linked with the desired gene, an efficient means of isolating DNA from large breeding populations, and a marker screening technique that is reproducible, efficient, economical, and user-friendly. Molecular markers are especially useful for traits that are difficult and/or expensive to evaluate, traits with low heritability, and traits that are highly influenced by the environment. This would include resistance to pathogens, tolerance to abiotic stresses, quality traits, and quantitative traits (Mohan et al., 1997). Molecular markers are independent of the environment and with appropriate markers and genetic variation, genetic gain can be made for many traits such as drought tolerance, freezing tolerance, heat tolerance, and disease resistance, without exposing genotypes to the environmental stresses (Mohan et al., 1997). For disease resistance, MAS can be used as a complementary method for selecting linked resistance genes, and gene pyramiding (Young 1996). Identification of disease resistance is conducted in the field, greenhouse, and laboratory. Some disease screens are difficult to conduct in the field because of the variability in aggressiveness or availability of the pathogen, or sensitivity of the disease reaction to environmental conditions. Some disease screens are time-consuming or can be conducted only at particular locations, times of the

- 17 -

year, or stages of plant development (Varshney et al., 1998). However, it is not necessary to test the resistance reaction by inoculating with a pathogen or evaluating the resistance reaction in marker assisted selection. This can be accomplished much later in the selection process and with a reduced population size. MAS is used for the pyramiding of major genes, the combination of major and minor genes, not only for one disease but for multiple diseases (Varshney et al., 1998).

#### 2.3 Fusarium head blight

Fusarium head blight (FHB) is currently the most serious disease affecting the grain industry in Canada (Gilbert and Tekauz 2000; Tekauz et al., 2000). Large annual economic losses averaging \$50 million have occurred since the FHB epidemic of 1993 in Manitoba (Gilbert and Tekauz 2000). Fusarium graminearum Schwabe [teleomorph Gibberella zeae (Schwein.) Petch] (F.graminearum) is the predominant species causing FHB in North America (Wilcoxson et al., 1988; McMullen et al., 1997). Although there are differences in aggressiveness among isolates (Dusabenyagasani et al., 1997), there is no evidence of host-isolate specificity in populations of this pathogen (Wang and Miller 1988; Van Eeuwijk et al. 1995). The species involved in FHB are facultative parasites capable of infecting all plant parts. Fusarium is found on a wide range of hosts including wheat, barley, oat, corn, rye, and wild grass species. Infection of the crown and root tissues may often coincide with head blight. Yield losses are due to floret sterility and poor seed filling; seed germination is also impaired. Infection by Fusarium graminearum, Fusarium avenaceum, and Fusarium culmorum alters milling and baking qualities, primarily by digestion of endosperm proteins. As a result, both domestic and export markets have low

tolerances for fusarium damaged kernels (FDK) in grain and their presence may result in grade reductions. Fusarium species produce many different mycotoxins (Leonard and Bushnell, 2003). Fusarium graminearum and Fusarium culmorum produce deoxynivalenol (DON, vomitoxin), one of the many toxins produced by Fusarium species that renders grain unfit for human food or animal feed. Food and Agriculture Organization of the United Nations Codex Alimentarius (2002) recommends Canada has a maximum level of 5,000  $\mu$ g/kg for DON in feeding stuffs for cattle and poultry and a maximum of 1,000  $\mu$ g/kg for DON in feeding stuffs for swine, young calves and lactating dairy animals. The CODEX code also has maximum guideline for human food. Canada has a guideline level of 2000  $\mu$ g/kg of DON in unclean soft wheat, corresponding to 1200  $\mu$ g/kg in the flour portion (for the manufacture of non-staple foods such as cakes, cookies, biscuits). With respect to unclean soft wheat intended for use in infant foods, the guideline is 1000  $\mu$ g/kg corresponding to  $600 \mu g/kg$  in the flour portion. Since levels of DON in hard wheat, the major Canadian wheat, are sometimes high, no guidelines have been established for DON for this type of wheat, nor other grains.

#### 2.3.1 Symptoms

The first visible symptoms or signs of infection by *F. graminearum* usually occur on the first florets to flower, generally near the middle of the spike. Under conditions highly favorable for infection, such as continuous wetness at 25°C, visible lesions develop within 2 to 4 days (Andersen 1948; Atanasoff 1920). Lesions may be brown, purplishbrown, or brown with a bleached centre (Bennett 1931; Tu 1930). Under moist conditions, lesions may appear more water soaked and darker olive green than the interveinal areas (Atanasoff 1920). Sites of spikelet attachment to the rachis also can become water soaked. Infection of the rachis may result in seeds above the point of infection not filling, and grain from blighted spikes may be shriveled, lightweight and chalky white or occasionally pink (FDK or tombstone kernels).

In some cases, the first indication of infection may be the fungus itself giving a pinkish tint to partially exposed anthers (Pugh et al., 1933) or chalky, slightly pinkish mycelium on spikelet surfaces (Atanasoff, 1920). In more advanced infections, the fungus can produce macroconidia in association with a pink color on margins and surfaces of florets and glumes (Atanasoff 1920; Pugh et al., 1933). Eventually, sporodochia or perithecia may be visible (Pugh et al., 1933). As lesions enlarge and coalesce, entire florets become bleached or brown in color.

Pathway of FHB infection and concomitant plant reactions are unknown and need to be studied to better understand FHB and possible control measures (Leonard and Bushnell 2003).

#### 2.3.2 Disease cycle

*Fusarium graminearum* overwinters on crop residues and produces perithecia and sporodochia that give rise to spores (wind-dispersed ascospores and rain-splashed conidia). The pathogen also survives on weeds and native plants. Infection is rare in seedlings and is generally restricted to senescing tissue around the crown. FHB develops when spores are carried by wind or rain splash from crop residue to the spikes. The disease cycle and epidemiology of FHB was reviewed by Sutton (1982) and Miller (1994) but the importance of ascospores as primary inoculum was not understood. A clearer understanding of the role

- 20 -

of ascospores in eastern Canada has emerged (Paulitz 1996, 1999). Tschanz et al., (1976) reported that UV light in the 300-320 nm range for several hours was necessary to stimulate the sexual cycle, although many isolates formed perithecia in culture without UV treatment in the laboratories at the Cereal Research Centre, Winnipeg, Manitoba. Optimum temperatures for perithecial production range from 15 to 28.5°C (Tschanz et al., 1976), and for ascospore production from 25 to 28°C (Sutton 1982). Ascospore discharge is triggered by a drop in air temperature and a rise in relative humidity (RH), and spores are released with diurnal periodicity, with peak numbers usually trapped between 16:00 and midnight (Paulitz 1996; Paulitz and Seaman 1994). While perithecial drying during the day followed by an increase in RH appears to trigger ascospore discharge, release is inhibited by more than 5 mm rain, intermittent rain, or days with continuous high (>80%) humidity. This apparent contradiction suggests that there is a threshold RH beyond which release slows or stops. Hourly spore counts ranged from 600 to 9000 ascospores m<sup>-3</sup> and release occurred over a range of temperatures (11-30°C) and RH values (60-95%). Wheat is most susceptible to infection by F. graminearum at anthesis (Sutton 1982). The timing of ascospore release has yet to be established in western Canada. Presumably ascospore release in western Canada must occur in July to coincide with flowering of the majority of the spring wheat cultivars. The optimum temperature range of 28 to 32°C for production of macroconidia is higher than that for perithecia and ascospores; macroconidial production decreases sharply at temperatures below 16°C and above 36°C (Tschanz et al., 1976). The relative importance of either ascospores or conidia in the disease cycle of FHB is unknown (Francl et al., 1999). Wind appears to play only a small role in dispersal of macroconidia (Fernando et al., 1997).

#### 2.3.3 Control

#### 2.3.3.1 Cultural control

*F. graminearum* readily survives in crop residues; therefore, cultural practices may have an effect on the development of FHB. Evidence has shown that rotations away from corn, wheat, and barley may reduce disease levels (MacInnes and Fogelman 1923; Sutton 1982; Parry et al.. 1995; Dill-Macky and Jones 1999). Following the 1993 epidemic in Manitoba, Tekauz et al.. 2000 found no significant impact of cultivar, rotation, and tillage practices on percent weight of FDK in harvested samples, although the authors acknowledged that high infection pressure may have masked possible differences. *F. graminearum* persists for unknown periods of time, mainly on the debris in no-till plots; however, Miller et al.. (1998) concluded that weather conditions are more influential than tillage practices in the development of FHB.

To reduce the potential for downgrading, current recommendations include setting the combine so that lightweight FDK are not harvested. Little is known about the ultimate fate of seed-borne *F. graminearum*, its survival in the seed, development of *G. zeae* perithecia on seed, or infestation of soil in the proximity of the seed. The role of seedborne Fusarium and its contribution to subsequent outbreaks of FHB require additional study.

#### 2.3.3.2 Chemical control

Fungicide control is a management option to reduce both DON accumulation and incidence of diseases associated with *Fusarium* spp. Many fungicides applied as seed

treatments have proven to be effective against seedling blight and reduce fungal growth sufficiently to permit successful plant establishment (Gilbert and Tekauz 1995). Foliar fungicides have given variable levels of control of FHB (Parry et al., 1995; McMullen et al., 1997).

#### 2.3.3.3 Biological control

In addition to chemical control, there is increasing interest in the use of biological agents to manage FHB. *Trichoderma* spp. are known to have a high antagonist potential against several plant pathogens, including *Fusarium* spp. and *T. harzianum* was effective in colonizing wheat straw and reducing incidence of *F. graminearum* (Fernandez 1992). Such biological control agents may be effectively used as part of an integrated pest management program, although more research is required to learn precisely what effect such inoculation of straw residues has on the new crop (Fernandez 1992).

#### 2.3.3.4 Genetic control

Genetic resistance remains the most desirable management option for FHB. Resistance to FHB in wheat is quantitative, controlled by 2 to 5 genes (Snijders 1994; Buestmayr et al., 1999; Miedaner 1997; Van Ginkel et al., 1996). The large number of putative chromosomal locations containing resistance genes is indicative of the difficulty of screening cultivars and lines for FHB resistance (Buerstmayr et al., 1999). Problems associated with greenhouse- and field-based screening for FHB resistance include dependence on the environment for symptom development, the high cost of phenotyping, and significant time and resource requirements (Yang 1994; Bai and Shaner 1994;

- 23 -

Campbell and Lipps 1998). Development of DNA marker-based screening for the presence of resistance genes may make selection for resistance more efficient in breeding programs (Bai et al., 1999; Kolb et al., 2001).

Five mechanisms of resistance to FHB have been proposed: type I is resistance to initial infection as manifested in the cultivar Frontana; type II prevents spread within the spike following infection which is the form of resistance expressed by the Chinese resistant varieties such as Sumai 3; type III is resistance to kernel infection; type IV is tolerance whereby yields are maintained despite the presence of disease; and type V is ability of hosts to degrade toxins (Mesterhazy 1995). Type II is most reliably assessed under controlled conditions. Inoculum is introduced directly into a floret, either using a pipette or inserting a small piece of cotton soaked in a spore suspension. Disease symptoms are restricted to the inoculated florets in resistant varieties but spread up and down the spike in susceptible lines. Macroconidial spray inoculation methods are used to screen lines for type I resistance. Wheat lines that incorporate both type I and II qualities, resist both initial infection and spread within the spike are being employed in Canadian breeding programs. Rates of seed infection can differ at a given level of resistance as measured by disease severity with Type III resistance (Mesterhazy 1995). Tolerance against FHB has been established in the past decade (Mesterhazy 1989; Mesterhazy 1995). Tolerant wheat maintains yield despite the presence of disease. Tolerance is independent from the other traits (it can be found at different resistance and susceptibility levels). Type V resistance to toxins can be the result of toxin decomposition by the plant, plant tolerance (insensitivity) to toxin, or limitation in the amount of toxin that accumulates in spike tissues. Reduction in DON concentrations in maturing heads suggests that the plant can degrade toxin (Miller
and Young 1985). Resistance types III, IV, and V are more difficult to manipulate and/or more expensive to screen and are not being used in current breeding programs.

Molecular mapping and marker assisted selection (MAS) are innovative tools that have been used in research programs to aid in the manipulation and pyramiding of several resistance genes in a short period of time. Molecular markers have been linked to QTLs associated with various types of FHB resistance, particularly in Sumai 3 (Bai et al., 1999, Anderson et al., 2001, Buerstmayr et al., 2002; Yang et al., 2003; Yang et al., 2005; Zhou et al., 2002). Other sources of FHB resistance include: Ning derivatives, Wangshuibai, Frontana, Wuhan and Nyubai (Zhou et al., 2002; Buerstmayr et al., 2002, Somers et al., 2003; Steiner et al., 2004; Han et al., 2005).

Extensive efforts have been made to map QTLs for FHB resistance in the Chinese wheat cultivar Sumai 3, a source of FHB resistance which has been used successfully worldwide. A major QTL designated *Qfhs.ndsu-3BS* was originally mapped by RFLP analysis (Waldron et al., 1999) and verified later by several research groups (Bai et al., 1999, Anderson et al., 2001; Zhou et al., 2002; Somers et al., 2003; Yang et al., 2003; Yang et al., 2005). *Qfhs.ndsu-3BS* is located on chromosome 3BS between microsatellite markers GWM493 and GWM533 (Anderson et al., 2001). Liu and Anderson (2003) increased the marker density in this chromosome region using sequence-tagged site (STS) markers developed from wheat ESTs near *Qfhs.ndsu-3BS*, which facilitated the fine mapping of the resistance gene.

Exploitation of molecular markers associated with FHB resistance genes has focused on Type II FHB resistance (Bai et al., 1999; Waldron et al., 1999; Anderson et al., 2001; Buestmayr et al., 2002; Zhou et al., 2002; Yang et al., 2003). Previous QTL

- 25 -

mapping studies have revealed major Sumai 3-derived Type II FHB resistant QTLs on chromosomes 3BS (Waldron et al., 1999; Anderson et al., 2001; Buerstmayr et al., 2002; Somers et al.. 2003; Zhou et al.. 2002; Yang et al.. 2003), 5A (Ban and Suenaga 1998; Xu et al. 2001; Buesrstmayr et al. 2002) and 6B (Anderson et al. 2001; Yang et al. 2003) using segregating populations. These studies seemed to show pronounced epistasis and genotype by environment (G x E) interactions affecting type II FHB resistance. Prominent QTLs with main effects such as the QTL on chromosome 6B were only occasionally detected in QTL mapping studies. The most prominent and consistent 3BS QTL detected in different mapping populations segregating for "Sumai 3-derived" resistance genes still had significant GxE effects, explaining 15 to 60% of the phenotypic variation for type II resistance in different replicates or test environments. To date, there is little evidence for QTL associated with other mechanisms of resistance (Xu et al., 2001; Somers et al., 2003). There is a strong need to identify beneficial genes and alleles for all types of resistance and to develop superior wheat cultivars by introgressing and pyramiding a full complement of FHB resistance genes.

### 2.4 Wheat leaf rust (brown rust)

Wheat leaf rust is a major disease of wheat and is caused by the basidiomycete *Puccinia triticina* Eriks. (Div. Amastigomycota, Class Basidiomycetes, Subclass Teliomycetidae, Order Uredinales, Family Pucciniaceae) (Bold et al., 1997) (synonym *P. recondita* Rob. Ex Desmaz. f.sp. *tritici*). Leaf rust is an obligate parasite and is macrocyclic which can result in a large economic impact under ideal environmental conditions. In the eastern prairies of Canada, yield losses are frequently 5 to 15% when

susceptible cultivars are grown, but can exceed that amount if environmental conditions permit (Samborski 1985). In 1999, the Canadian prairies experienced one of the most severe years of infection in the past 20 years with yield losses of 5 to 20% (McCallum et al.. 2000). This increase in severity was caused by environmental conditions, large amounts of inoculum from the United States, and the leaf rust susceptibility of the cultivar grown on the largest acreage in western Canada.

There are many distinct strains of leaf rust known as virulence phenotypes or physiologic races. Wheat cultivars vary in their resistance to the different phenotypes. A new cultivar may be classified as resistant when it is first released, but with each year as it is more widely grown; virulence phenotypes that can attack it become more prevalent, until the cultivar is reclassified as susceptible. Host resistance is an effective control measure that has been used in numerous breeding programs (Kolmer 1998). However, a gene-forgene interaction exists between host resistance genes and pathogen avirulence genes in the wheat-*P*:*triticina* pathosystem (Samborski and Dyck 1968).

Annual surveys of physiologic specialization in *P. triticina* in Canada have been conducted at the Cereal Research Centre in Winnipeg since 1931 (Kolmer 1998). The continual surveys have generated a unique database to examine selection and evolution in a plant pathogen population over a comparatively long period of time and in a large geographic area. A system of nomenclature using a set of 12 near isogenic lines (NILs) was reported by Long and Kolmer (1989). Races, or virulence phenotypes, are defined by their differential virulence on the NILs carrying single leaf rust resistance (Lr) genes, and are assigned a three letter code. Virulence phenotypes evolve by change in their virulence

on different Lr genes. This occurs mainly by mutation, and asexual recombination may play a minor role (Samborski 1985).

### 2.4.1 Host-pathogen interaction

The co-existence of host plants and the pathogen in nature indicates that the two have evolved together over time. Changes in virulence of pathogens appear to be continually balanced by changes in the resistance of the host, and vice versa. Therefore, a dynamic equilibrium of resistance and virulence is maintained, and both host and pathogen survive over considerable periods of time.

The gene-for-gene concept was first demonstrated using flax (*Linum usitatissimum* L.) and flax rust (*Melamspora lini* Desm.) (Flor 1956). There are other examples which include other rusts, smuts, powdery mildew, apple scab, late blight of potatoes, and other diseases causes by fungi, bacteria, viruses, parasitic higher plants, nematodes, and insects (Agrios 1997).

The gene-for-gene theory asserts that for avirulence genes in the pathogen, there are corresponding resistance genes in the host. An incompatible interaction (failure to infect) occurs if any corresponding set of avirulence and resistance genes interact between the pathogen and host. The host is then said to be resistant. This interaction can be visualized using the quadratic check (Agrios 1997).

The gene-for-gene model holds true most of the time for the wheat-*P.triticina* hostpathosystem (Samborski and Dyck 1968, 1976; Dyck and Samborski 1970). However, the genetic states (homozygosity or heterozygosity) of resistance and avirulence genes affect the interaction in this system (Kolmer and Dyck 1994). Infection type may vary with

- 28 -

zygosity of the resistance and/or avirulence genes. Therefore, some of these genes will display incomplete dominance. In fact, compatible reactions were seen in some cases where both the resistance gene and avirulence gene were putatively heterozygous. Kolmer and Dyck (1994) suggest a three-by-three table that accounts for the effects that heterozygosity can have on the host-pathogen interaction and is more complete than the quadratic check. The expression of resistance genes can be altered as a result of background effects, suppressors of resistance genes, temperature effects and the complementation and gene interactions that occur with some of the Lr genes.

### 2.4.2 Symptoms

Leaf rust pustules are small, oval fruiting bodies (uredia) of the rust fungus (*Puccinia triticina* Eriks.) (Kolmer 1998; Bailey et al.. 2003; Agrios 1997; Anikster et al.. 1997; Singh 1992a). Reddish-orange urediniospores (summer spores) develop within the uredia and rupture the epidermis of the leaf as the spores mature (Bailey et al.. 2003; Agrios 1997). Pustules can be either scattered or clustered on the leaves and leaf sheaths of infected plants. Each pustule contains thousands of urediniospores which are disseminated by the wind. Rust pustules can be distinguished from other leaf spot diseases because rust pustules will smear on the leaf surface when rubbed (Bailey et al.. 2003). The color is due to hundreds of orange red urediniospores within each pustule. Usually, infections occur first on the upper leaves due to spores that have been deposited out of the air during spore showers. The interior of the leaf is infected via the stomata. In the cavity beneath the stomata, the tip of the penetration hypha dilates. From this vesicle new hypha emerge, forming mycelium that grows between the plant's cells. Haustoria are formed to feed on

- 29 -

host cells. Leaf rust differs from stem rust in that it does not usually infect the peduncle (Anikster et al., 1997). During favorable weather for rust development, pustules increase in number until 30 to 50 percent of the leaf surface is covered. As the plant matures, black, submerged pustules develop on the leaves and leaf sheaths (Bailey et al., 2003; Anikster et al., 1997; Samborski 1985). These pustules (telia) contain the winter spores (teliospores) (Bailey et al., 2003). Teliospores germinate to produce basidiospores which infect meadow rue (*Thalictrum speciosimum*). Telia may not develop when plants become infected near maturity.

#### 2.4.3 Disease cycle

*P. triticina* is an obligate parasite and macrocyclic (Kolmer 1998). The alternate host of P. *triticina* is meadow rue *Thalictrum speciosissimum* L. (Anikster et al., 1997). Wheat leaves are usually infected by urediospores and rarely by aeciospores. The sexual cycle does not occur frequently in nature, and therefore does not play an important role in the epidemiology, or in the origin of new races (Samborski 1985). In North America, the persistence of leaf rust from year to year is due to the overwintering of the uredinial stage on wheat in the southern United States and Mexico (Kolmer 1998; Kolmer and Liu 2001). The disease progresses northward each spring, producing cycles of urediniospores on sequentially maturing wheat crops. In western Canada, the first leaf rust infections of the growing season are usually observed in June, and the disease reaches a peak in August. The teliospores survive over winter in Canada on straw and germinate in the spring. Each teliospore produces four basidiospores. These spores cannot infect wheat but can infect other plants, such as species of meadow rue (*Thalictrum*) that grow in Europe or Asia

(Anikster et al., 1997; Samborski 1985). Acciospores formed on meadow rue can, in turn, infect wheat to produce urediniospores. The species of *Thalictrum* that are native to North America are highly resistant to leaf rust and do not contribute to its spread.

#### 2.4.4 Control

Leaf rust can be effectively controlled by the following registered foliar fungicides for spring wheat: Tilt<sup>®</sup>, Folicur<sup>®</sup>, Dithane DG<sup>®</sup>, Bravo<sup>®</sup>, Bumper 418 EC<sup>®</sup>, Headline EC<sup>®</sup>, Manzate DF<sup>®</sup>, Pennocozeb 75 DF<sup>®</sup>, Pivot<sup>®</sup>, Stratego 250EC<sup>®</sup> (Crop Protection Guide 2007). Early seeding may also help to reduce disease severity as losses are greatest on lateseeded crops. However, the most efficient, cost-effective, and environmentally friendly approach to prevent the losses caused by rust epidemics is through host genetic resistance. The use of cultivars with single-gene resistance permits the selection of mutations in the pathogen at a single locus and can render the resistance ineffective in a relatively short period of time. Due to selection pressure and evolution, new virulent races of the fungus appear which increase the need to develop durable resistance. Hence the use of combinations of genes irrespective of whether they are major or minor has been suggested as the best method for genetic control of leaf rust (Roelfs 1988). This can be achieved by pyramiding effective resistance genes, but it is difficult to monitor the expression of individual resistance genes against the background of other resistance genes. With the advent of molecular marker technology it is now possible to tackle such complex problems.

To date, more than 50 leaf rust resistance genes have been characterized (Schnurbusch et al.. 2003); Knott 1989; McIntosh et al.. 1995). Most of the genes are effective from the seedling stage through the whole life of the plant whereas a few of them

are only effective at the adult stage. Resistance during this latter period is called adult plant resistance (APR) and was defined by Zadoks (1961) as a resistance that is effective in the advanced plant growth stage but not at the seedling stage. An attack of the pathogen during heading (adult plant stage) can cause severe grain yield losses due to reduced floret set (Roelfs et al., 1992). Therefore, resistance at the adult stage is of considerable economic significance in wheat breeding. Seven genes which are only effective at the adult plant stage have been described (McIntosh et al., 1995). Although these genes are inherited in a monogenic fashion the type of resistance differs between these adult resistance genes.

### 2.4.4.1 *Lr34*

The adult plant resistance gene Lr34 expresses resistance in a quantitative way (Drijepondt and Pretorius 1989; German and Kolmer 1992; Singh 1992a). Lr34 causes an increased latency period, fewer small to moderate-sized uredinia/leaf area, and significantly smaller sized uredinia compared to susceptible lines (Drijepondt and Pretorius 1989). The characteristics of resistance conditioned by Lr34 are typical of slow rusting or partial resistance are identical. Many wheat cultivars characterized as having slow rusting or partial resistance (Ohm and Shaner 1976) have Chinese Spring or Frontana – derived parents in their pedigrees.

To date, Lr34 is probably the most important Lr gene in terms of widespread distribution and durability. This gene has been found in a number of wheat accessions collected from diverse locations. Dyck (1977) identified Lr34 from wheat from Iran, China, Afghanistan, and Lebanon. Dyck (1994) further identified Lr34 from a number of wheats from Russia, Argentina, Tunisia, and France. Shang et al.. 1986 and Simmonds and Rajaram 1988 found Lr34 in wheats from Manchuria and India. It is remarkable that Lr34 has continued to condition an effective level of resistance despite being in cultivars that have been extensively grown for extended periods of time in many wheat growing areas of the world. There is no clear explanation for the longevity of Lr34's effectiveness. Wheat cultivars with Lr34 have maintained effective levels of resistance in all regions despite the large number of yearly uredinial generations that should give ample opportunity for isolates with virulence to this gene to increase within the *P. triticina* population.

Lr34 is tightly linked with, or is pleiotropic for other traits and disease resistance in wheat. Singh (1992b) reported tight linkage of leaf-tip necrosis (Ltn) in flag leaves with Lr34 in several different lines. Leaf tip necrosis, however, is influenced by environmental effects and genetic background and can be too variable to be considered a reliable marker. Lr34 is also linked to the stripe rust resistance gene (P. striiformis) (McIntosh 1992, Singh 1992b), and resistance barley yellow dwarf virus (BYDV) (Singh 1993). Selection for Lr34 would also select resistance to both stripe rust and BYDV. Lr34 also contributes to stem rust (P. graminis) resistance in North American hard red spring wheat. Dyck (1993) showed that Lr34 segregated with higher stem rust resistance in crosses with the cultivar Roblin.

Lr34 may also be present at more than one location in the wheat genome. Thatcher line RL6077 has leaf rust resistance, stripe rust resistance (Singh 1992a), and leaf tip necrosis (Singh 1992a) similar to Thatcher line RL 6058 and other lines with Lr34. Dyck (1987) suggested that RL 6077 probably has Lr34. RL 6077 and RL 6058 were intercrossed, and F<sub>3</sub> progeny lines segregated in a two-gene ratio for resistance. The stem and leaf rust resistance of five of the F<sub>3</sub> lines was slightly more effective than that of either

- 33 -

of the parents (Dyck et al., 1994). It is possible that these lines are homozygous for resistance from both RL 6077 and RL 6058. Cytogenetic evidence from RL6077/RL 6058 hybrids indicated that the Lr34 gene in RL 6077 may be translocated onto another chromosome.

#### 2.4.5 Mapping leaf rust resistance genes

The development of molecular markers has permitted the location of various wheat genes, including Lr genes, to be mapped with increased precision. Molecular markers including RAPD, RFLP, and SSR markers linked to race-specific resistance genes have been identified in wheat and can be used in marker-assisted selection. Three RAPD and one RFLP marker showed complete linkage to leaf rust resistance gene Lr9 (Schachermyr et al., 1994). Robert et al., (1999) identified one RAPD and one RFLP marker that are closely linked to the stripe rust resistance gene Yr17. More recently, SSR markers have been developed (Pestsova et al., 2000; Roder et al., 1998; Song et al., 2002; Stephenson et al., 1998) and utilized in the tagging of rust resistance genes such as Lr16 (McCartney et al., 2005), Lr39 (Raupp et al., 2001), and YrH52 (Peng et al., 1999).

Genes Lr34 and Yr18 confer slow rust resistance to leaf and stripe rust, respectively, and are known to be pleiotropic or completely linked to each other (McIntosh 1992; Singh 1992a). Although genes Lr34 and Yr18 may not provide adequate resistance in some environments under high disease pressure when present alone (Ma and Singh 1996, Singh and Gupta 1992) they could contribute to achieving acceptable levels of resistance in combination with other slow rusting genes (Singh et al.. 2001; Singh and Rajaram 1991). Leaf tip necrosis (Ltn), a morphological trait, shows complete linkage or pleiotropism with

Lr34 and Yr18 (Singh 1992) and could be used in some environments as a marker to identify wheat lines carrying these genes. Since slow rusting resistance is quantitatively inherited, quantitative trait loci (QTL) analysis has been applied to map genes conferring resistance to Lr34 and Yr18 and other resistance genes effective at the adult plant stage have been mapped by QTL analysis using molecular markers (Bariana et al., 2001; Boukhatem et al.. 2002; Messamer et al.. 2000; Nelson et al.. 1995; Singh et al.. 2000). The QTL for the gene complex Lr34/Yr18 has been mapped to chromosome 7D and positioned within confidence intervals for QTLs delineated by molecular markers (Singh et al.. 2000; Suenaga et al.. 2003; Schnurbusch et al.. 2004). Spielmeyer et al.. (2005) confirmed Lr34 and Yr18 fine mapped to a single locus flanked by microsatellite markers Xgwm295 and Xgwm1220. In this study, both genes were separated by two recombinants on the distal side from Xgwm1220 (0.9 cM) and by six recombinants on the proximal side from Xgwm295 (2.7 cM). Spielmeyer et al. (2005) also found that adult plant resistance to powdery mildew co-segregated with both rust resistance genes. Results obtained from several studies indicate that the genomic region linked to microsatellite markers Xgwm1220 and Xgwm295 on chromosome 7DS confers: durable adult plant resistance to leaf rust (Lr34) (Singh and Gupta 1991); durable adult plant resistance to stripe rust (Yr18)(McIntosh 1992; Singh 1992); adult plant resistance to powdery mildew (Spielmeyer et al.. 2005); tolerance to barley yellow dwarf virus (*Bdv1*) (Singh 1993); enhanced expression of stem rust resistance (Dyck 1987); and leaf-tip necrosis of flag leaves (*Ltn*) (Singh 1992b).

All resistance specificities located within this 7DS region are expressed during the adult plant stage, while at least some are associated with a slow increase of disease infections. Rust resistance genes Lr34 and Yr18 and possibly the gene for BYDV (Bdv1)

have played an important role in providing durable disease resistance in a wide range of CIMMYT-generated spring wheat and also in many wheat in the USA and Canada (Kolmer 1998). Quantitative leaf rust resistance has also been mapped in European winter wheat to the Lr34/Yr18 region on chromosome 7DS, indicating that these resistance genes may also be prevalent in European winter wheat gene pool (Messmer et al.. 2006; Schnurbusch et al.. 2004). The study completed by Spielmeyer et al.. (2005) links the genomic region of Lr34/Yr18 with adult plant resistance to powdery mildew, further adding to the value of this region as a source of valuable and durable disease resistance in wheat.

Successful tagging of these adult plant resistance genes with molecular markers should not only help to identify their presence in wheat cultivars but also to pyramid the most desirable gene combinations in new cultivars. Identifying markers associated with adult plant resistance genes could also provide the basis for saturating these important chromosomal regions with more markers, thereby facilitating fine mapping of the regions and cloning of these useful genes, which have shown durability.

Map-based cloning is a universal strategy to clone genes that have been finely mapped. However, gene cloning based on fine genetic and physical mapping must deal with a large genome size and a high ratio of physical to genetic distance resulting from low levels of recombination or polymorphism in wheat. To date, there have been three leaf rust resistance genes Lr1, Lr21, and Lr10 that researchers have successfully cloned (Huang et al. 2003; Cloutier et al. 2005 and Feuillet et al. 1997). The cloned genes have not been explored extensively in terms of manipulating the sequence to determine what portion of the sequence conditions specificity and whether it is possible to create new specificity from changes in the cloned gene. However, the successful map-based cloning approach as

- 36 -

demonstrated now opens the door for cloning of many crop-specific agronomic and other disease resistance traits located in the gene-rich regions of bread wheat.

# 3.0 FINE MAPPING *FHB1*, A MAJOR GENE CONTROLLING FUSARIUM HEAD BLIGHT RESISTANCE IN BREAD WHEAT (*TRITICUM AESTIVUM* L.)

## 3.1 Abstract

A major Fusarium head blight (FHB) resistance gene *Fhb1* (syn. *Ofhs.ndsu-3BS*) was fine mapped on the distal segment of chromosome 3BS of spring wheat (Triticum aestivum L.) as a Mendelian factor. FHB resistant parents, Sumai 3 and Nyubai, were used as sources of this gene. Two mapping populations were developed to facilitate segregation of *Qfhs.ndsu*-3BS in either a fixed resistant (Thatcher/5\*Sumai 3) (T/S) or fixed susceptible (HC374/3\*98B69-L47) (HC/98) genetic background (HC374=Wuhan1/Nyubai) for Type II resistance. Type II resistance (disease spread within the spike) was phenotyped in the greenhouse using single floret injections with a mixture of macro-conidia of three virulent strains of Fusarium graminearum. Due to the limited heterogeneity in the genetic background of the crosses and based on the spread of infection, fixed recombinants in the interval between molecular markers XGWM533 and XGWM493 on 3BS could be assigned to discrete "resistant" and "susceptible" classes. The phenotypic distribution was bimodal with progeny clearly resembling either the resistant or susceptible parent. Marker order for the two maps was identical with the exception of marker STS-3BS 142, which was not polymorphic in the HC/98 population. The major gene *Fhb1* was successfully fine mapped on chromosome 3BS in the same location in the two populations within a 1.27 cM interval (T/S) and a 6.05 cM interval (HC/98). Fine mapping of *Fhb1* in wheat provides tightly linked markers that can reduce linkage drag associated with marker-assisted selection of

*Fhb1* and assist in the isolation, sequencing and functional identification of the underlying resistance gene.

# **3.2 Introduction**

Fusarium head blight (FHB), also known as scab, is a disease of small grain cereals and is caused by several species of *Fusarium*. *Fusarium graminearum* Schw. (Giberella zeae Schw. & Petch) is the Fusarium species that primarily causes FHB in wheat in eastern Saskatchewan, Manitoba, eastern Canada and the United States (Gilbert and Tekauz 2000). FHB infection is favoured by warm humid conditions during flowering and early stages of kernel development (Gilbert and Tekauz 2000). This fungal disease reduces yield and grain quality through shrivelled kernels, and contaminates the grain with mycotoxins (such as deoxynivalenol) rendering it unsuitable for food or feed (Gilbert and Tekauz 2000).

Several types of resistance have been identified in spring wheat (Schroeder and Christensen 1963). Resistance to initial infection (Type I) is assessed as the incidence of infection in the presence of natural or augmented inoculum (e.g., spray inoculations). Resistance to spread within the spike (Type II) is assessed as the spread of infection within the spike following single floret injections (SFI). Other types of resistance are not as well established. Disproportionate reductions in the accumulation of DON (i.e., statistically uncorrelated with Type I or II resistance) are described as Type III resistance (Mesterhazy 1995).

FHB resistance is polygenic (Bai and Shaner 1994) and expression of resistance is highly influenced by the environment. Together, these factors make it very challenging to reproduce phenotypic results. Therefore, researchers have attempted to perform genetic analysis of FHB resistance through chromosomal manipulation (e.g. intervarietal substitutions) or through the mapping of quantitative trait loci based on high-density genetic maps.

Molecular mapping and marker assisted selection (MAS) are innovative tools that have been used in research programs to aid in the manipulation and pyramiding of several resistance genes in a short period of time. Molecular markers have been linked to QTLs associated with various types of FHB resistance, particularly in Sumai 3 (Bai et al., 1999, Anderson et al., 2001, Buerstmayr et al., 2002; Yang et al., 2003; Yang et al., 2005; Zhou et al., 2002). Other sources of FHB resistance include: Ning derivatives, Wangshuibai, Frontana, Wuhan and Nyubai (Zhou et al., 2002; Buerstmayr et al., 2002, Somers et al., 2003; Steiner et al., 2004; Han et al., 2005). The most prominent QTL for FHB resistance have been associated with a specific type of resistance: Type II resistance on chromosome 3BS (Waldron et al., 1999; Anderson et al., 2001; Bai et al., 1999; Ban et al., 2000), and 6B (Anderson et al., 2001; Yang et al., 2003); Type 1 resistance on 3A (Steiner et al., 2004), and 5A (Buerstmayr et al., 2002).

Extensive efforts have been made to map QTL for FHB resistance in the Chinese wheat cultivar Sumai 3, a source of FHB resistance which has been used successfully worldwide. A major QTL designated *Qfhs.ndsu-3BS* was originally mapped by RFLP analysis (Waldron et al., 1999) and verified later by several research groups (Bai et al., 1999, Anderson et al., 2001; Zhou et al., 2002; Somers et al., 2003; Yang et al., 2003; Yang et al., 2005). *Qfhs.ndsu-3BS* is located on chromosome 3BS between microsatellite markers GWM493 and GWM533 (Anderson et al., 2001). Liu and Anderson (2003) increased the marker density in this chromosome region using sequence-tagged site (STS)

markers developed from wheat ESTs near *Qfhs.ndsu-3BS*, which facilitated the fine mapping of the resistance gene.

To determine the map location of individual genes controlling quantitative traits, substitution lines or near-isogenic lines (NILs) can be developed to isolate the gene of interest as a Mendelian factor. The objective of this research was to isolate and fine map *Fhb1* (*Qfhs.ndsu-3BS*) using sequence-tagged sites (STS) markers in both a fixed resistant (Sumai 3) and fixed susceptible (98B69-L47) genetic background.

#### **3.3 Materials and methods**

### **3.3.1 Mapping populations**

Two large fine mapping populations were developed at Agriculture and Agri-Food Canada containing different sources of type II FHB resistance, Sumai 3 and HC374 (Wuhan1/Nyubai). The pedigrees of the two mapping populations (Fig. 3.1) were (Thatcher/5\*Sumai 3) and (HC374/3\*98B69-L47) where 98B69-L47 = Augusta/HWAlpha//3\*Superb. The populations are identified in this study as (T/S) and (HC/98), respectively.

The T/S mapping population was developed using a backcrossing program, each time selecting the BCF<sub>1</sub> plants that were heterozygous for microsatellite markers GWM493 and GWM533 previously identified to flank *Qfhs.ndsu.3BS* (Liu and Anderson 2003). Two BC<sub>3</sub>F<sub>1</sub> plants were pollinated by 14 Sumai 3 plants to generate 508 BC<sub>4</sub>F<sub>1</sub> seeds. A subset of 467 plants was genotyped using GWM533 and GWM493 to select recombinant plants. The BC<sub>4</sub>F<sub>1</sub> recombinant plants were self-pollinated and ten BC<sub>4</sub>F<sub>2</sub> progeny were genotyped with GWM533 and GWM493 to select fixed recombinant plants in the 3BS marker

interval, i.e., plants that showed a recombination event in the interval and were homozygous for both microsatellite markers.  $BC_4F_1$  recombinant plants were also genotyped with markers from 5A (BARC117, GWM293, GWM304, and WMC705) and 6B (BARC146, GWM508, GWM191, GWM608, GWM644, WMC397, andWMC398) to verify the plants were homozygous resistant for other reported FHB resistance QTLs for Type II resistance (Somers et al.. 2003; Yang et al.. 2003).

The HC/98 population is a derivative from an on-going molecular breeding program at Agriculture and Agri-Food Canada to introgress FHB resistance into spring wheat. Three BC<sub>2</sub>F<sub>1</sub> plants were selected to be heterozygous between markers GWM533 and GWM493 on 3BS, and homozygous susceptible for the following markers at other reported FHB resistance QTLs for Type II resistance 4B (WMC710, WMC238, GWM149), 5A (GWM154, GWM304), and 6B (WMC494, GWM644, GWM219) (Somers et al.. 2003; Yang et al.. 2003). The three plants were self-pollinated to generate a large population of approximately 2,000 BC<sub>2</sub>F<sub>2</sub> seeds. A subset of 420 BC<sub>2</sub>F<sub>2</sub> plants was genotyped using GWM533 and GWM493 to select recombinant plants. The recombinant plants were selfpollinated and ten progeny from each recombinant plant were genotyped with GWM533 and GWM493 to select fixed recombinant plants in the 3BS marker interval (Fig. 3.1).

Thirty non-recombinant, homozygous plants carrying the resistant parental allele and thirty non-recombinant, homozygous plants carrying the susceptible parental allele from both populations were selected genotypically using markers GWM533 and GWM493 and were used as checks for phenotyping.  $\downarrow \\ \mathbf{F}_1 \quad \mathbf{x} \quad \mathbf{98B69*L47}$ 

Ţ

 $BC_1F_1 = x = 98B69*L47$ Select heterozygotes from  $BC_1F_1$  for interval 3BS

using GWM493 & GWM 533

Ļ

3 BC<sub>2</sub>F<sub>1</sub>

Select heterozygotes from  $BC_2F_1$  for interval 3BS using GWM493 & GWM 533 and

homozygous susceptible at other loci 4B, 5A and 6B<sup>1</sup>

# ↓⊗

#### 2000 BC<sub>2</sub>F<sub>2</sub>

420 BC<sub>2</sub>F<sub>2</sub> plants genotyped within markers GWM533 & GWM493

79 recombinants plants identified from 420 plants<sup>2</sup>

66 of 79 recombinants used in study<sup>3</sup>

#### ↓⊗

 $66 \ BC_2F_3$ 

10 progeny from each recombinant screened

Genotyping: 40 fixed recombinants identified using GWM533 & GWM493

Phenotyping:

20 resistant and 20 susceptible

 $F_{1} \quad x \quad Sumai 3$   $\downarrow$   $BC_{1}F_{1} \quad x \quad Sumai 3$   $\downarrow$   $BC_{2}F_{1} \quad x \quad Sumai 3$   $\downarrow$   $BC_{2}F_{1} \quad x \quad Sumai 3$ Select heterozygotes from BC\_{2}F\_{1} for interval 3BS
using GWM493 & GWM 533 and  $\downarrow \otimes$   $2 BC_{3}F_{1} \quad x \quad 14 Sumai 3$   $\downarrow \otimes$   $508 BC_{4}F_{1}$ 467 of 508 BC\_{3}F\_{1} plants genotyped for interval 3BS within markers
GWM533 & GWM493 and homozygous resistant at intervals 5A and 6B.<sup>4</sup>
55 recombinants identified from 467 plants<sup>2</sup>

Thatcher / Sumai 3

51 recombinants used in study

 $\downarrow \otimes$ 51 BC<sub>4</sub>F<sub>2</sub>

10 progeny from each recombinant screened

Genotyping:

51 fixed recombinants identified using GWM533 & GWM493

Phenotyping: 30 resistant and 21 susceptible

<sup>1</sup> Three BC<sub>2</sub>F<sub>1</sub> plants were selected to be heterozygous between markers GWM533 and GWM 493 on 3BS, and homozygous susceptible for the following markers at other reported FHB resistance QTLs for Type II resistance 4B (WMC710, WMC238, GWM149), 5A (GWM154, GWM304), and 6B (WMC494, GWM644, GWM219) (Somers et al. 2003; Yang et al. 2003)

<sup>2</sup> Recombinants were selected through genotyping using two flanking markers (GWM533 and GWM493) around *Qfhs.ndsu-3BS* determined by Anderson et al. 2001.

<sup>3</sup> Thirteen plants were lost due to pests in the greenhouse prior to selection of fixed recombinants. Twenty six plants did not have complete genotyping data and resulted in the inability to select a fixed recombinant.

<sup>4</sup> BC<sub>4</sub>F<sub>1</sub> recombinant plants were genotyped with markers from 5A (BARC117, GWM293, GWM304, and WMC705) and 6B (BARC146, GWM508, GWM191, GWM608, GWM644, WMC397, and WMC398) to verify the plants were homozygous resistant for other reported FHB resistance QTLs for Type II resistance (Somers et al. 2003; Yang et al. 2003).

Figure 3.1: Development of two large mapping populations for fine mapping *Fhb1* on the distal end of chromosome 3BS in spring wheat crosses: Thatcher/5\*Sumai 3 (left) and HC374/3\*98B69-L47 (right)

#### **3.3.2 Genotyping and selection of recombinants**

The populations were germinated and leaf tissue was harvested and lyophilized for DNA extraction with the Qiagen DNeasy 96 Plant Kit (Qiagen, Mississauga, Ont.). DNA was quantified by fluorimetry using Hoechst 33258 stain. Genotypic data for the two populations was collected using M13-tailing and fluorescent capillary electrophoresis on an ABI3100 genotyper (Applied Biosystems Inc., Foster City, Calif.). M13-tailing required adding the M13 sequence (CACGACGTTGTAAAACGAC) to the 5' end of the forward primer during primer synthesis (Schuelke 2000). The PCR conditions were: 24 ng DNA, 1.5 mM MgCl<sub>2</sub>, 50 mM KCl, 0.8 mM dNTPs, 2 pmol reverse primer, 0.2 pmol forward primer, and 1.8 pmol M13 primer (CACGACGTTGTAAAACGAC) fluorescently labelled with 6-FAM, HEX, or NED (Applied Biosystems Inc.), and 0.5 U Tag DNA polymerase (Promega, Madison, Wis.). Thermal cycling included:  $94^{\circ}C - 2 \min 30$  cycles of  $95^{\circ}C - 1$ min,  $(0.5^{\circ}C/s \text{ to } 61/51^{\circ}C)$ ,  $61/51^{\circ}C - 50 \text{ sec}$ ,  $(0.5^{\circ}C/s \text{ to } 73^{\circ}C)$ ,  $73^{\circ}C - 1 \text{ min}$ , 1 cycle  $73^{\circ}C$ -5 min. The internal molecular weight standard for the ABI3100 was Genescan 500-ROX (Applied Biosystems Inc.). Data collected by fluorescent capillary electrophoresis was first converted to а gel-like image using Genographer available at http://hordeum.oscs.montana.edu/genographer.

### 3.3.3 Fusarium head blight phenotyping

Fixed recombinant, and non-recombinant resistant and susceptible check plants from each mapping population were phenotyped using single floret injection (SFI) in the greenhouse. The inoculum used throughout the phenotyping process was a mixture of three virulent strains of *Fusarium graminearum* Schwabe (EEI #20/6, EEI #23/6, and JM

- 44 -

#6) provided by Dr. Jeannie Gilbert, Agriculture and Agri-Food Canada – Cereal Research Centre. The inoculum was produced as described by Sung and Cook (1981). Recombinant lines from each mapping population were grown in the greenhouse at an average daytime temperature of 23°C and a night time temperature of 16°C. Four or five spikes per recombinant plant were inoculated when each spike reached 50% anthesis. Each spike was inoculated by injecting a 10  $\mu$ l macroconidial suspension (50,000 spores/mL) between the lemma and palea of a floret positioned at the inoculation point (Appendix 8.1). To determine the inoculation point on each spike, the total number of spikelets/per spike was multiplied by two-thirds (i.e., total number of spikelets =  $12 \times \frac{2}{3} = 8$ ). The inoculation point was the 8<sup>th</sup> and 9<sup>th</sup> spikelet from the base of the spike on opposite sides of the spike in this example (Fig. 3.2). Primary or secondary florets were inoculated but not the tertiary floret. Following point inoculation, a 10 x 5cm clear, Bitran S Series (Fisher Scientific) liquid tight specimen bag was placed over the spike to increase the humidity around the spike (Appendix 8.2). Moisture from transpiration was visible in the bag covering the spike within 30 minutes of placement over the spike. The bags were left on the spikes for an incubation period of 48 hours. Plants remained on the bench in the greenhouse and ratings were performed at 7, 14, and 21 days post-inoculation. Ratings were assessed by counting the number of infected florets directly below the inoculated florets and excluding the inoculated florets. The percentage of infected florets was averaged for each plant and plants were classified as resistant or susceptible. Resistant and susceptible classes were determined based on the bimodal distribution of ratings.

The inoculation procedure was repeated on five progeny plants derived from the original fixed recombinant plants in both populations to verify FHB ratings and classify

- 45 -

resistant and susceptible plants. Four or five spikes per progeny plant were phenotyped and the average percentage of infected florets was recorded as previously described.



Figure 3.2: Infection phenotype 21 days post-inoculation in 4 spikes from a susceptible plant (left) and 4 spikes from a resistant plant (right). A pair of florets on opposite sides of the spike (arrow heads), were injected with *F. graminearum*. The inoculation point was set at 2/3 of the distance from the base of the spike. The spike was enclosed in a polypropylene bag for 48 hours post-inoculation

## **3.3.4** Construction of the genetic map

A total of 15 markers on chromosome 3BS including STS (Liu and Anderson 2003) and microsatellite markers (Somers et al.. 2004) were screened for polymorphism between the parents of the two populations. Polymorphic marker primer sequences, annealing temperatures, and allele sizes are listed in Table 3.1. The polymorphic markers were used to genotype the fixed recombinant plants in both populations. JoinMap, version 3.0 (Biometris, Wageningen, The Netherlands, http://www.joinmap.nl) was used to determine the marker order and map distances.

# **3.4 Results**

#### 3.4.1 Genotyping

There were 79 recombinant plants identified in the HC/98 population (BC<sub>2</sub>F<sub>2</sub>) and 55 recombinant plants in the T/S population (BC<sub>4</sub>F<sub>1</sub>) using flanking markers GWM533 and GWM493. The HC/98 population was reduced from 79 to 66 recombinant plants due to pests in the greenhouse. Genotypic data was fully characterized for 51 of the 55 T/S BC<sub>4</sub>F<sub>2</sub> families and 40 of the 66 HC/98 BC<sub>2</sub>F<sub>3</sub> families (Fig. 3.1). The interval distance between GWM533 and GWM493 was 10.63 and 11.05 cM in the T/S population and HC/98 populations, respectively.

#### **3.4.2** Phenotyping

Four or five spikes/plant for fixed recombinants and non-recombinant resistant and susceptible checks were inoculated in the greenhouse using SFI to assess Type II resistance. The range in infection ratings for T/S fixed recombinants was 0-5% (resistant) and 70 to 100% (susceptible); HC/98 fixed recombinants 5-25% (resistant) and 90 to 100% (susceptible); and for non-recombinant checks 0-8% (resistant) and 83-100% (susceptible) (Fig. 3.3). There were no intermediate ratings, all inoculated spikes showed either a clear resistant or susceptible infection phenotype and thus all plants could be clearly classified (Fig. 3.2). Darkening of the inoculation point was visible by day 7, and susceptible fixed recombinant and check plants had 83 to 100% infected spikes by day 14. There was no change in infection ratings between day 14 and 21 post-inoculation. Disease development progressed basally from the inoculation point of the spike and was similar in both populations according to ratings completed 7, 14, and 21 days post-inoculation.



Figure 3.3: Phenotypic distribution of Type II resistance to FHB in fixed recombinant plants measured by single floret injection. The data was collected in a greenhouse for the two crosses Thatcher/5\*Sumai 3 (T/S) and HC374/3\*98B69-L47 (HC/98) and is represented as disease severity (% of infected florets). Resistant and susceptible parent and check lines showed infection phenotypes within the respective resistant ( $\Box$ ) and susceptible (**m**) modes of the distribution.

The T/S population segregated 30 resistant to 21 susceptible plants, fitting a 1:1 chisquare ratio (p>0.10). The HC/98 population segregated 20 resistant to 20 susceptible plants, fitting a 1:1 chi-square ratio (p>0.975). Progeny plants derived from each initial fixed recombinant showed infection ratings to be the same as the previous generation.

### 3.4.3 Genetic map

The two maps were constructed with three microsatellite and five STS markers. Marker order was identical on each map, with the exception of marker, STS3BS-142, which was not polymorphic in the HC/98 population. Allele size for three of the eight markers was two base pairs smaller in Sumai 3 than in HC374 (Table 3.1). The total map length for the two populations was 10.63 (T/S) and 11.05 cM (HC/98) (Fig. 3.4). The major gene *Fhb1* was successfully mapped to syntenic intervals in the two populations flanked by STS3B-80 and STS3B-142 (T/S) and STS3B-80 and STS3B-66 (HC/98). *Fhb1* was mapped to a 1.27 and 6.05 cM interval in the T/S and HC/98 populations, respectively (Fig. 3.4).

- 49 -

Table 3.1: Primer sequences, annealing temperatures, and allele sizes of markers used in the Thatcher/5\*Sumai 3 (T/S) and HC374\*3/98B69-L47 (HC/98) populations for fine mapping *Fhb1* in spring wheat

Marker	Forward Primer $(5' \rightarrow 3')$	Reverse Primer (5'→3')	Anneal (°C)	Allele Size Nyubai/Sumai	References
BARC133	AGCGCTCGAAAAGTCAG	GGCAGGTCCAACTCCAG	51	125/125	http://www.scabusa.org
GWM493	TTCCCATAACTAAAACCGCG	GGAACATCATTTCTGGACTTTG	61	197/195	Röder et al 1998
GWM533	AAGGCGAATCAAACGGAATA	GTTGCTTTAGGGGAAAAGCC	61	139/141	Röder et al 1998
STS3B-66	AGTCAGGCGAAGAGCGATAA	AGCACTGCACAATGAGCATC	55	192/192	Liu and Anderson 2003
STS3B-80	AGAAGAAGGAAGCCCCTCTG	GCCATGTCTTTTGTGCCTTT	55	Null/Null	Liu and Anderson 2003
STS3B-138	CAAGATCAAGAAGGCCAAGC	AGGTACACCCCGTTCTCGAT	55	355/355	Liu and Anderson 2003
STS3B-142	CGAGTACTACCTCGGCAAGC	CATAGAATGCCCCGAAACTG	50	156/156	Liu and Anderson 2003
STS3B-163	TTCATGGACGAGTACGACGA	AAGGTTGCCATTGCTCTCAC	55	470/468	Liu and Anderson 2003



Figure 3.4: Fine map of the major FHB resistance gene, *Fhb1*, in two large bread wheat populations containing different sources of FHB resistance: Sumai 3 (left) and HC374 (Wuhan1/Nyubai) (right). The crosses of the populations were Thatcher/5\*Sumai 3 (T/S) and HC374\*3/98B69-L47 (HC/98)

# **3.5 Discussion**

There are inherent difficulties associated with phenotypic characterization of FHB due to methodological problems of inoculation, the variability of the fungus, and confounding effects of the environment (Andersen 1948; Hanson et al., 1950; Scott 1927). Researchers have had great difficulty achieving reliable and reproducible FHB infection data. Reproducible phenotypic data is essential to create a reliable fine map of

QTL candidate genes. The present study successfully decreased the variability in phenotyping FHB resistance and increased the reproducibility by focusing on variables that could be managed through the development of two large mapping populations with selected fixed resistant and fixed susceptible backgrounds, use of a mixture of isolates of the pathogen, proper inoculation techniques, multiple replication and progeny testing, and a temperature monitored indoor environment.

The SFI phenotyping completed in a greenhouse during this study showed similarity and consistency of infection rating within plants and generations. The use of four or five spikes per fixed recombinant plant provided replication and allowed the plants to be classified as resistant or susceptible (Fig. 3.2). There was a high level of confidence and reproducibility in the phenotypic data based on the consistency in infection ratings of the non-recombinant resistant and susceptible checks from both populations (LSD ( $\alpha = 0.05$ ) T/S = 4.36; HC/98 = 8.02) and the similarity in infection data between the fixed recombinant plants and progeny testing.

Resistance to FHB is a complicated quantitative trait; however, the limitation of the spread of symptoms in a spike is a major component of resistance and may be controlled by a few major genes (Gu 1983; Bai and Xiao 1989; Bai et al., 1990). In this study, a coefficient of determination ( $\mathbb{R}^2$ ) was calculated for both populations with an ANOVA based on the percentage infection of the initial fixed recombinants. The  $\mathbb{R}^2$ value for both populations was 0.99 and indicated there was a major effect of *Fhb1* on infection. The major effect of *Fhb1* on Type II resistance was clearly evident in the susceptible fixed recombinant plants of the T/S population even in a fixed resistant background. Although Sumai 3 alleles were present at FHB QTLs on 5A and 6B in the

T/S population, 21 out of 51 plants homozygous for Thatcher alleles at *Fhb1* were still fully susceptible to spread of the disease (Fig. 3.3). In the HC/98 population, 98B69-L47 alleles were present at the 5A and 6B locus. Phenotypic data from the study demonstrated that 20 of the 40 recombinant plants were fully susceptible when Nyubai alleles were not present at Fhb1, and 20 of the 40 recombinant plants were fully resistant when Nyubai alleles were present. When the infection range for phenotypic data is analyzed, the degree of resistance in phenotypic data is greater in the T/S fixed recombinant plants (0-5%) versus the HC/98 fixed recombinant plants (5-25%) and nonrecombinant resistant check plants (0-8%) (Fig. 3.3). These data from both populations suggests that *Fhb1* is functionally essential to provide Type II resistance and that *Fhb1* is an additive gene relative to other resistance loci. For example, Yang et al. (2003) detected strong Type II resistance derived from the 6B locus of Sumai 3 ( $R^2=0.21$ ) in a spring wheat cross (HY368/Sumai3//AC Foremost), yet this level of Type II resistance is not apparent in the present study when the Sumai 3 alleles of *Fhb1* are substituted for Thatcher alleles.

This study provided a novel approach to fine map the gene *Fhb1* from two different sources of FHB resistance (Sumai 3 and Nyubai) in two independent, large populations. The Sumai 3 and Nyubai sources of resistance had different allele sizes for three of the eight markers suggesting the sources of resistance present in the populations were genetically diverse. The two sources of FHB resistance mapped to syntenic marker intervals in independent populations (Fig. 3.4). Identification of original recombinant plants showed the interval distance between the two flanking markers GWM493 and GWM533 was 10.63 and 11.05 cM in the T/S and HC/98 populations, respectively.

Comparative mapping between the present populations and Liu and Anderson (2003) showed the same marker order; however, the GWM493 to GWM533 distance was 6.8 map units based on results from Liu et al.. (2003). The difference in genetic distance between GWM493 and GWM533 could be due to the difference in population size and structure. The increased recombination surrounding *Fhb1* in the HC/98 population compared to the T/S population could be due to population size, genetics of the two different FHB resistance sources of *Fhb1*, and/or the difference between the generations that were phenotyped and genotyped in this study.

The interval distance surrounding *Fhb1* indicated there was a higher recombination frequency in the HC/98 population compared to the T/S population immediately distal to *Fhb1* (Fig. 3.4). Comparisons of physical and genetic maps of wheat indicate that most genetic recombination occurs in gene-rich regions (Gill et al., 1996; Faris et al., 2000). In the study by Liu and Anderson (2003), QTL analysis of chromosome 3BS for FHB resistance in the Sumai 3 x Stoa population was completed using two greenhouse FHB evaluations. The present results indicate the *Fhb1* gene maps to a similar position as the peak of *Qfhs.ndsu-3BS* identified by Liu and Anderson (2003). However, Liu and Anderson (2003) indicate due to the small population size used, the exact order of markers and precise QTL position could not be determined.

In summary, *Fhb1* was successfully fine mapped to the same location in two populations with two genetically diverse sources of FHB resistance in spring wheat using an innovative strategy. This strategy combined unique population design to fix resistant and susceptible backgrounds, large population sizes, and the use of microsatellite markers to select fixed recombinants, robust phenotypic data, good marker density and

- 54 -

comparative mapping to attain a precise map position of *Fhb1*. This study suggests *Fhb1* is functionally essential to provide Type II resistance and *Fhb1* is an additive gene relative to other resistance loci. This information should aid plant breeders worldwide to reduce linkage drag associated with marker-assisted selection and assist in the cloning of the functional resistance gene.

# 4.0 MAPPING OF *FHB2* ON CHROMOSOME 6BS: A GENE CONTROLLING FUSARIUM HEAD BLIGHT FIELD RESISTANCE IN BREAD WHEAT (*TRITICUM AESTIVUM* L.)

# 4.1 Abstract

Fusarium head blight (FHB) is one of the most important fungal wheat diseases worldwide. Understanding the genetics of FHB resistance is key to facilitate the introgression of different FHB resistance genes into adapted wheat. The objective of this project was to study the FHB resistance QTL on chromosome 6B, quantify the phenotypic variation, and qualitatively map the resistance gene as a Mendelian factor. The FHB resistant parent BW278 (AC Domain\*2/Sumai 3) was used as the source of the resistance allele. A large recombinant inbred line (RIL) mapping population was developed from the cross BW278/AC Foremost. The population segregated for three known FHB resistance QTL located on chromosomes 3BSc, 5A, and 6B. Molecular markers on chromosome 6B (WMC104, WMC397, GWM219), 5A (GWM154, GWM304, WMC415), and 3BS (WMC78, GWM566, WMC527) were amplified on approximately 1,440 F<sub>5:7</sub> RILs. The marker information was used to select 89 RILs that were fixed homozygous susceptible for the 3BSc and 5A FHB QTLs and were recombinant in the 6B interval. Disease response was evaluated on 89 RILs and parental checks in the greenhouse and field nurseries. Dual floret injection (DFI) was used in greenhouse trials to evaluate disease severity (DS). Macroconidial spray inoculations were used in field nurseries conducted at two locations in southern Manitoba (Carman and Glenlea) over two years 2003 and 2004, to evaluate disease incidence, disease severity, visual rating index, and Fusarium-damaged kernels. The phenotypic distribution for all five disease infection measurements was bimodal, with lines resembling either the resistant or susceptible checks and parents. All of the four field traits for FHB resistance mapped qualitatively to a coincident position on chromosome 6BS, flanked by GWM133 and GWM644, and is named *Fhb2*. The greenhouse-DS trait mapped 2 cM distal to *Fhb2*. Qualitative mapping of *Fhb2* in wheat provides tightly linked markers that can reduce linkage drag associated with marker assisted selection of *Fhb2* and aid the pyramiding of different resistance loci for wheat improvement.

# **4.2 Introduction**

Fusarium head blight (FHB), caused primarily by *Fusarium graminearum* Schwabe (teleomorph *Gibberella zeae* (Schwein.) Petch), has become the most serious fungal disease of small cereal grains in Manitoba, eastern Saskatchewan, and eastern Canada. FHB infection is favoured by warm humid conditions during flowering and early stages of kernel development (Gilbert and Tekauz 2000). Lightweight Fusariumdamaged kernels (FDK) may contain high concentrations of mycotoxins, such as deoxynivalenol (DON), rendering the grain unsuitable for food or feed (Gilbert and Tekauz 2000).

Producers can follow a number of management practices to help control FHB; however, the most efficacious and economical strategy to this devastating problem is to breed genetic disease resistance into adapted cultivars. Breeding for resistant cultivars is difficult given the complexity of FHB resistance, the need to screen host plants at maturity, and the large environmental effects on disease expression. Several types of FHB resistance in wheat have been proposed (Bai and Shaner 1994; Yang 1994;

- 57 -

McMullen et al.. 1997). Resistance to initial infection (Type I) is assessed as the incidence of infection in the presence of natural or augmented inoculum (e.g. spray inoculations). Resistance to spread within the spike (Type II) is most accurately assessed as the spread of infection within the spike following single/dual floret injections (SFI/DFI). Other types of resistance are not as well characterized. Disproportionate reductions in the accumulation of DON (i.e., statistically uncorrelated with Type I or II resistance) are described as Type III resistance (Mesterhazy 1995).

The most common sources of genetic resistance are derived from the Chinese wheat cultivar Sumai 3 (Yang 1994; Bai and Shaner 1994; Wan et al., 1997) and resistance to FHB is quantitatively inherited (Chen 1983; Lin et al., 1992; Yang 1994; Bai et al., 2000). Problems associated with greenhouse- and field-based screening for FHB resistance include dependence on the environment for symptom development, the high cost of phenotyping, and significant time and resource requirements (Yang 1994; Bai and Shaner 1994; Campbell and Lipps 1998). Development of DNA marker-based screening for the presence of resistance genes may make selection for resistance more efficient in breeding programs (Bai et al., 1999; Kolb et al., 2001).

Exploitation of molecular markers associated with FHB resistance genes has focused on Type II FHB resistance (Bai et al., 1999; Waldron et al., 1999; Anderson et al., 2001; Buestmayr et al., 2002; Zhou et al., 2002; Yang et al., 2003). Previous QTL mapping studies have revealed major Sumai 3-derived Type II FHB resistant QTL on chromosomes 3BS (Waldron et al., 1999; Anderson et al., 2001; Buerstmayr et al., 2002; Somers et al., 2003; Zhou et al., 2002; Yang et al., 2003), 5A (Ban and Suenaga 1998; Xu et al., 2001; Buesrstmayr et al., 2002) and 6B (Anderson et al., 2001; Yang et al., 2003). Understanding the genetics of FHB resistance and mapping the location of genes will be necessary to facilitate the introgression and pyramiding of different FHB resistance genes into adapted wheat.

To determine the map location of individual genes controlling quantitative traits, substitution lines, recombinant inbred lines (RILs) or near-isogenic lines (NILs) can be developed to isolate the gene of interest as a Mendelian factor. The objective of this research was to study the FHB resistance QTL on chromosome 6B (Yang et al., 2003) by quantifying the phenotypic variation in disease incidence (DI), disease severity (DS), visual rating index (VRI), and Fusarium-damaged kernels (FDK) and qualitatively map the resistance gene.

### 4.3 Materials and methods

#### **4.3.1** Population, genotyping and selection of recombinants

Single-seed descent was used to develop a recombinant inbred population of 1,440 F<sub>5:7</sub> lines from the cross BW278 (AC Domain\*2/Sumai 3, FHB resistant) and AC Foremost (HY320\*5/BW553//HY320\*6/7424-BW5B4, FHB susceptible). Sumai 3 was the source of FHB resistance in the population, which segregated for three known FHB resistance QTL on chromosomes 3BSc, 5A, and 6B. The QTL identified on 3BSc is located proximal to the centromere (Somers et al.. 2003). The 1,440 F<sub>5:7</sub> recombinant inbred lines (RILs) were genotyped using microsatellite markers on chromosome 6B (WMC104, WMC397, GWM219), 5A (GWM154, GWM304, WMC415), and 3BS (WMC78, GWM566, WMC527) (Somers et al., 2004) to facilitate selection of RILs homozygous susceptible for QTL intervals on 3BSc, 5A, and recombinant for the interval

on 6B carrying the FHB resistance gene (Table 4.1). BW278 is known to lack resistance alleles at *Fhb1* (Cuthbert et al.. 2006) on 3BS near GWM493 (data not shown).

Seed from the 1,440 RILs were germinated on moist filter paper in Petri dishes for one week in the dark at 21°C. The leaf tissue was harvested and lyophilized, then DNA was extracted using the Qiagen DNeasy 96 Plant Kit (Qiagen, Mississauga, Ont.) and quantified by fluorimetry using Hoechst 33258 stain. DNA from five plants per line was collected and bulked for initial genotyping of the 1,440 lines. Lines showing heterogeneity or heterozygosity were eliminated from the project. DNA was collected from five new plants per line for genotyping of the selected recombinant lines, and no heterogeneity or heterozygosity was detected within the families of the final selected recombinant population. Genotypic data for the population was collected using M13tailing and fluorescent capillary electrophoresis on an ABI3100 genotyper (Applied Biosystems Inc., Foster City, Calif.). M13-tailing required adding the M13 sequence (CACGACGTTGTAAAACGAC) to the 5' end of the forward primer during primer synthesis (Schuelke 2000). The PCR conditions were: 24 ng DNA, 1.5 mM MgCl<sub>2</sub>, 50 mM KCl, 0.8 mM dNTPs, 2 pmol reverse primer, 0.2 pmol forward primer, and 1.8 pmol M13 primer (CACGACGTTGTAAAACGAC) fluorescently labelled with 6-FAM, HEX, or NED (Applied Biosystems Inc.), and 0.5 U Taq DNA polymerase (Promega, Madison, Wis.). Thermal cycling included:  $94^{\circ}C - 2 \min$ , 30 cycles of  $95^{\circ}C - 1 \min$ , (0.5°C/s to  $61/51^{\circ}$ C),  $61/51^{\circ}$ C - 50 sec, (0.5°C/s to 73C), 73°C - 1 min, 1 cycle 73°C - 5 min. The internal molecular weight standard for the ABI3100 was Genescan 500-ROX (Applied Biosystems Inc.). Data collected by fluorescent capillary electrophoresis was first
converted to a gel-like image using Genographer (available at http://hordeum.oscs.montana.edu/genographer).

-

Marker	Forward Primer $(5' \rightarrow 3')$	Reverse Primer (5'→3')	Chromosome	Anneal (°C)	Allele Size Sumai	References
110 (0104				<i>(</i> 1	1.50	· · · · · · · · · · · · · · · · · · ·
WMC104	TCTCCCTCATTAGAGTTGTCCA	ATGCAAGTTTAGAGCAACACCA	6B	61	153	http://wheat.pw.usda.gov
GWM132	TACCAAATCGAAACACATCAGG	CATATCAAGGTCTCCTTCCCC	6B	61	134	Röder et al (1998)
CFD13	CCACTAACCAAGCTGCCATT	TTTTTGGCATTGATCTGCTG	6B	61	Null	http://wheat.pw.usda.gov
GWM518	AATCACAACAAGGCGTGACA	CAGGGTGGTGCATGCAT	6B	61	208	Röder et al (1998)
GWM494	ATTGAACAGGAAGACATCAGGG	TTCCTGGAGCTGTCTGGC	6B	61	217	Röder et al (1998)
GWM508	GTTATAGTAGCATATAATGGCC	GTGCTGCCATGATATTT	6B	51	156	Röder et al (1998)
WMC398	GGAGATTGACCGAGTGGAT	CGTGAGAGCGGTTCTTTG	6B	61	173	http://wheat.pw.usda.gov
GWM133	ATCTAAACAAGACGGCGGTG	ATCTGTGACAACCGGTGAGA	6B	61	120	Röder et al (1998)
GWM644	GTGGGTCAAGGCCAAGG	AGGAGTAGCGTGAGGGGC	6B	61	206	Röder et al (1998)
WMC397	AGTCGTGCACCTCCATTTTG	CATTGGACATCGGAGACCTG	6B	61	175	http://wheat.pw.usda.gov
WMC756	TTCCGTGGCCTCTCGTTC	CATTGCCATCAGTCACCCTC	6B	51	231	http://wheat.pw.usda.gov
GWM88	CACTACAACTATGCGCTCGC	TCCATTGGCTTCTCTCTCAA	6B	61	248	Röder et al (1998)
WMC179	CATGGTGGCCATGAGTGGAGGT	CATGATCTTGCGTGTGCGTAGG	6B	61	217	http://wheat.pw.usda.gov
WMC182	GTATCTCACGAGCATAACACAA	GAAAGTGTATGGATCATTAGGC	6B	61	180	http://wheat.pw.usda.gov
GWM608	ACATTGTGTGTGCGGCC	GATCCCTCTCCGCTAGAAGC	6B	61	163	Röder et al (1998)
GWM107	ATTAATACCTGAGGGAGGTGC	GGTCTCAGGAGCAAGAACAC	6B	61	209	Röder et al (1998)
WMC152	CTATTGGCAATCTACCAAACTG	TCTCTTCTTGCCACATATTCGT	6B	61	256	http://wheat.pw.usda.gov
BARC24	CGCCTCTTATGGACCAGCCTAT	GCGGTGAGCCATCGGGTTACAAAG	6B	51	211	http://www.scabusa.org
GWM219	GATGAGCGACACCTAGCCTC	GGGGTCCGAGTCCACAAC	6B	61	198	Röder et al (1998)
GWM154	TCACAGAGAGAGAGGGAGGG	ATGTGTACATGTTGCCTGCA	5A	51	121	Röder et al (1998)
GWM304	AGGAAACAGAAATATCGCGG	AGGACTGTGGGGGAATGAATG	5A	61	234	Röder et al (1998)
WMC415	AATTCGATACCTCTCACTCACG	TCAACTGCTACAACCTAGACCC	5A	61	176	http://wheat.pw.usda.gov
WMC78	AGTAAATCCTCCCTTCGGCTTC	AGCTTCTTTGCTAGTCCGTTGC	3BSc	61	285	http://wheat.pw.usda.gov
WMC527	ACCCAAGATTGGTTGCAGAA	GCTACAGAAAACCGGAGCCTAT	3BSc	61	402	http://wheat.pw.usda.gov
GWM566	TCTGTCTACCCATGGGATTTG	CTGGCTTCGAGGTAAGCAAC	3BSc	61	142	Röder et al (1998)

Table 4.1: Primer sequences, annealing temperatures, and allele sizes of markers on chromosome 6B used in the BW278/AC Foremost population for mapping *Fhb2* in spring wheat

### 4.3.2 Fusarium head blight phenotyping – greenhouse

The 89 RILs and parents from the mapping population were randomly arranged and grown in the greenhouse with supplemental lighting set for 16 hour daylight. The greenhouse temperature was monitored and recorded daily and averaged 22°C during the day with a range of 18 to 25°C and 18°C at night with a range of 17 to 21°C. The inoculum used throughout the experiment was a mixture of virulent strains of Fusarium graminearum Schwabe (JM-6-00; EEI-23-00; RK-9-02; RK-16-02) provided by Dr. Jeannie Gilbert, Agriculture and Agri-Food Canada – Cereal Research Centre, Winnipeg, Manitoba. The inoculum was produced as described by Sung and Cook (1981). Ten single spikes from 10 plants for each F<sub>5:7</sub> RIL and parent were inoculated to assess FHB resistance. A single primary spike on each recombinant plant was inoculated when the spike reached 50% anthesis. Each spike was inoculated by injecting a 10 µl macroconidial suspension (50,000 spores/mL) between the lemma and palea of the primary and secondary florets positioned at the inoculation point. The inoculation points on each spike were the spikelet positioned 2/3 of the way from the base of the spike and the spikelet immediately above that point. For example, the inoculation points were the adjacent 8<sup>th</sup> and 9<sup>th</sup> spikelets on a spike that had a total of 12 spikelets  $(12 \times 2/3 = 8)$ . Following point inoculation, plants were incubated in a chamber at 100% relative humidity for 24 hours and then returned to the greenhouse bench. Ratings were performed at 7, 14, and 21 days post-inoculation. Disease severity (DS) ratings were assessed by counting the number of infected spikelets directly below the inoculated florets and excluding the inoculated florets. The number of infected spikelets was only counted below the point of inoculation since infection can restrict the flow of water to distal florets and cause early senescence. The percentage of infected florets was averaged for each plant and RILs were classified as resistant or susceptible based on the bimodal distribution of ratings.

### **4.3.3 Fusarium head blight phenotyping - field trials**

The field trials included 97 entries (89 RILs and 8 check varieties: AC Barrie, AC Foremost, AC Morse, AC Vista, Alsen, BW278, CDC Teal, and FHB 37) which were screened in Fusarium head blight nurseries at two locations in southern Manitoba (Glenlea and Carman) during the 2003 and 2004 field seasons (Appendix 8.3). Trial entries and checks were replicated four times in a randomized complete block design. Selected checks (AC Morse [susceptible], CDC Teal [susceptible], Alsen [moderately resistant], and FHB37 [resistant]) were placed every 50 rows to monitor disease development throughout the field. Plots in Carman 2003 and 2004 consisted of a single 1 meter row with 17 cm row spacings. Plots at Glenlea consisted of a single 1.5 meter row in 2003 and 0.9 meter row in 2004 row both with 30 cm row spacings. Sowing density was approximately 60 seeds per row. The spikes of the entire row were spray-inoculated at 50% anthesis with a 50 ml inoculum solution of virulent strains of Fusarium graminearum (2003 [JM-6-00, EEI-23-00, RK-9-02, RK-16-02] and 2004 [RK-16-02, MS/DS-15-03, MS/DS-3-03, EM/MB-19-03, MB/DS/DB-47-03]) using a CO<sub>2</sub> backpack sprayer calibrated at 30 psi. Re-inoculation of the same rows was performed two and three days following the first inoculation in the Glenlea and Carman nurseries, respectively. The inoculum solution was a suspension of 50,000 macro-conidia spores / mL in water and Tween 20. There was a difference in isolates used for the inoculation

procedure from year to year, which is standard procedure to ensure current isolates are being used for testing of breeding material. The nursery at Glenlea in 2003 and 2004 was irrigated with a sprinkler system for 30 minutes following each inoculation to favor development of the disease. Plots at the Carman nursery were irrigated two hours postinoculation for five minutes every hour for twelve hours. All plots at Carman were mistirrigated on alternate days for a period of ten days.

Disease incidence (DI - initial infection) and disease severity (DS - disease spread within the spike) of each row were rated 18 to 21 days post-inoculation using a 1 (resistant) -10 (susceptible) scale. Visual rating index (VRI) was calculated (VRI = DI x DS) for each line. Twenty-five spikes per row were harvested at random and stored at -  $20^{\circ}$ C the day visual field ratings were performed to later verify the visual rating in the field.

Nurseries were hand harvested at the end of the season when they reached physiological maturity using a Whitecapper – offset double row thresher (Glenlea 2003) or Wintersteiger Elite combine (Carman 2003/2004, Glenlea 2004). The threshing mechanism was set at a normal setting on the combine; however, the wind speed was decreased and sieves were opened to ensure the Fusarium-damaged kernels were maintained in the harvested samples. Harvested seed samples were placed in paper bags and dried for one week at 36°C using a forced air system. A 50 gram sample from each plot was visually assessed to determine the % of FDK (Appendix 8.4). Fusarium-damaged kernels were identified as shriveled, light-weight and chalky white kernels with occasional pink colouration. These kernels were distinguishable from plump visually disease free kernels within a sample.

### 4.3.4 Construction of the genetic map

A total of 40 microsatellite markers on chromosome 6B (Somers et al.. 2004) were screened for polymorphism between the parents of the population. Polymorphic marker primer sequences, annealing temperatures, and allele sizes are listed in Table 4.1. Nineteen polymorphic markers on 6B were used to genotype the RILs and create the genetic map. JoinMap, V3.0 (Biometris, Wageningen, The Netherlands, http://www.joinmap.nl) was used to determine the marker order and map distances.

### 4.3.5 Statistical analysis

Analysis of variance (ANOVA) for DI, DS, VRI, and FDK for each site and a combined analysis across the four site years were performed using the "PROC GLM" procedure of the SAS software package (SAS Institute Inc., Version 8.2). A homogeneity test was conducted to ensure the data could be combined over site years. The model statement used in the combined analysis was "variables = env rep(env) entry entry\*env. All factors in this statement with the exception of entry were considered to be random. The F-test values were considered approximate since the trait values were not normally distributed.

### 4.4 Results

There were 89 RILs identified from the 1,440  $F_{5:7}$  mapping population to be homozygous susceptible for FHB resistance QTL on 3BSc and 5A and recombinant near the FHB resistance 6B QTL interval. The interval distance between flanking markers WMC104 and GWM219 on 6BS was 32 cM (Figure 4.2).



Figure 4.2: Phenotypic distribution based on FHB infection of 89  $F_{5:7}$  RILs from the cross Domain\*2/Sumai 3//AC Foremost. Percent infection was measured from one replicated greenhouse (GH) experiment and replicated field disease nurseries combined over four site-years. Traits included disease severity (DS), disease incidence (DI), visual rating index (VRI = DS x DI), and Fusarium damaged kernels (FDK). Resistant and susceptible parent and check lines showed infection phenotypes within the respective modes of the distributions. Small black arrows indicate the division point to classify lines as resistant or susceptible.

# 4.4.1 Fusarium head blight phenotyping

### 4.4.1.1 Greenhouse trials

Ten plants of each RIL and parental check were inoculated in the greenhouse using DFI on a single primary spike to assess disease severity. The range in GH-DS (1-10) infection ratings showed a bimodal distribution (Fig 4.1 GH-DS). The range in GH-DS of RILs classified as resistant was 1.5-5.3 and for lines classified as susceptible was 7.6-10.0. There was very low variability (standard error: resistant RILs 4.1  $\pm$  0.25 and susceptible RILs 9.3  $\pm$  0.25) within the ten plants of each RIL and no overlap between the resistant and susceptible classes (Fig. 4.1). Resistant parental checks showed a disease severity range of 2.1-2.3 (resistant) and the susceptible check was 9.3. Darkening of the inoculation point was visible by day 7; however, disease progression was minimal by day 14 for the susceptible RILs and susceptible parental check. Disease development progressed basally from the inoculation point and there was a substantial change in infection ratings for susceptible RILs between day 14 and 21 post-inoculation. The population segregated 41 resistant to 48 susceptible plants, fitting a 1:1 chi-square ratio (p<0.10).



Figure 4.2: Genetic map position of *Fhb2* on chromosome 6BS in the cross AC Domain\*2/Sumai 3//AC Foremost. A population of 89 RILs recombinant for a segment of chromosome 6B was phenotyped for FHB infection symptoms and RILs were classified as resistant or susceptible based on replicated greenhouse and field disease nurseries combined over four site years. Recombination distance is shown on the left in cM and the Chinese Spring deletion bin assignment for GWM133 and GWM644 (Sourdille et al. 2004) are shown on the right.

# 4.4.1.2 Field trials

Environmental data was collected and conditions differed between the 2003 and 2004 field seasons. Mean temperature and precipitation during inoculation and prior to rating (July and August) are the most critical for infection and disease development.

High amounts of precipitation and flooding in the spring of 2004 affected plant establishment at Glenlea and led to loss of one of the replicates. Despite the differences in temperature and precipitation between years, the mean infection level for all field traits did not differ between the years.

The ANOVA for combined site-years indicated all sources of variation for Field -DI, -VRI, -FDK, and –DS were significant with the exception of environment for Field -DS (Table 4.2). The phenotypic distribution of all field traits over site years was bimodal (Fig. 4.1). All 89 RILs that were classified showed the same classification for all four field traits. The population segregated 45 resistant to 44 susceptible RILs for all field traits, fitting a 1:1 chi-square ratio (p<0.05) (Fig. 4.1). The correlation between the averaged greenhouse and field data measurements for the 89 RILs showed all correlations were high and statistically significant ( $\alpha$ =0.01) (Table 4.3).

There were 12 RILs that were recombinant between the GH-DS and field traits. Four RILs were resistant for GH-DS and susceptible for field traits with a range of 47-52% and a mean rating of 50% infection. There were 8 RILs susceptible for the GH-DS rating and resistant for the field traits with a range of 78 - 100% and a mean rating of 89% infection. The correlation based on DS between the visual assessment of each row in the FHB field nurseries and the harvested spike analysis (25 heads) was also high: Carman 2003 (r=0.91), Glenlea 2003 (r=0.89), Carman 2004 (r=0.88), and Glenlea 2004 (r=0.85).

Source	df	Type III SS	Mean	F-value	P-value				
			square						
Variable: Dise	ase Incidence	(DI)		ren .					
Env.	3	49.7	16.6	16.0	<0.0001				
Rep(env)	11	25.7	2.3	2.3	0.0102				
Entry	102	6301.9	61.8	59.8	<0.0001				
Env*Entry	304	510.7	1.7	1.6	<0.0001				
Error	1,115	1152.9	1.0						
Variable: Dise	ase Severity (I	DS)							
Env.	3	4.2	1.4	1.2	0.3118				
Rep(env)	11	29.1	2.6	2.3	0.0095				
Entry	102	7712.3	75.6	65.1	< 0.0001				
Env*Entry	304	607.0	2.0	1.7	< 0.0001				
Error	1,115	1295.8	1.2						
Variable: Visu	al Rating Inde	ex (VRI)							
Env.	3	835.0	278.3	4.2	0.0055				
Rep(env)	11	1862.2	169.3	2.3	0.0031				
Entry	102	759439.1	7445.5	113.3	< 0.0001				
Env*Entry	304	36144.5	118.9	1.8	< 0.0001				
Error	1,115	73281.5	65.7						
Variable: Fusa	Variable: Fusarium-damaged kernels (FDK)								
Env.	3	13106.7	4368.9	36.5	<0.0001				
Rep(env)	11	2221.5	202.0	1.7	0.0708				
Entry	102	924706.9	9065.8	75.8	<0.0001				
Env*Entry	304	83535.5	274.8	2.3	<0.0001				
Error	1,115	133352.1	119.6						

Table 4.2: Analysis of variance for four phenotypic field variables (disease incidence, disease severity, visual rating index, and Fusarium-damaged kernels) from two field locations (Glenlea and Carman, MB) over two years (2003 and 2004).

	Greenhouse/Field Data Measurements						
•••	Greenhouse - DS	Field - VRI	Field - DI	Field - DS	Field - FDK		
Greenhouse DS							
Field - VRI	0.75						
Field - DI	0.74	0.99					
Field - DS	0.78	0.94	0.92				
Field - FDK	0.73	0.97	0.97	0.91			

Table 4.3 Correlation coefficients (r) calculated using averaged values for greenhouse and field data measurements from two field locations (Glenlea and Carman, MB) over two years (2003 and 2004).

### 4.4.2 Genetic map

A genetic map was constructed by genotyping the 89 RILs with 19 polymorphic microsatellite markers on chromosome 6B (Table 4.1). The marker order was identical to the wheat consensus map (Somers et al. 2004), with the exception of markers GWM518 and CFD13; and GWM608 and WMC182, which were inverted. The total map length for the population was 32 cM (Fig. 4.2) compared to 42 cM on the wheat consensus map (Somers et al. 2004). The 89 RILs were classified as resistant or susceptible using five disease infection measurements (Field-DI, Field-DS, Field-VRI, Field-FDK and GH-DS). All of the four field traits mapped to a coincident genetic position on chromosome 6BS flanked by GWM133 and GWM644. This gene controlling field resistance to FHB is here named *Fhb2*. The GH-DS mapped 2 cM distal to *Fhb2* due to the presence of 12 RILs which were recombinant between GH-DS and field traits (Fig. 4.2).

# 4.5 Discussion

There are inherent difficulties associated with phenotypic characterization of FHB due to methodological problems of inoculation and confounding effects of the environment (Andersen 1948; Hanson et al., 1950; Scott 1927). The present study decreased this variability and increased the reproducibility in phenotyping FHB resistance by focusing on variables that could be controlled. This included the development of a large RIL mapping population that segregated for a single major FHB resistance gene, multiple site-years of field phenotyping, and indoor DFI phenotyping.

The significant level of variation for all field traits (Table 4.2) may be due to the varying level of disease pressure for combined site years, differences in isolates used and differences in the type of irrigation system used between locations. Statistically, the most significant factors affecting FHB ratings in the population were entry and env\*entry interaction. The env\*entry interaction for Field-DI, Field-DS, Field-VRI and Field-FDK compared to the entry effect is relatively small since the sum of squares for entry is more than 10 fold higher than the sum of squares for the env\*entry interaction (Table 4.2). There is no overlap in the two classes of RILs (Fig. 4.1) therefore the env\*entry interaction is more a function of annual differences in values as opposed to overlapping phenotypic classes.

The ANOVA showed that the entries were highly significant for all field traits and using the phenotype distribution each RIL could be classified as either resistant or susceptible (Table 4.2, Fig. 4.1). The experimental design and data collection methods were effective in removing these sources of variation from the entry effects. The DS was measured both in the greenhouse using DFI and in the field using spray inoculation to ensure consistency amongst ratings and proper characterization of the RILs. There were 12 RILs that were recombinant between the GH-DS and field traits. The overall rating results indicated the GH-DS levels were greater than the field DS (Fig. 4.1); however, the GH-DS and field DS were highly correlated (r=0.78). The data showed all four field measurements of FHB resistance mapped to one coincident location on chromosome 6BS, represented by *Fhb2*. The GH-DS measurement of FHB resistance mapped 2 cM distal to *Fhb2* due to the presence of the 12 recombinant lines between GH-DS and *Fhb2*.

This study provided an approach to qualitatively map the *Fhb2* gene using the Sumai 3 source of resistance in a large mapping population by collecting phenotypic data from both the field and greenhouse. The total genetic distance between the two flanking markers WMC104 and GWM219 on the wheat consensus map is 42 cM and 32 cM in the present population. The main difference in genetic distance between the two populations is attributed to the map distance between markers CFD13 and GWM518 in the wheat consensus map of 10 cM versus 1 cM in the BW278/AC Foremost map. The increased genetic distance may be due to differences in background genetics, population types, and population sizes used to create the wheat consensus map.

The distance between flanking markers GWM133 and GWM644, and *Fhb2* is 2 and 4 cM, respectively. Comparisons of physical and genetic maps of wheat indicate that most genetic recombination occurs in gene-rich, telomeric regions (Gill et al., 1996; Faris et al., 2000). *Fhb2* is shown to map to 6BS, proximal to the centromere, since the flanking markers GWM133 and GWM644 are assigned to deletion bin C-6BS5-0.76

(Sourdille et al., 2004) (Fig. 4.2). In the study by Yang et al., 2003, QTL analysis of chromosome 6B for FHB resistance in the population DH181 (Sumai 3 derivative)/AC Foremost was completed evaluating Type II resistance in the greenhouse using SFI. The results of the research revealed a major QTL on chromosome 6B contributes to FHB resistance. The most important microsatellite marker in the study located on 6B was GWM644 and explained 21% of the phenotypic variation in DH181(Sumai 3 derivative)/AC Foremost population. An additional study conducted by Shen et al.. (2003) developed a RIL population from the cross of Ning 894037 and Alondra. Type II resistance was evaluated in the field and greenhouse using SFI. The QTL on 6B was found to be closest to marker GWM644. Based on the location of markers on the wheat consensus map, these intervals in these two studies are coincident on 6BS proximal to the centromere (Somers et al. 2004; Sourdille et al. 2004). The present results indicated there was one gene, *Fhb2*, controlling FHB field resistance on 6BS and an additional locus 2 cM distal to Fhb2 controlling FHB Type II resistance. Fhb2 was estimated to map within 2 cM of the QTL intervals reported by Yang et al.. (2003) and Shen et al.. (2003), suggesting the FHB resistance QTL on 6BS and *Fhb2* are likely coincident.

In summary, *Fhb2* was successfully mapped to 6BS and confers field resistance to FHB. The large population design with a fixed susceptible background, qualitative mapping and comparative mapping were used to attain a precise map position of *Fhb2*. *Fhb2* provides FHB field resistance as a single gene present in a susceptible background. Yang et al.. (2003) reported a coefficient of determination on GH-DS of 21% for the microsatellite marker GWM644 on 6BS which reduced FHB severity by 52%. In the present study the resistant allele on 6BS reduced FHB GH-DS by 56% when compared to

the RILs carrying the susceptible allele. A more precise map location should reduce linkage drag associated with marker-assisted selection and assist with efficient and effective pyramiding of different FHB resistance genes for wheat improvement.

# 5.0 MAPPING OF ADULT LEAF RUST RESISTANCE GENE, *LR34*, AND THE INTERACTION WITH FUSARIUM HEAD BLIGHT RESISTANCE GENE, *FHB2*, IN SPRING WHEAT (*TRITICUM AESTIVUM* L.).

# 5.1 Abstract

Leaf rust and Fusarium head blight (FHB) are two of the most destructive spring wheat diseases. The ability for breeders to develop spring wheat varieties with enhanced resistance levels simultaneously may involve the resistance genes Lr34 and Fhb2; and therefore, understanding their interaction is important. A large mapping population of 2,300 recombinant inbred lines (RILs) was developed from the cross BW278/AC Foremost. The population segregated for two known leaf rust resistance genes Lr16, Lr34 and one unidentified resistance gene tentatively named LrF; and the FHB resistance gene Fhb2 on 6BS. There were 253 of these RILs that carried the susceptible allele for Lr16 and LrF and were recombinant between markers GWM1220 and GWM130 flanking Lr34 and segregated for Fhb2. These RILs were evaluated for leaf rust severity in the field during 2003 and 2004 to develop a high density map for Lr34. A second group of 89 RILs from the same cross were genotypically selected to be fixed resistant (45 RILs) or susceptible (44 RILs) for *Fhb2* and segregated for varying combinations of Lr16, LrF, and Lr34. This group of RILs was evaluated for leaf rust using growth cabinet inoculation and for Fusarium head blight in the field during 2003 and 2004. The phenotypic distribution for leaf rust was bimodal for both groups of RILs and Lr34 was mapped on 7D in both RIL populations. Leaf rust resistance gene Lr34 is known to enhance the level of disease resistance to many diseases in addition to its effects on leaf

rust. However, there was no clear positive enhancement of FHB resistance when Lr34 and the unlinked gene, *Fhb2* resistance alleles were present together.

# 5.2 Introduction

Leaf rust, caused by Puccinia triticina Eriks. (Anikster et al., 1997) (synonym P. recondita Rob. Ex Desmaz f.sp. tritici), is a major disease of wheat (Triticum aestivum L.) worldwide. The disease severity ranges from year to year; however, annual average yield losses in western Canada were estimated at \$88 million annually between 2001 and 2005 (McCallum et al., 2007). Genetic rust resistance offers a cost-effective strategy to reduce losses in wheat from attack by rust pathogens. To date, more than 50 leaf rust resistance genes have been characterized (Knott 1989; McIntosh et al., 1995). Most of them are effective from the seedling stage through the whole life of the plant, whereas only seven of them are monogenic and primarily effective at the adult stage (McIntosh et al. 1995). Wheat genes Lr34 and the completely linked Yr18 have provided durable resistance to leaf rust and stripe rust (Puccinia striiformis), respectively (Dyck et al.. 1966; Singh and Rajaram 1992; Ma and Singh 1996). Lr34 typically expresses partial resistance quantitatively (Drijepondt and Pretorius 1989; German and Kolmer 1992; Singh 1992) with an increased latency period, and a decreased infection frequency and uredium size (Drijepondt and Pretorius 1989).

Using monosomic analysis, Dyck (1987) mapped Lr34 to chromosome 7DS. In the study by Spielmeyer et al.. (2005), Lr34 and Yr18 were mapped to a single locus flanked by microsatellite markers GWM295 and GWM1220 on chromosome 7DS. The Lr34/Yr18 region has been associated with many traits and disease resistance in wheat including durable, adult plant resistance to leaf rust (Lr34) (Singh and Gupta 1991); durable adult plant resistance to stripe rust (Yr18) (McIntosh 1992; Singh 1992a); adult plant resistance to powdery mildew (Spielmeyer et al. 2005); tolerance to barley yellow dwarf virus (Bdv1) (Singh 1993); enhanced expression of stem rust resistance (Dyck 1987, Vanegas et al. 2007)); and leaf tip necrosis of flag leaves (Ltn) (Singh 1992b).

Although Lr34 is present in wheat worldwide, it is still not known whether some or all of the disease resistance traits are controlled by single genes or by several tightly linked resistance genes. Fusarium head blight is one disease that has not been studied to determine if there is an enhancement in the level of FHB resistance when Lr34 is present. Sumai 3, an FHB resistant Chinese spring wheat variety possesses FHB resistance genes *Fhb1* and *Fhb2* found on chromosomes 3BS and 6BS, respectively (Cuthbert et al.. 2006a; Cuthbert et al.. 2006b) and another gene on 5AS (*Qfhs.ifa-5A*) (Bai et al.. 1999, Anderson et al.. 2001; Zhou et al.., 2002; Somers et al.. 2003; Yang et al.. 2003; Yang et al.. 2005). Sumai 3 also contains the adult leaf rust resistant gene Lr34 (Brent McCallum, unpublished data). Although *Fhb1* and *Fhb2* are not linked with Lr34, it may be possible that there is a gene interaction occurring resulting in an increased level of FHB resistance when resistance alleles for Lr34 and *Fhb1* or *Fhb2* are present in the same line.

There were two major objectives for this research: 1) to map the Lr34 locus using microsatellite markers; and 2) to measure any gene interaction between Lr34 and Fhb2.

#### **5.3 Materials and methods**

# 5.3.1 Genetic mapping of *Lr34*

# 5.3.1.1 Mapping population, genotyping and selection of lines

The line BW278 is a backcross derived doubled haploid (DH) line (AC Domain\*2/Sumai 3) and carries resistance alleles for *Lr16*, *Lr34*, and *Fhb2* (Cuthbert et al., 2006b). Single seed descent was used to develop a recombinant inbred line (RIL) population of 2,300  $F_{5:7}$  lines from the cross BW278 x AC Foremost (HY320\*5/BW553//HY320\*6/7424-BW5B4). AC Foremost carries an unidentified seedling leaf rust resistance gene, tentatively named *LrF*.

The leaf rust reactions of the 2,300  $F_{5:7}$  RILs were determined in the greenhouse using leaf rust races (MBDS [avirulent to Lr16/virulent to LrF]; TJBJ [virulent to Lr16/avirulent to LrF]; TDT [avirulent to Lr16 and LrF] to select 253 seedling susceptible lines that carried the susceptible alleles for both Lr16 and LrF, but segregated for the adult leaf rust resistance gene Lr34. The selected 253  $F_{5:7}$  lines were grown in the field to evaluate disease resistance to leaf rust.

# 5.3.1.2 Genotyping

Five seeds of each of the recombinant lines were germinated on filter paper in petri dishes and leaf tissue was harvested and lyophilized for DNA extraction with the Qiagen DNeasy 96 Plant Kit (Qiagen, Mississauga, Ont.). DNA was quantified by fluorimetry using Hoechst 33258 stain. Genotypic data for the population was collected using M13tailing and fluorescent capillary electrophoresis on an ABI3100 genotyper (Applied

Biosystems Inc., Foster City, Calif.). M13-tailing required adding the M13 sequence (CACGACGTTGTAAAACGAC) to the 5' end of the forward primer during primer synthesis (Schuelke 2000). The PCR conditions were: 24 ng DNA, 1.5 mM MgCl<sub>2</sub>, 50 mM KCl, 0.8 mM dNTPs, 2 pmol reverse primer, 0.2 pmol forward primer, and 1.8 pmol M13 primer (CACGACGTTGTAAAACGAC) fluorescently labelled with 6-FAM, HEX, or NED (Applied Biosystems Inc.), and 0.5 U Taq DNA polymerase (Promega, Madison, Wis.). Thermal cycling included:  $94^{\circ}C - 2 \min$ , 30 cycles of  $95^{\circ}C - 1 \min$ , (0.5°C/s to  $61/51^{\circ}$ C),  $61/51^{\circ}$ C - 50 sec, (0.5°C/s to 73C),  $73^{\circ}$ C - 1 min, 1 cycle  $73^{\circ}$ C - 5 min. The internal molecular weight standard for the ABI3100 was Genescan 500-ROX (Applied Biosystems Inc.). Data collected by fluorescent capillary electrophoresis was first converted gel-like using Genographer available to а image at http://hordeum.oscs.montana.edu/genographer.

### 5.3.1.3 Leaf rust phenotyping – field

The field trials tested 257 entries including 253 RILs and 4 check varieties: AC Barrie, AC Foremost, BW278, and Thatcher, which were screened in the leaf rust nursery at Glenlea, Manitoba during the 2003 and 2004 field seasons. Trial entries and checks were replicated three times in a randomized complete block design with susceptible spreader rows of 'Little Club'. Spreader rows were placed every alternating third and fourth row to increase and monitor disease development throughout the field. Plots consisted of a single 1 meter row spaced 17 cm apart. Sowing density was approximately 60 seeds per row. The inoculum used throughout the phenotyping process was an epidemic mixture of an equal proportion of the most prominent isolates collected during

the 2002 and 2003 leaf rust disease survey and used in the 2003 and 2004 leaf rust nurseries, respectively (McCallum and Seto-Goh 2004, 2005). Spreader rows were first inoculated at the stem elongation phase. The inoculum mixture was a suspension of 1.5 grams of urediospores / L of light mineral oil (Bayol, Esso Canada, Toronto Ontario). A hand held low volume herbicide applicator was used to apply the inoculum to the spreader rows only. Inoculation was conducted in the afternoon as relative humidity was rising to ensure adequate moisture for spore germination and infection. Spreader rows were re-inoculated twice after heading until disease pressure increased and symptoms were present on the spreader rows.

When pustules became visible on the check and spreader rows, disease ratings were performed every five days on the flag leaves using the modified Cobb Scale (% infection of flag leaf) and pustule type (R=resistant; MR=moderately resistant; MS=moderately susceptible; S=susceptible) (Petersen et al., 1948). There were a total of four ratings conducted per growing season which allowed area under the disease progress curve (AUDPC) to be calculated for each growing season. Days to heading, and leaf tip necrosis (*Ltn*) notes were also taken during the growing seasons to determine if leaf senescence was due to maturity or susceptibility. *Ltn* is a phenotypic marker linked to *Lr34* (Singh 1992b).

# 5.3.1.4 Construction of the genetic map of 7DS

A total of 71 microsatellite markers on chromosome 7DS (Somers et al., 2004) were screened for polymorphism between the parents of the mapping population.

Polymorphic marker primer sequences, annealing temperatures, and allele sizes are listed in Table 5.1. The polymorphic markers were used to genotype the 253 RILs in the population. JoinMap V3.0 (Biometris, Wageningen, The Netherlands, http://www.joinmap.nl) was used to determine the marker order and map distances.

#### 5.3.1.5 Statistical analysis

Analysis of variance (ANOVA) for AUDPC for each site year and a combined analysis across the two site years were performed using the "PROC GLM" procedure of the SAS software package (SAS Institute Inc., Version 8.2). A Bartlett's test for homogeneity was conducted to determine if the data could be combined over site years. The model statement used in the combined analysis was AUDPC= Entry Rep Year Rep(Year) Entry\*Year. All factors in this statement with the exception of entry were considered to be random.

# 5.3.2 Interaction between *Lr34* on *Fhb2*

### 5.3.2.1 Genetic population development

A subset of 1,440 lines of the original 2,300 RILs was randomly selected from the cross between BW278 and AC Foremost. BW278 was the source of FHB resistance in the population, which segregated for three known FHB resistance QTL or genes including QFhb.crc-3BSc, *Fhb.crc-5A* and *Fhb2*. The QTL identified on 3BSc is located proximal to the centromere (Somers et al.. 2003). The 1,440 F<sub>5:7</sub> recombinant inbred lines (RILs) were genotyped using microsatellite markers on chromosome 6B (WMC104, WMC397, GWM219), 5A (GWM154, GWM304, WMC415), and 3BS (WMC78, GWM566,

WMC527) (Somers et al., 2004) which facilitated selection of 89 RILs that were homozygous susceptible for FHB QTL intervals on 3BSc, 5A, and recombinant for the interval on 6B carrying *Fhb2* (Cuthbert et al., 2006b). BW278 is known to lack resistance alleles at *Fhb1* (Cuthbert et al., 2006a) on 3BS near GWM493. These 89 RILs were segregating for *Lr16*, *LrF*, and *Lr34*.

The epistatic effect between Lr34 and Fhb2 was determined by evaluating the leaf rust reaction of the 89 RILs in the growth cabinet using an isolate virulent to Lr16 and LrF; and the FHB reaction of the lines in the greenhouse and field.

# 5.3.2.2 Fusarium head blight phenotyping.

Fusarium head blight disease response was evaluated on the 89 RILs and parental checks in the greenhouse and field to classify each line as resistant or susceptible (Cuthbert et al.. 2006b). Dual floret injection (DFI) was used in the greenhouse to evaluate disease severity (DS). Macroconidial spray inoculations were used in the field nurseries conducted at two locations in southern Manitoba (Carman and Glenlea) over two years 2003 and 2004 to evaluate disease incidence (DI), disease severity (DS), visual rating index (VRI) and Fusarium damaged kernels (FDK).

### 5.3.2.3 Leaf rust phenotyping – growth cabinet

The 89 RILs and resistant (BW278), intermediate resistant (AC Barrie) and susceptible checks (Thatcher, AC Foremost) were arranged in a completely randomized design and grown in a growth cabinet with lighting set for 16 hour daylight and temperature during the day of 18°C and 15°C. RILs and checks were replicated seven times over two cycles

within the same growth cabinet. The relatively low temperature was used to promote tillering. Once the flag leaves emerged the plants were inoculated with *P. triticina* virulence phenotype MGBJ (Long and Kolmer 199; McCallum and Seto-Goh 2003) by suspending urediospores in light oil (Bayol 55, Imperial Oil Canada, Toronto, ON) and spraying the oil/spore mixture on the flag leaves of the adult plants using an air powered inoculator. This isolate was virulent to the seedling resistance genes Lr16 and LrF but avirulent to Lr34. After inoculation, plants were allowed to dry for four hours and were placed overnight in a dew chamber (Percival, model 160D, Perry Iowa) for 16 hours with the following chamber conditions: water reservoir 30°C, chamber wall 5°C, and air temperature 20°C. Plants were placed back into the growth cabinet for infection to develop and flag leaves were rated 14 days post-inoculation using the modified Cobb Scale (1-100%) and pustule type (R/MR/MS/S) (Petersen et al.. 1948).

### 5.3.2.4 Genetic map

A total of 71 microsatellite markers on chromosome 7DS (Somers et al.. 2004) were screened for polymorphism between the parents of the mapping population. Polymorphic marker primer sequences, annealing temperatures, and allele sizes are listed in Table 5.1. The polymorphic markers were used to genotype the 89 RILS. JoinMap V3.0 (Biometris, Wageningen, The Netherlands, http://www.joinmap.nl) was used to determine the marker order and map distances.

# 5.3.2.5 Statistical analysis

A two-way ANOVA was conducted to determine if there was any significant gene interaction between the expression of adult leaf rust resistance from *Lr34* and FHB resistance from *Fhb2* in the 89 RILs. The main variable is represented by FHB and sources of variation include the resistance and susceptibility ratings for leaf rust and FHB. FHB resistance was measured in the 89 RILs using greenhouse disease severity (GH-DS); and field disease incidence (DI), disease severity (DS), visual rating index (VRI), and Fusarium damaged kernels (FDK) data (Cuthbert et al.. 2006b).

Marker	Forward Primer $(5' \rightarrow 3')$	Reverse Primer $(5' \rightarrow 3')$	Anneal	Allele Size	References
			(°C)	BW278	
WMC506	CACTTCCTCAACATGCCAGA	CTTTCAATGTGGAAGGCGAC	61	241	http://wheat.pw.usda.gov
GWM635	TTCCTCACTGTAAGGGCGTT	CAGCCTTAGCCTTGGCG	61	115	Röder et al (1998)
CFD41	TAAAGTCTCAGGCGACCCAC	AGTGATAGACGGATGGCACC	61	296	http://wheat.pw.usda.gov
CFD31	GCACCAACCTTGATAGGGAA	GTGCCTGATGATTTTACCCG	61	243	http://wheat.pw.usda.gov
CFD66	AGGTCTTGGTGGTTTTTGGTG	TTTTCACATGCCCACAGTTG	61	194	http://wheat.pw.usda.gov
WMC463	GATTGTATAGTCGGTTACCCCT	ATTAGTGCCCTCCATAATTGTG	61	178	http://wheat.pw.usda.gov
CFD30	AATCGCACAACAATGGTTCA	GCCTCTCCTCTCTGCTCCTT	61	224	http://wheat.pw.usda.gov
GWM1220	CACGACGTTGTAAAACGACAG	GAATAGAAGTCATCGCGCGT	61	159	Dr. Ganal - Trait genetics
GWM130	AGCTCTGCTTCACGAGGAAG	CTCCTCTTTATATCGCGTCCC	61	148	Röder et al (1998)
GWM44	GTTGAGCTTTTCAGTTCGGC	ACTGGCATCCACTGAGCTG	61	194	Röder et al (1998)
CFD46	TGGTGGTATAGTCGTTGGAGC	CCACACACACACACCATCAA	61	198	http://wheat.pw.usda.gov
WMC121	GGCTGTGGTCTCCCGATCATTC	ACTGGACTTGAGGAGGCTGGCA	61	303	http://wheat.pw.usda.gov

Table 5.1 Primer sequences, annealing temperatures, and allele sizes of markers used in the BW278/AC Foremost population for mapping Lr34 in spring wheat

.

#### 5.4 Results

# 5.4.1 Genetic mapping of Lr34

# 5.4.1.1 Leaf rust field trials

Environmental data were collected and conditions differed substantially between the 2003 and 2004 field seasons. Mean temperature and precipitation during inoculation (June) and prior to rating (July and August) are the most critical for infection and disease development. High amounts of precipitation and flooding in the spring of 2004 affected plant establishment at Glenlea. Temperatures were below normal for the 2003 = 765.7; 2004 = 389.1). Leaf tip necrosis ratings were difficult to distinguish in this population in the field due to the variability in the weather in 2003 and 2004. As a result, this trait was not included in the mapping analysis.

The ANOVA for individual site years indicated entry was highly significant in both 2003 and 2004; however, the replicate effect was non-significant in 2003 and significant in 2004 due to variability of disease development (Table 5.2). Due to the heterogeneity of the error variances the data were presented separately by year.

There were 253 RILs from the 2,300  $F_{5:7}$  line mapping population identified to be susceptible for seedling genes *Lr16* and *LrF* and recombinant in the *Lr34* region. The phenotypic distribution of leaf rust AUDPC over site years was bimodal for this major gene (Fig. 5.1A). The range in % infection of flag leaf of RILs classified as resistant was 12 - 51% and for lines classified as susceptible was 82 - 100%. The population

segregated 115 resistant to 138 susceptible RILs for AUDPC, fitting a 1:1 chi-square ratio ( $X^2 = 2.08$ ; P>0.10). (Fig. 5.1A).

Table 5.2.	Analysis	of variance	for le	eaf rust	area	under	disease	progress	curve	(AUDPC)
collected in g	years (200	3, 2004) in C	Henlea	i, Manito	oba.					

Source	DF	Type III SS	Mean	<b>F-Value</b>	P-value			
			Square					
AUDPC - Year: 2003								
Model	258	159,817,475.4	619,447.6	38.41	<.0001			
Entry	256	159,797,201.8	624,207.8	38.70	<.0001			
Rep	2	300.2	150.1	0.01	0.9907			
Error	442	7,128,923.8	16,128.8					
AUDPC - Yea	r: 2004		1990 West of Party of					
Model	258	90,817,061.0	352,004.1	20.29	<.0001			
Entry	256	90,185,460.3	352,287.0	20.31	<.0001			
Rep	2	601,550.9	300,775.5	17.34	<.0001			
Error	442	8,811,727.2	17,345.9					
AUDPC - Yea	r: Combine	d 2003 & 2004						
Model	517	302,602,169.9	585,304.0	34.88	<.0001			
Entry	256	232,753,716.8	909,194.2	54.18	<.0001			
Rep	2	268,116.6	134,058.3	7.99	0.0004			
Year	1	52,385,469.0	52,385,469.0	3,121.97	<.0001			
Rep(Year)	2	288,738.5	144,369.3	8.60	0.0002			
Entry*Year	256	17,180,020.3	67,109.5	4.00	<.0001			
Error	950	15,940,651.0	16,779.6					



Fig. 5.1 Phenotypic distribution of leaf rust infection (% infection of flag leaf) collected in the field and growth cabinet for the 253 RILs (A) and 89 RILs (B), selected from the population BW278/AC Foremost. The rating scale was based on the modified Cobb Scale (% infection of flag leaf) and pustule type (R=resistant; MR=moderately resistant; MS=moderately susceptible; S=susceptible)

#### 5.4.1.2 Genetic map on 7DS

There were 12 polymorphic markers out of 71 tested on 7DS which were used to genotype the 253 RILs and construct a genetic map of chromosome 7DS (Table 5.1). The marker order of the map was identical to the ITMI map (Roder et al., 1998). The total map length for the population was 52.8 cM (Fig. 5.2A) which is similar to 72 cM on the ITMI map (Roder et al., 1998). The 253 RILs were classified as resistant or susceptible to leaf rust using AUDPC measurements from field data collected over two

site years. Lr34 mapped to a 13.0 cM interval on chromosome 7DS. This gene controlling resistance to adult leaf rust resistance was flanked by GWM1220 and GWM130 (Fig. 5.2A).



Fig. 5.2 Genetic map of the adult leaf rust resistance gene, Lr34 on chromosome 7DS, based on field data (253 RILs) (A) and controlled environment data (89 RILS) (B), of the population BW278/AC Foremost. Rating scale based on the Modified Cobb Scale (% infection of flag leaf).

# 5.4.2 Interaction between effects of Lr34 on Fhb2

### 5.4.2.1 Leaf rust growth cabinet test

Seven plants per RIL of the population containing 89 RILs and checks were inoculated with *P. triticina* in the growth cabinet using adult plant inoculations to assess % infection. The infection ratings showed a bimodal distribution (Fig. 5.1B). The range in % infection of the flag leaf of RILs classified as resistant was 6 - 38% and for lines classified as susceptible was 62 - 94%. There was very low variability (standard error: resistant RILs  $15.3 \pm 0.75$  and susceptible RILs  $76.3 \pm 1.02$ ) within the seven plants of each RIL and no overlap between the resistant and susceptible classes (Fig. 5.1B). Parental checks showed a % flag leaf infection range of 19% (resistant); 50% (intermediate) and 82-86% (susceptible). The population segregated 49 resistant to 40 susceptible plants, fitting a 1:1 chi-square ratio ( $X^2 = 0.72$ ; P<0.5).

#### 5.4.2.2 Fusarium head blight tests

The phenotypic distribution for all five FHB disease infection measurements in the greenhouse and field was bimodal with lines resembling either the resistant or susceptible checks and parents (Fig. 5.3). The 89 RILs in the greenhouse experiment segregated 42 resistant to 47 susceptible plants, fitting a 1:1 chi-square ratio (p<0.90). Phenotypic classification for the 89 RILs in the field for all traits (DI, DS, VRI, FDK) showed the same classification. The population segregated 45 resistant to 44 susceptible RILs for all traits, fitting a 1:1 chi-square ratio (p>0.90). The correlation between the averaged greenhouse and field data measurements for the 89 RILs showed all correlations were high and statistically significant ( $\alpha$ =0.05)

#### 5.4.2.3 Genetic map

A genetic map was constructed by genotyping the 89 RILs with 12 polymorphic microsatellite markers on chromosome 7DS (Table 5.1). The marker order of the map was identical to the ITMI map (Röder et al., 1998). The total map length for the 7DS segment in the 89 RILs was 76.9 cM (Fig. 5.2B) which is similar to 72 cM on the ITMI map (Roder et al., 1998) and 52.8 of the genetic map created using the 253 RILs population in this study. The 89 RILs were classified as resistant or susceptible to leaf rust using AUDPC measurements from growth cabinet data collected over seven replications. Lr34 mapped to a 3.0 cM interval on chromosome 7DS. This gene controlling resistance to adult leaf rust resistance was flanked by GWM1220 and GWM130 (Fig. 5.2B).

### 5.4.2.4 Gene interaction

The presence or absence of Lr34 had no significant effect on the expression of FHB resistance parameters (Table 5.3). However, there was a significant effect (P = 0.05) of *Fhb2* in lowering all the FHB parameters measured (Table 5.3). There was a clear bimodal distribution between those lines that carried *Fhb2* and those that did not for all the FHB parameters (Figure 5.3). *Fhb2* is the only FHB gene segregating in the population; therefore, *Fhb2* would be expected to be significant. The interaction between Lr34 and *Fhb2* was not statistically significant for any of the FHB parameters measured (Table 5.3) based on a two-way ANOVA.

Table 5.3. Two-way analysis of variance to measure the effect of the interaction between Fusarium head blight (FHB) resistance gene Fhb2 and leaf rust resistance gene Lr34. Data for FHB was collected in the greenhouse (disease severity) (GH-DS) and at two field locations (Carman, Glenlea, MB) over two years (2003, 2004) (disease severity, disease incidence, visual rating index, Fusarium damaged kernels). Leaf rust data was collected in growth cabinet using the rating scale of modified Cobb Scale.

Source	DF	Type III SS	Mean Square	F-Value	P-value					
Greenhouse - Di	Greenhouse - Disease Severity (GH-DS)									
Model	3	355.63	118.54	34.90	< 0.0001					
Lr34	1	2.72	2.72	0.80	0.3732					
Fhb2	1	295.30	295.30	86.94	< 0.0001					
Lr34*Fhb2	1	2.70	2.70	0.79	0.3754					
Error	85	288.71	3.40							
Field - Disease S	everity (Field	d-DS)								
Model	3	520.63	173.54	261.65	< 0.0001					
Lr34	1	1.95	1.95	2.94	0.0901					
Fhb2	1	442.76	442.76	667.55	< 0.0001					
Lr34*Fhb2	1	0.01	0.01	0.01	0.9282					
Error	85	56.38	0.66							
Field – Disease I	ncidence (Fi	eld-DI)								
Model	3	375.70	125.23	639.85	< 0.0001					
Lr34	1	0.35	0.35	1.80	0.1829					
Fhb2	1	326.81	326.81	1669.73	<0.0001					
Lr34*Fhb2	1	0.16	0.16	0.80	0.3739					
Error	85	16.64	0.20							
Field –Visual Ra	ting Index (I	Field-VRI)								
Model	3	44495.93	14831.98	649.66	< 0.0001					
Lr34	1	61.47	61.47	2.69	0.1045					
Fhb2	1	38464.15	38464.15	1684.78	< 0.0001					
Lr34*Fhb2	1	40.15	40.15	1.76	0.1883					
Error	85	1940.58	22.83							
Field – Fusarium Damaged Kernels (Field – FDK)										
Model	3	53558.73	17852.91	408.19	< 0.0001					
Lr34	1	1.69	1.69	0.04	0.8445					
Fhb2	1	47363.03	47363.03	1082.91	<0.0001					
Lr34*Fhb2	1	61.93	61.93	1.42	0.2374					
Error	85	3717.62	43.74							

# 5.5 Discussion

Leaf rust infection data were collected from the field on the 253 RILs to map the Lr34 resistance gene on 7DS. The ANOVA for combined site years indicated a significant level of variation arising from year, rep and entry (Table 5.2). The significant level of variation over years was due to the varying level of disease pressure between the two field seasons as a result of the differences in environmental conditions in 2003 and 2004. The ANOVA showed the entries were highly significant and using the distribution of disease reaction each RIL could be classified as either resistant or susceptible.

To determine the interaction between Lr34 and Fhb2 a different subset of 89 RILs were phenotyped for both leaf rust and FHB. Leaf rust infection data were collected on the 89 RILs segregating at both the Fhb2 and Lr34 loci. To reduce the confounding effect of multiple leaf rust resistance genes, phenotyping of Lr34 was conducted in a growth cabinet using adult plant inoculations with a *P. triticina* culture that was virulent to Lr16and LrF (Fig. 5.1).

This study provided a unique approach to map the gene Lr34 using the BW278 source of resistance in two groups of RILs from the same spring wheat mapping population. The data collected in the field and growth cabinet for leaf rust resistance mapped to one coincident location on chromosome 7DS (Fig. 5.2). The total genetic distance between the two flanking markers CFD66 and CFD46 on the wheat ITMI map is 72 cM (Somers (data unpublished)) and was 52.8 and 76.9 cM in the 253 RILs and 89 RILs population, respectively. The difference in genetic distance between the two groups of RILS from the same population may be attributed to the distortion in the segregation of the 89 RILs possibly caused by the difference in sample size of the initial population

(2,300 and 1,400 lines) and sampling of RILs to create the genetic map for the 253 and 89 RILs, respectively (Fig.5.2). The segregation ratios for the two genes in the population were Fhb2+Lr34+25: Fhb2+Lr34-20: Fhb2-Lr34+15: Fhb2-Lr34-29. A chi-square test of independence was conducted for the two genes, Fhb2 and Lr34, and the results indicated that data does not differ significantly from the expected 1:1:1:1 ratio for the 89 RILs (X<sup>2</sup> = 3.3, P>0.05).

The distance to flanking markers surrounding Lr34 in the mapping population with 253 RILs is 3.8 and 9.5 cM; 89 RILs is 0.6 and 2.4 cM (Fig. 5.2). Comparisons of physical and genetic maps of wheat indicate that most genetic recombination occurs in gene-rich, telomeric regions (Gill et al., 1996; Faris et al., 2000). In previous studies, researchers have been able to map Lr34 to 7DS and indicate the closest microsatellite locus is GWM295 (Dyck et al., 1966; Singh et al., 2000; Boukhatem et al., 2002; Ramburan et al. 2004; Schnurbusch et al. 2004a). In a recent study by Spielmeyer et al. 2005, Lr34 was mapped to a single locus flanked by microsatellite loci GWM1220 and GWM295 on chromosome 7DS. Microsatellite marker GWM130 is coincident to GWM295. The distance to flanking markers GWM1220 and GWM130 in the study by Spielmeyer et al.. (2005) is 0.9 and 5.4 cM. The present study differed from that by Spielmeyer et al.. (2005) in that the group of 253 RILs mapped the gene Lr34 in isolation with no other background effects provided by secondary leaf rust genes. The infection ratings from the growth cabinet (89 RILS) were lower for the resistant RILs, compared to the 253 RILs from the same cross that were rated for leaf rust in the field. This could be due to the presence of other leaf rust seedling resistance genes (Lr16 and LrF) in combination with Lr34 versus the 253 RILs segregating for only Lr34 (Fig. 5.1); or
reflect the inherent differences between artificial inoculation in the growth cabinet and natural infection from inoculated field nurseries.

Based on statistical data provided in this study (Table 3), there was no indication that the RILs containing Lr34 resistance were more resistant to FHB than those lines that did not contain Lr34. The *Fhb2* effect was significant (P < 0.05) for all FHB parameters; whereas Lr34 had no significant effect on the expression of all FHB resistance parameters measured (Table 5.3). Figure 5.3 also demonstrates the strong effect of *Fhb2* segregating within the 89 RILs where those lines with *Fhb2* are clearly distinguished from those without *Fhb2* for all the FHB parameters measured. This effect is significant because *Fhb2* is the only FHB resistance gene segregating in the 89 RILs, whereas Lr34was not significant at the 0.05 level and there was no interaction between *Fhb2* and *Lr34*.

FHB data tends to be quite variable in nature due to the influence of environmental factor on disease symptoms making it difficult to determine smaller difference in levels of resistance. The effect of the gene interaction may be less dramatic since *Fhb2* is the only FHB resistance gene present in the population and the fact that the population size is small. FHB resistance is polygenic (Bai and Shaner 1994) and is strongest when multiple resistance genes are present within a population (Berzonsky et al.. 2007). Although the objective of this study was reached, it would be interesting to conduct this same study using a population segregating for multiple FHB resistance genes, including the major FHB resistance gene *Fhb1* to see if the results would be different.



Fig. 5.3 Distribution of the number of lines for 89 RILs from the cross BW278/AC Foremost carrying various combinations of resistance genes *Fhb2* and *Lr34* to demonstrate the effect of interaction of the two resistance genes. Percent of infection was measured from one replicated greenhouse (GH) experiment and four replicated field disease nurseries over four site-years. FHB resistance and susceptibility is displayed as a percent infection (1 to 100%) on individual histograms for each trait measured: (A) disease severity (GH-DS); (B) field - disease severity (DS); (C) field - disease incidence (DI); (D) field - visual rating index (VRI); and (E) field - Fusarium damaged kernels (FDK). Classes of 89 RILs carrying  $Lr34 + (\blacksquare)$ ,  $Lr34 - (\Box)$  are represented by bars on graph.

Lr34 is a very important disease resistance gene in wheat breeding programs worldwide because it provides enhancement of resistance to multiple diseases. Based on data from this study, there was no clear enhancement of FHB resistance in RILs containing both Lr34 and Fhb2. The use of Lr34 instead of other leaf rust resistance genes will have little effect on the progress that can be made in the FHB reaction. This indicates development of cultivars resistant to these two major wheat diseases in Canada will require pyramiding disease resistance genes to leaf rust and FHB.

## 6.0 GENERAL DISCUSSION

Fusarium head blight and leaf rust are two of the most devastating diseases of spring wheat in western Canada. There are many mechanisms for managing the two fungal diseases; however, pyramiding genetic host resistance is considered to be the most efficient and cost effective over the long term. Plant breeders can use classical and molecular plant breeding to pyramid *Fhb1* (Type II FHB resistance), *Fhb2* (FHB field resistance), and *Lr34* (slow rusting adult leaf rust resistance) along with other disease resistance genes to develop cultivars of spring wheat with suitable levels of FHB and leaf rust resistance.

The ability to select desirable individuals in a breeding population based on genotype is an extremely powerful application of DNA markers and QTL mapping. DNA markers in genomic regions of interest enable breeders to select on the basis of genotype rather than phenotype. This can be especially helpful for target traits such as Fusarium head blight and leaf rust that are time consuming and laborious to score. By simply eliminating or reducing the need for field trials early in a breeding program, marker assisted breeding may revolutionize the process of cultivar development.

Quantitatively inherited traits have a strong genetic component but under normal conditions of measurement and population design, cannot be shown to be controlled by individually recognizable loci. There are many reasons for the inability to recognize individual loci. Some disease reactions are difficult to score reliably and others are highly sensitive to the environment. Environmentally sensitive traits are difficult to measure accurately, resulting in lowered estimates of heritability and a reduced likelihood

of segregation in a Mendelian fashion unless special experimental precautions have been taken.

FHB is a complex, quantitative trait. The present study allowed the design of spring wheat populations with an FHB resistance QTL to be expressed as a Mendelian factor using genotyping and phenotyping to isolate and map the locus of interest in a fixed resistant and/or susceptible background. There were two sources of FHB resistance (Sumai 3 and Nyubai) used in the study and phenotyping was performed in two different environments (greenhouse and field). The design and development of populations using genotyping and phenotyping to isolate the QTL of interest as a Mendelian factor allowed a complex quantitative trait to be mapped qualitatively.

*Fhb1* was fine mapped to the syntenic interval on 3BS as a Mendelian factor in both a fixed resistant (Thatcher/5\*Sumai3) (T/S) and susceptible (HC374/3\*98B69-L47) (HC/98) background. The major effect of *Fhb1* on Type II resistance was clearly evident in the susceptible fixed recombinant plants of the T/S population and even in a fixed resistant background. When the infection range for phenotypic data was analyzed, the degree of resistance in phenotypic data is greater in the T/S fixed recombinant plants (0-5%) versus the HC/98 fixed recombinant plants (5-25%) and non-recombinant resistant check plants (0-8%). These data from both populations suggests that *Fhb1* provides a significant degree of Type II resistance and *Fhb1* is an additive gene relative to other FHB resistance loci.

*Fhb2* was qualitatively mapped to chromosome 6BS in a fixed susceptible population (BW278/AC Foremost) which differed from the mapping of *Fhb1*. Although, the source of resistance was similar, Sumai 3, the phenotypic data was collected using

double floret injections (DFI) in the greenhouse to measure disease severity (DS) and also using conidial spray inoculations in the field to measure field disease incidence (DI), severity (DS), visual rating index (VRI) and Fusarium damaged kernels (FDK). Fhb1 only used greenhouse single floret injection (SFI) for phenotypic mapping data because Type II FHB resistance that was being mapped. For the *Fhb2* study, the DS was measured in the greenhouse and field to ensure consistency amongst ratings and proper characterization of recombinant inbred lines (RILs). There were 12 RILs that were recombinant between the GH-DS and field traits. The overall rating results indicated that GH-DS were greater than Field DS; however the GH-DS and Field-DS were highly correlated. The data also showed that all four field measurements of FHB resistance mapped to one coincident location on chromosome 6BS, represented by Fhb2. The GH-DS measurement mapped to 2 cM distal to Fhb2 due to the presence of the 12 recombinant lines between GH-DS and *Fhb2*. These results indicate that field resistance is controlled by Fhb2 and an additional locus 2 cM distal from Fhb2 provides Type II Based on the size of the population (89 RILs), this result should be resistance. interpreted with caution and the experiment repeated using a larger population size. However, in studies by Yang et al. (2003) and Shen et al. (2003), Fhb2 was estimated to map within 2 cM of the QTL intervals suggesting the FHB resistance QTL on 6BS and *Fhb2* are likely coincident. Yang et al. (2003) reported a coefficient of determination on GH-DS of 21% for the microsatellite marker GWM644 on 6BS, which reduced DS by 52%. In the present study the resistant allele on 6BS reduced FHB GH-DS by 56% when compared to the RILs carrying the susceptible allele. *Fhb2* provides field resistance as a single gene present in a susceptible background.

The use of molecular markers and marker assisted selection facilitates the mapping of specific loci in genetically simple and complex traits and allows genetic interactions between resistance genes to be discovered. QTL mapping has uncovered a number of examples of epistatic interactions in quantitative resistance. The ability to develop spring wheat varieties with enhanced resistance levels simultaneously may involve the resistance genes Lr34 and Fhb2; and therefore, understanding their genetic interaction may be important for breeders.

In the final chapter of this thesis, the objective was two fold, to map the adult leaf rust resistance gene Lr34 and to measure any gene interaction between Lr34 and Fhb2. Lr34 was successfully mapped in two sub-populations of the cross BW278/AC Foremost. Lr34 mapped to a coincident interval in both sub-populations (GWM1220 and GWM130) with a difference in the mapping interval. The one population, 89 RILs, that was used to map Fhb2 was also used to map Lr34 and determine if there was any gene interaction between Fhb2 and Lr34. Statistical analysis was conducted and the result of the ANOVA indicated that there was no clear positive effect of gene interaction when the two genes were present in the same lines together.

There was no clear phenotype improvement in the population when Lr34 and Fhb2 were present in the same lines, therefore, breeders will have to select for the genes and disease resistance individually and use gene pyramiding to develop durable resistant lines for the two diseases.

Information from this thesis regarding the location and genetic interaction of key disease resistance genes such as *Fhb1*, *Fhb2* and *Lr34* should aid plant breeders worldwide to select for a small genetic interval around the gene of interest reducing

"linkage drag" that frequently occurs from a donor parent. This knowledge also lays the foundation that will be very useful in assisting in cloning of the functional resistance gene(s).

While the concept of QTL mapping seems clear and simple, there are still many practical limitations in practice. Many DNA marker maps are not sufficiently dense to achieve the potential of QTL mapping, since sparse marker maps severely limit the power of QTL mapping. Even under optimal conditions, multiple QTLs on a single linkage group are difficult or impossible to resolve. Populations must be relatively large to uncover some loci, and the biological relevance of loci and interactions depends on the cut-off chosen for statistical significance. Since the traits of interest are, by nature, genetically complex, environmental factors and genetic background potentially have an enormous impact on results. Of course, this is one of the most powerful applications of QTL mapping (i.e. analyzing gene x gene and gene x environment interactions) but it also means that many large, time-consuming experiments as the ones in this thesis need to be conducted.

QTL mapping like any genetic study is only as good as its phenotypic scoring method. In studies such as this one involving complex disease resistance, factors ranging from suitable inoculum source to difficulties in quantitative assessment of resistance make QTL mapping more challenging. Fortunately, powerful computer software and programs are now available to analyze QTL mapping results and better DNA marker systems have been developed to simplify the technique and increase marker density.

The application of marker assisted breeding for disease resistance takes on special roles. Obviously pyramiding resistance genes (*Fhb1*, *Fhb2*, *Lr34*) into valuable genetic

background is simplified through the use of marker based selection. This should be especially helpful when screening for one resistance gene interferes with the ability to screen for another, a frequent occurrence in disease resistance breeding. Rather than screen sequentially for the inheritance of single resistance (or simultaneously through progeny screens), individuals that have retained all of the genes of interest can be selected based solely on DNA marker genotypes. Similarly, gene deployment can be accelerated through the use of marker-assisted breeding. This approach, in which cultivars with complementary sets of resistance genes (*Fhb1*, *Fhb2*, *Lr34*) with differing race-specificities are grown by farmers, aims at achieving durable disease protection. However, there are few, if any, published reports of resistance gene pyramiding or deployment using DNA markers.

In theory, the capacity to pyramid or deploy genes of interest is not restricted to major, single locus resistance genes. With QTL mapping, partial resistance loci can be treated as Mendelian factors and manipulated just like any major gene, as seen in these studies. Quantitative resistance loci from diverse donors can be rapidly introduced into a desirable genetic background or deployed in a set of cultivars. The ultimate achievement of QTL mapping technology in the future will be the molecular cloning of the key resistance genes *Fhb1*, *Fhb2* and *Lr34* as well as other that confer partial resistance.

In conclusion, genes that control quantitative disease resistance in plants have been difficult to identify or characterize precisely. The need for new types of disease resistance in agriculture, especially those that hold the promise of long term duability, calls out to plant pathologists, breeders, geneticists, and molecular biologists to turn their attention to polygenic resistance phenotypes. It will be necessary in the future to discover new sources of *Fhb1*, *Fhb2* and *Lr34* before the resistance genes are overcome by new races/isolates of disease and these genes are no longer effective. Before the advent of QTL mapping, analyzing the genes that control complex disease resistance traits was an overwhelming task. With DNA markers and QTL mapping, complex forms of disease resistance and their underlying genes are now far more accessible. Someday soon, the distinction between qualitative and quantitative disease resistance may finally disappear.

## 7.0 REFERENCES

Agrios, G. 1997. Plant pathology, 4<sup>th</sup> edition. San Diego, Academic press.

Andersen, A.L. 1948. The development of *Gibberella zeae* head blight of wheat. Phytopathology 38:595-611

Anderson JA, Stack RW, Liu S, Waldron BL, Fjeld AD, Coyne C, Moreno-Sevilla B, Mitchell-Fetch J, Song QJ, Cregan PB, Frohberg RC. 2001. DNA markers for Fusarium head blight resistance QTLs in two wheat populations. Theor Appl Genet 102:1164-1168

Anikster, Y., Bushnell, W.R., Eilam, T., Manisterski, J., and Roelfs, A.P. 1997. *Puccinia recondite* causing leaf rust on cultivated wheats, wild wheats, and rye. Can. J. Bot. 75: 2082-2096.

Atanasoff, D. 1920. Fusarium-blight (scab) of wheat and other cereals. J. Agr. Res. 20: 1-40.

Bai G, Xiao QP. 1989. Genetic analysis on resistance to scab in six wheat cultivars. Acta Agr Shanghai 5:17-23

Bai G, Zhou CF, Qian CM, Ge YF. 1990. Genetic analysis on resistance genes to scab spread in wheat cultivars. In Zhu LH (ed) Advances in Genetic Researches on Resistance to Diseases in Major Crops, Jiangsu Sci Tech Publ House, Nanjing China, pp 171-177

Bai G, Shaner G. 1994. Scab of wheat: prospects for control. Plant Dis 78:760-766

Bai G, Kolb FL, Shaner G, Domier LL. 1999. Amplified fragment length polymorphism markers linked to a major quantitative trait locus controlling scab resistance in wheat. Phytopathology 89:343-348

Bailey, KL, Gossen, BD, Gugel, RK, and Morrall RAA. 2003. Diseases of Field Crops. Canadian Phytopathological Society. Pp: 98-99, 93.

Ban, T. 2000. Review studies on the genetics of resistance to Fusarium head blight caused by *Fusarium graminearum* in wheat. In Raupp J, Ma Z, Chen P, Liu D (eds), Proc Int Sym Wheat Improve Scab Resistance, May 5-11, 2000, Suzhou and Nanjing China, pp 82-93

Ban, T. and Suenaga, K. 1998. Genetic analysis of resistance to Fusarium head blight caused by *Fusarium graminearum* in wheat. In Proc 9<sup>th</sup> International Wheat Genet Symposium, 2-7 August 1998, Saskatoon, Sask. Edited by A. E Slinkard. University Extension Press, Saskatoon, Sask. pp. 192-199.

Bariana, HS., Hayden MJ., Ahmed, NU., Bell, JA, Sharp, PJ., and McIntosh, RA. 2001. Mapping of durable adult plant and seedling resistances to stripe rust and stem rust diseases in wheat. Aust. J. Agric. Res. 52: 1247-1255.

Bennett, FT. 1931. *Giberella saubinetii.* (Mont) Sacc. On British cereals. II: Physiological and pathological studies. Ann. Appl. Biol. 18: 158-177.

Berzonsky, W., Gebhard, BL, Gamotin, E., Leach, GD, And Ali, S. 2007. A reciprocal backcross monosomic analysis of the scab resistant spring wheat (*Triticum aestivum* L.) cultivar, 'Frontana'. Plant Breeding 126 (3), 234–239.

Bold, H.C., Alexopoulos, C.J., and Delevoryas, T. 1987. Morphology of plants and fungi: 5<sup>th</sup> edition. Pp. 791. Harper and Row, New York, USA.

Boukhatem, N., Baret, P.V., Mingeot, D., Jacquemin, J.M. 2002. Quantitative trait loci for resistance against yellow rust in two wheat-derived recombinant inbred line populations. Theor. Appl. Genet. 104:111-118

Bryan, G.J., Collins, AJ., Stephenson, P., Orry, A., Smith, JB. 1997. Isolation and characterization of microsatellites from hexaploid bread wheat. Theor. Appl. Genet. 94: 557-563.

Buestmayr, H., Lemans, M., Fedak, G., and Ruckenbauer, P. 1999. Back-cross reciprocal analysis of Fusarium head blight resistance in wheat (*Triticum aestivum* L.). Theor. Appl. Genet. 98: 76-85.

Buerstmayr H, Lemmens M, Hartl L, Doldi L, Steiner B, Stierschneider M, Ruckenbauer P. 2002. Molecular mapping of QTLs for Fusarium head blight resistance in spring wheat, I, Resistance to fungal spread (Type II resistance). Theor. Appl. Genet. 104:84-91.

Burr, B., Burr, F.A., Thompson, K.H., Albertson, M.C., and Stuber, C.W. 1988. Gene mapping with recombinant inbreds in maize. Genetics 118: 519-526.

Campbell, K.A.G and Lipps, P.E. 1998. Allocation of resources: sources of variation in Fusarium head blight screening nurseries. Phytopathology 88:1078-1086.

Chalmers, K.J., Campbell, A.W., Kretschmer, J., Karakousis, A., Henscheke, P.H., Pierens, S., Harker, N., Pallotta, M., Cornish, G.B., Shariflou, M.R., Rampling, L.R., McLauchlan, A., Daggard, G., Sharp, P.J., Holton, T.A., Sutherland, M.W., Appels, R., and Langridge, P. 2001. Construction of three linkage maps in bread wheat, *Triticum aestivum*. Aust. J. Agric. Res. 52: 1089-1119.

Chao, S.P., Sharp, P.J., Worland, A.J., Warham, E.J., Koebner, R.M.D., and Gale, M.D. 1989. RFLP-based genetic maps of wheat homeologous group 7 chromosomes. Theor. Appl. Genet. 78: 493-504.

Chen, CH (1983) A study on the inheritance of scab-resistance in wheat. Acta Agric. Univ. Zhejiangensis 9:115-126

Cloutier S, McCallum, BD, Loutre, C, Banks, TW, Wicker, T, Feuillet, C, Keller B, and Jordan, M. Leaf rust resistance *Lr1*, isolated from bread wheat (*Triticum aestivum* L.) is a member of the large psr567 gene family. Plant Molecular Biology. 65: 102-106.

Crop Protection Guide 2007.

Cuthbert P.A, Somers D.J, Thomas J, Cloutier S, and Brule-Babel, A. 2006a. Fine mapping *Fhb1*, a major gene controlling Fusarium head blight resistance in bread wheat (*Triticum aestivum* L.). Theor Appl Genet. 112: 1462-1472

Cuthbert, P.A., Somers, D.J., and Brule-Babel, A. 2006b. Mapping of *Fhb2* on chromosome 6BS: a gene controlling Fusarium head blight resistance in bread wheat (*Triticum aestivum* L.) Theor Appl Genet. 114: 429-437

D'Ovidio, R., Tanzarella OA, Porceddu, E. 1990. Rapid and efficient detection of genetic polymorphisms in wheat through amplification by the polymerase chain reaction. Plant. Mol. Biol. 15: 169-171.

Devos, KM., and Gale, MD. 1992. The use of random amplified polymorphic DNA markers in wheat. Theor. Appl. Genet. 84: 567-572.

Devos, KM, and Gale, MD. 1993a. Extended genetic maps of the homoeologous group 3 chromosomes of wheat, rye, and barley. Theor. Appl. Genet. 85: 649-652.

Devos, KM, and Gale, MD. 1993b. The genetic maps of wheat and their potential in plant breeding. Outlook Agric. 22: 93-99.

Devos, KM, and Gale, MD. 1997. Comparative genetics in the grasses. Plant Mol. Biol. 35: 3-15.

Devos, KM., Atkinson, MD, Chinoy, CN, and Gale MD. 1992. RFLP based genetic map of the homoeologous group 3 chromosomes of wheat and rye. Theor. Appl. Genet. 83: 931-939.

Devos, KM., Bryan GJ, Collins AJ., Stephenson, P., Gale, MD. 1995. Application of two micrsatellite sequences in wheat storage proteins as molecular markers. Theor. Appl. Genet. 83: 931-939.

Dill-Macky, R., and Jones, R.K. 1999. Effects of previous crop and tillage on Fusarium head blight of wheat. Phytopathology 89: S21 (abstract).

Dreijepondt, S.C., and Pretorius, Z.A. 1989. Greenhouse evaluation of adult-plant resistance conferred by the gene *Lr34* to leaf rust of wheat. Plant Dis. 73:669-671

Dusabenyagasani, M., Hamelin, RC., Collin, J., Dostaler, D. 1997. Effect of cultivar and strain interactions in the screening of resistance against wheat scab caused by *Fusarium graminearum*. Phytoprotection 78: 53-60.

Dyck, PL. 1977. Genetics of leaf rust reaction in three introductions of common wheat. Can. J. Genet.Cytol. 19: 711-716.

Dyck PL. 1987. The association of a gene for leaf rust resistance with the chromosome 7D suppressor of stem rust resistance in common wheat. Genome 29:467-469

Dyck, P.L. 1993. Inheritance of leaf rust resistance in the wheat cultivar Pasqua. Can. J. Plant Sci. 73: 903-906.

Dyck, P.L. 1994. Genetics of leaf rust resistance in 13 accessions of the Watkins wheat collection. Euphytica 80: 151-155.

Dyck, P.L, Samborski, D.J, Anderson, R.G. 1966. Inheritance of adult-plant leaf rust resistance derived from the common wheat varieties Exchange and Frontana. Can J Genet Cytol: 665-671

Dyck, P.L., and Samborski, D.J. 1970. The genetics of two alleles for leaf rust resistance at the *Lr14* locus in wheat. Can. J. Genet. Cytol. 12: 689-694.

Faris, J.D., Haen, K.M, and Gill, B.S. 2000. Saturation mapping of a gene-rich recombination hot spot region in wheat. Genetics 154:823-835

Fernandez, M.R. 1992. The effect of *Trichoderma harzianum* on fungal pathogens infesting wheat and black oat straw. Soil Biol. Biochem. 24: 1031-1034.

Fernando, W.G.D., Paulitz, T.C., Seaman, W.L. Dutilleul, P., and Miller, JD. 1997. Head blight gradients caused by *Gibberella zeae* from area sources of inoculum in wheat field plots. Phytopathology 87: 414-421.

Feuillet, C, Schachermayr, G, and Keller, B. 1997. Molecular cloning of a new receptor like kinase gene encoded at the Lr10 disease resistance locus of wheat. The Plant Journal 11: 45-52.

Flor, H.H. 1956. The complementary genic systems in flax and flax rust. Adv. Genet. 8: 29-54.

Food and Agriculture Organization of the United Nations Codex Alimentarius. 2002. November newsletter. http://www.scabusa.org/pdfs/02-03\_CODEX\_DON.pdf

Francl, L., Shaner, G., Bergstrom, G., Gilbert, J., Pederson, W., Dill-Macky, R., Sweets, L., Corwin, B., Jin, Y., Gallenberg, D., and Wiersma, J. 1999. Daily inoculum levels of *Gibberella zeae* on wheat spikes. Plant Dis. 83: 662-666.

German, S.E., Kolmer, J.A. 1992. Effect of gene *Lr34* in the enhancement of resistance to leaf rust of wheat. Theor Appl Genet 84:97-105.

Gilbert, J., and Tekauz, A. 1995. Effects of Fusarium head blight and seed treatment on germination, emergence, and seedling vigour of spring wheat. Can J. Plant Pathol. 17: 252-259.

Gilbert J, Tekauz A. 2000. Review: Recent developments in research on Fusarium head blight of wheat in Canada. Can J Plant Pathol 22:1-8

Gill, K.S., Gill, B.S., Endo, T.R., Taylor, T. 1996. Identification and high-density mapping of gene-rich regions in chromosome group I of wheat. Genetics 144:1883-1891

Gu, J.Q. 1983. A study on genetics of wheat scab resistance. Sci Agr Sin 6:61-64

Gupta, P.K., Balyan, H.S., Sharma, P.C., and Ramesh, B. 1996. Microsatellites in plants: a new class of molecular markers. Curr. Sci. 70: 45-54.

Gupta, P.K., Varshney, R.K., Sharma, P.C., and Ramesh, B. 1999. Molecular markers and their application in wheat breeding. Plant Breed. 118: 369-390.

Gupta, PK., and Varshney, RK. 2000. The development and use of microsatellite markers for genetic analysis and plant breeding emphasis on bread wheat. 113: 163-185.

Gupta, P, Balyan, H., Edwards, K., Isaac, P Isaac, Korzun, V., Röder, MS., Gautier, MF, Joudrier, P., Schlatter, A, Dubcovsky, J., De la Pena, R., Khairallah, M., Penner, G., Hayden, M., Sharp, P., Keller, B., Wang, R., Hardouin, J., Jack, P. and Leroy, P. 2002. Genetic mapping of 66 new microsatellite (SSR) loci in bread wheat. 105: 1432-2242.

Han, F.P., Fedak, G., Ouellet, T., Dan, H., Somers, D.J. 2005. Mapping of genes expressed in *Fusarium graminearum*-infected heads of wheat cultivar 'Frontana'. Genome 48:88-96

Hanson EW, Ausemus ER, Stakman EC. 1950. Varietal resistance of spring wheats to fusarial head blight. Phytopathology 40:902-914

He, S., Ohm, H., and Mackenzie, S. 1992. Detection of DNA sequence polymorphisms among wheat varieties. Theor. Appl. Genet. 84: 573-578.

Helentjaris, T., Slocum, M., Wright, S., Schaefer, A., and Nienhuis, J. 1986. Construction of genetic linkage maps in maize and tomato using restriction fragment length polymorphisms. Theor. Appl. Genet. 72: 761-769.

Huang, X., Hsam, SLK., Zeller, FJ., Wenzel, G., and Mohler, V. 2000. Molecular mapping of wheat powdery mildew resistance gene Pm24 and marker validation for molecular breeding. 101: 407-414.

Huang, X., Borner, A., Roder, MS., and Ganal, M. 2002. Assessing genetic diversity of wheat (*Triticum aestivum* L.) germplasm using microsatellite markers. Theor. Appl. Genet. 105: 699-797.

Huang L, Brooks S, Wanlong L, Fellers JP, Trick HN, and Gill BS. 2003. Map-based cloning of leaf rust resistance gene *Lr21* from the large and polyploidy genome of bread wheat. Genetics 164: 655-664.

Kimber, G., and Sears, E.R. 1987. Evolution in the Genus Triticum and the Origin of Cultivated Wheat. Pp: 154-164. *In:* Wheat and Wheat Improvement, Second Edition. E.G. Heyne ed. ASA, CSSA, and SSSA, Madison, WI. 765 pp.

Kolb FL, Bai GH, Muehlbauer GJ, Anderson JA, Smith KP, and Fedak G. 2001. Host plant resistance genes for Fusarium head blight: mapping and manipulation with molecular markers. Crop Sci. 41:611-619

Kolmer, J.A., and Dyck, P.L. 1994. Gene expression in the *Triticum aestivum-Puccinia recondita* f.sp. *tritici* gene-for-gene system. Phytopathology 84: 437-440.

Kolmer, JA. 1998. Physiologic specialization of *Puccinia recondita* f.sp. *tritici* in Canada in 1996. Can. J. Plant. Pathol. 20: 176-181.

Knott, D.R. 1989. The wheat rusts: breeding for resistance. Monographs on Theoretical and Applied Genetics 12. Springer, Berlin

Leonard, W.H., and Martin, J.H. 1963. Cereal crops. The Macmillan Co., New York. 825 pp.

Leonard, KJ., and Bushnell, WR. 2003. Fusarium head blight of Wheat and Barley. American Phytopathological Society, St. Paul, Minnesota.

Lin, Y.B., Yan, Z.P., and Wu, Z.S. 1992 Genetic analysis of resistance to scab (*Gibberella zeae*) in wheat varieties from different regions. Acta Agric. Shanghai 8:31-36

Lin, J.J., Kuo, J. Ma J., Saunders, J.A., Beard, H.S., MacDonald, M.H., Kenworthy, W., Ude, GN., Matthews, BL. 1996. Identification of molecular markers in soybeans: Comparing RFLP, RAPD, and AFLP DNA mapping techniques. Plant Mol. Biol. Rep. 14: 156-169.

Liu, B. 1998. Statistical genomics. CRC Press L.L.C, Boca Raton, FL.

Liu, YG, Mori, N, and Tsunewaki, K. 1990. Restriction fragment length polymorphism (RFLP) analysis in wheat. I. Genomic DNA library construction and RFLP analysis in common wheat. Jpn J. Genet. 65: 367-380.

Liu S, and Anderson, J.A. 2003. Targeted molecular mapping of a major wheat QTL for Fusarium head blight using ESTs and synteny with rice. Genome 46:817-823

Liu S, Griffey, CA, and Saghai Maroof, MA. 1998. Preliminary report on molecular marker analysis of adult plant resistance to pwdery mildew in winter wheat massey. In: A.E. Slinkard (ed) Proc. 9<sup>th</sup> Int. Wheat Genet. Symp. Vol 3: 132-134, Univ. Extension Press., Univ. of Saskatchewan, Saskatoon.

Liu YG, and Tsunewaki K. 1991. RFLP analysis in wheat II. Linkage maps of the RFLP sites in common wheat. Jpn. J. Genet. 66: 617-633.

Liu S, Pumphrey M, and Anderson JA. 2003. Fine mapping a major wheat QTL for Fusarium head blight resistance. In 10<sup>th</sup> Int Proc Wheat Genetics Sym, Paestum, Italy 1-6 September 2003, Volume 1, Section (6)368-371

Long, D.L., and Kolmer, J.A. 1989. A North American system of nomenclature for *Puccinia recondite* f.sp. *tritici*. Phytopathology 79: 525-529.

Ma, H., and Singh, R.P. 1996. Contribution of adult plant resistance gene *Yr1* in protecting wheat from yellow rust. Plant Dis 80:66-69

MacInnes, J., and Fogelman, R. 1923. Wheat scab in Minnesota. Univ. Minn. Agric. Exp. Stn. Tech. Bull. 18.

McCallum, B., Seto Goh, P., Dunsmore, K., and Hiebert, C. 2000. Leaf rust of wheat in western Canada in 1999. Can. Plant Dis. Surv. 80: 65 (http://www.cps-scp.ca/cpds.htm)

McCallum, B.D., Fetch, T., and Chong, J. 2007. Cereal rust control in Canada. Australian Journal of Agricultural Research 58: 639-647.

McCartney, CA., Somers, DJ., McCallum, BD., Thomas, J, Humphreys, DG., Menzies, JG., Brown, PD. 2005. Microsatellite tagging of the leaf rust resistance gene Lr16 on wheat chromosome 2BSc. Molecular breeding 15: 329-337.

McCouch, SR, Kochert, G., and Yu. ZH. et al.. 1988. Molecular mapping of rice chromosomes. Theor Appl. Genet, 76, 815-829.

McIntosh, R.A. 1992 Close genetic linkage of genes conferring adult plant resistance to leaf rust and stripe rust in wheat. Plant Pathol 41:523-527

McIntosh, R.A., Wellings, C.R., and Park, R.F. 1995. Wheat rusts: An atlas of resistance genes. CSIRO Publications, East Melbourne, Australia.

McMullen M.P., Jones R. and Gallenberg, G. 1997. Scab of wheat and barley: A reemerging disease of devastating impact. Plant Dis. 81:1340-1348 Messamer, MM., Seyfarth, R., Keller, M., Schachermary, G., Winzeler, M., Zanetti., S., Feuillet., C., and Keller B. 2000. Genetic analysis of durable leaf rust resistance in winter wheat. Theor. Appl. Genet. 100: 419-431.

Mesterhazy, A. 1989. Progress in breeding of wheat and corn genotypes not susception to infection by Fusaria. In: Fusarium: Mycotoxins, Taxonomy, and Pathogenicity. Topics in Secondary Metabolism 2. J. Chelkowski, ed. Elsevier Amsterdam. Pp: 492.

Mesterhazy, A. 1995. Types and components of resistance to Fusarium head blight of wheat. Plant Breeding 114:377-386

Michelmore, R.W., Paran, I., Kesseli, R.V. 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. USA, 88: 9828-9832.

Miedaner, T. 1997. Breeding wheat and rye for resistance to *Fusarium* diseases. Plant Breed. 116: 201-220.

Miller, JD. 1994. Epidemiology of Fusarium ear diseases of cereals. *In:* Mycotoxins in grain: compounds other than aflatoxin. *Edited by* JD Miller and HL Trenholm. Eagan Press, St. Paul, Minn. Pp: 19-36.

Miller, JD., and Young, JC. 1985. Deoxynivalenol in an experimental *Fusarium* graminearum infection of wheat. Can. J. Plant Pathol. 7: 132-134.

Miller, JD, Culley, J, Fraser, K, Hubbard, S, Meloche, F, Ouellet, T, Seaman, WL, Turkington, K, and Voldeng, H. 1998. Effect of tillage practice on Fusarium head blight of wheat. Can J Plant Pathol. 20: 95-103.

Mohan, M., Nair, S., Bhagwat, A., Krishna, T.G., Yano, M., Bhatia, C.R., and Saski, T. 1997. Genome mapping, molecular markers and markers and marker-assisted selection in crop plants. Mol. Breed. 3: 87-103.

Muehlbauer, G.J., Specht, J.E., Thomas-Compton, M.A., Staswick, P.E., and Bernard, R.L. 1988. Near-isogenic lines – a potential resource in the integration of conventional and molecular marker linkage maps. Crop Sci. 28: 729-735.

Nelson, JC., Sorrels, ME., van Deynze, AE., Lu, YH., Atkinson, M, Bernard, M., Leroy, P., Faris, JD., and Anderson JA. 1995. Molecular Mapping of wheat: Major genes and rearrangements in homoeologous groups 4, 5, and 7. Genetics 141: 721-731.

Nkongolo, K.K., Dostaler, D and Couture, L. 1993. Effet de la betaine, de la choline, et d'estraits d'antheres dub le sur la croissance due *Fusarium graminearum*. Rev. Can. Phytopathol. 15: 81-84.

Nuttonson, N.Y. 1955. Wheat-climatic relationships and the use of phenology in ascertaining the thermal and photo-thermal requirements of wheat. American Institute of Crop Ecology, Washington, DC. 338 pp.

Ohm, HW., and Shaner, GE. 1976. Three components of slow leaf rusting at different growth stages in wheat. Phytopathology 11: 1356-1360.

Parry, D., Jenkinson, P., and McLeod, L. 1995. *Fusarium* ear blight (scab) in small grain cereals – a review. Plant Pathol. 44: 207-238.

Paterson, AH, Lander ES, Hewitt, JD, Peterson, S, Lincoln, SE, and Tanksley, SD. 1991. Resolution of quantitative traits into Mendelian factors by using a complete linkage map of restriction length polymorphisms. Nature (London) 335: 721-726.

Paulitz, T.C. 1996. Diurnal release of ascospores by *Gibberella zeae* in inoculated wheat plots. Plant Dis. 80: 674-678.

Paulitz, T.C, and Seaman, WL. 1994. Temporal analysis of ascospore release by *Gibberella zeae* in artificially-inoculated field plots of wheat. Phytopathology 84: 1070-1071.

Peng, J.H., Fahima, T., Roder, M.S., Li, Y.C., Dahan, A., Grama, A., Ronin YI., Korol, AB, and Nevo, E. 1999. Microsatellite tagging of the stripe rust resistance gene *YrH52* derived from wild emmer wheat, *Triticum dicoccoides*, and suggestive negative crossover interference on chromosome 1B. Theor. Appl. Genet. 98: 862-872.

Percival, J. 1921. The wheat plant, a monograph. E.P. Dutton and Co., New York. 463pp.

Pestsova, E., Ganal, M.V., and Röder, M.S. 2000. Isolation and mapping of microsatellite markers specific for the D genome of bread wheat. Genome 43: 689-697.

Peters, JL., Cnudde, F., Gerats, T. 2003. Forward genetics and map-based cloning approaches. Trends Plant Sci. 8: 484-491.

Peterson, R.F., Campbell, A.B., and Hannah, A.E. 1948. A diagrammatic scale for estimating rust intensity on leaves and stems of cereals. Can. J. Res. 26: 496-500.

Plascheke, JM, Ganal, MW., and Roder, MS. 1995. Detection of genetic diversity in closely related bread wheat using microsatellite markers. Theor. Appl. Genet. 91: 1001-1007.

Poehlman, J.M., and Sleper, D.A. 1995. Breeding Field Crops, 4<sup>th</sup> Edition. Iowa State University Press, Ames, Iowa. 494 pp.

Powell, WM, Morgante, C., Andre, M., Hanafey, J., Vogel, S., Tingey, S., and Rafalsky A. 1996. Identification of a white fly species by genomic and behavioural studies. Science 259: 74-77.

Pritsch, C., Bushnell, WR, Somers, DA, Muehlbauer, G., and Vance, CP. 1998. *Fusarium graminearum* infection of wheat spikes: early events. Phytopathology 88:S73.

Pugh, GW., Johann, H., and Dickson, JG. 1933. Cytotoxic effects of DON and T-2 toxin on plant cells. Mycopathologia 124: 95-97.

Ramburan, V.P., Pretorius, Z.A., Louw, J.H., Boyd, L.A., Smith, P.H., Boshoff, W.H.P., Prins, R. 2004. A genetic analysis of adult plant resistance to stripe rust in the wheat cultivar Kariega. Theor Appl Genet 108:1426-1433

Raupp, W.J., Sukhwinder-Singh; Brown-Guedira, GL., and Gill, BS. 2001. Cytogenic and molecular mapping of the leaf rust resistance gene Lr39 in wheat. Theor. Appl. Genet. 102: 347-352.

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

Ribaut, JM, Jiang, C., and Hoisington, D. 2002. Simulation Experiments on Gene Introgression by Backcrossing. Crop Sci: 42: 557-565.

Robert, O., Abelard, C., Dedryver, F. 1999. Identification of molecular markers for the detection of the yellow rust gene *Yr17* in wheat. Molecular breeding 5: 167-175.

Röder, M.S., Plascheke, S.J., Konig, S.U., Borner, A., Sorrells, ME. 1995. Abundance, variability, and chromosomal location of microsatellites in wheat. Mol. Gen. Genet. 246: 327-333.

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

Roelfs, AP. 1988. Genetic control of phenotypes in wheat stem rust. Annual Review of Phytopathology. 26: 351-367.

Roelfs, A.P., Singh, R.P., Saari, E.E. 1992. Rust diseases of wheat: concepts and methods of disease management (Translated by G.P. Hettel). CIMMYT, Mexico, DF

Samborski, D.J. 1985. Wheat leaf rust. pp. 39-59. *In:* A.P. Roelfs and W.R. Bushnell (ed.) The cereal rusts: Volume II. Academic Press Inc., Orlando, Florida.

Samborski, D.J., and Dyck, P.L. 1968. Inheritance of virulence in wheat leaf rust on the standard differential wheat varieties. Can. J. Genet. Cytol. 10:24-32.

Samborksi, D.J., and Dyck, P.L. 1976. Inheritance of virulence in *Puccinia recondita* on six backcross lines of wheat with single genes for resistance to leaf rust. Can. J. Bot. 54: 1666-1671.

Schachermayr, G., Siedler, H., Gale, MD., Winzeler, H., Winzeler, M, Keller B. 1994. Identification and localization of molecular markers linked to the Lr9 leaf rust resistance gene of wheat. Theor. Appl. Genet. 88: 110-115.

Schroeder, H.W., and Christensen, J.J. 1963. Factors affecting resistance of wheat to scab by Gibberella zeae. Phytopathology 53:831-838

Schnurbusch, T., Paillard, S., Schori, A., Messmer, M., Schachermayr, G., Winzeler, M., Keller, B. 2004 Dissection of quantitative and durable leaf rust resistance in Swiss winter wheat reveals a major resistance QTL in the *Lr34* chromosomal region. Theor. Appl. Genet. 108:477-484

Schuelke, M. 2000. An economic method for the fluorescent labelling of PCR fragments. Nat Biotechnol 18:233-234

Scott, I.T. 1927. Varietal resistance and susceptibility to wheat scab. University of Missouri College of Agriculture Agr Exp Stn Res Bull 111:14 pp

Shang, HS., Duck PL, Samborski, DJ. 1986. Inhertiance of resistance to *Puccinia recondite* in a group of resistant accessions of common wheat. Can. J. Plant Pathol. 8: 123-31.

Shen, X., Zhou, M., Lu, W., and Ohm, H. 2003. Detection of Fusarium head blight resistance QTL in a wheat population using bulk segregant analysis. Theor. Appl. Genet. 106: 1041-1047.

Simmonds, NW. and Rajaram S. eds. 1988. Breeding Strategies for Resitance to the Rusts of Wheat. Mexico. DF: CIMMYT.

Singh, R.P. 1992a. Genetic association of leaf rust resistance gene Lr34 with adult plant resistance to stripe rust in bread wheat. Phytopathology 82:835-838

Singh, R.P. 1992b. Association between gene Lr34 for leaf rust resistance and leaf tip necrosis in wheat. Crop Sci 32:874-878

Singh, RP., 1993. Resistance to leaf rust in 26 Mexican wheat cultivars. Crop Sci. 33: 633-637.

Singh, R.P., and Gupta, A.K. 1991. Genes for leaf rust resistance in Indian and Pakistani wheats tested with Mexican pathotypes of *Puccinia recondita* f.sp. *tritici*. Euphytica 57:27-36

Singh, R.P., and Gupta, A.K. 1992. Expression of wheat leaf rust resistance gene *Lr34* in seedlings and adult plants. Plant Dis. 76: 489-491.

Singh, RP., Huerta-Espino, J., and William, M. 2001. Slow rusting genes based resistance to leaf and yellow rusts in wheat: Genetics and breeding at CIMMYT. Pages 103-108 in Proc. 10<sup>th</sup> Assembly of the Wheat Breeding Soc. Of Australia, Mildura, Australia. Wheat Breeding Society of Australia Inc., Australia.

Singh, R.P., Nelson, J.C., Sorrells, M.E. 2000. Mapping *Yr28* and other genes for resistance to stripe rust in wheat. Crop Sci 40:1148-1155

Singh, R.P., and Rajaram, S. 1991. Resistance to *Puccinia recondite* f.sp. *tritici* in 50 Mexican bread wheat cultivars. Crop Sci. 31: 1472-1479.

Singh, R.P, Rajaram, S. 1992. Genetics of adult plant resistance of leaf rust in Frontana and 3 CIMMYT wheats. Genome 35:24-31

Snijders, CHA. 1994. Breeding for resistance to Fusarium head blight in wheat and maize. *In:* Mycotoxins in grain: compounds other than aflatoxin. *Edited by* JD Miller and HL Trenholm. Eagan Press. St. Paul. Minn. Pp. 37-58.

Somers, D.J., Fedak, G., Savard, M. 2003. Molecular mapping of novel genes controlling Fusarium head blight resistance and deoxynivalenol accumulation in spring wheat. Genome 46:555-564

Somers, D.J. 2004. Molecular marker systems and their evaluation for cereal genetics. In: Cereal Genomics, Gupta PK. and Varshney, RK. pp: 19-34.

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

Song, R., Llaca, V., Messing J. 2002. Mosaic organization of orthologous sequences in grass genomes. Genome Res. 12: 1549-1555.

Sourdille, P., Singh, S., Cadalen, T., Brown-Guedira, G.L., Gay, G., Qi, L., Gill, B.S., Dufour, P., Murigneux, A. and Bernard, M. 2004. Microsatellite-based deletion bin system for the establishment of genetic-physical map relationships in wheat (*Triticum aestivum* L.) Functional and Integrative Genomics. 4: 12-25.

Spielmeyer W, McIntosh RA, Kolmer J, Lagudah ES. 2005. Powdery mildew resistance and Lr34/Yr18 genes for durable resistance to leaf and stripe rust co-segregate at a locus on the short arm of chromosome 7D of wheat. Theor Appl Genet. 111: 731-735

Statistics Canada, 2007. http://www.statcan.ca/Daily/English/070424/d070424a.htm

Steiner, B., Lemmens, M., Griesser, M., Scholz, U., Schondelmaier, J., Buerstmayr, H. 2004. Molecular mapping of resistance to Fusarium head blight in the spring wheat cultivar Frontana. Theor Appl Genet 109:215-224

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

Suenaga, K., Singh, RP., Huerta- Espino, J., and William, HM. 2003. Microsatellite markers for genes Lr34/Yr18 and other quantitative trait loci for leaf rust and stripe rust resistance in bread wheat. Phytopathology 93: 881-890.

Sung, J.M., Cook, R.J. 1981. Effect of water potential on reproduction and spore germination by *Fusarium roseum 'Graminearum'*, '*Culmorum' and 'Avenaceum'*. Phytopathology 71:499-504

Sutton, J.C. 1982. Epidemiology of wheat head blight and maize ear rot caused by *Fusarium graminearum*. Can. J. Plant Pathol. 4: 195-209.

Tekauz, A., McCallum, B., Gilbert J. 2000. Review: Fusarium head blight of barley in western Canada. 22: 9-16.

Tschanz, AT, Horst RK, and Nelson, PE. 1976. The effect of environment on sexual reproduction of Gibberella zeae. Mycologia, 68: 327-369.

Tu, C. 1930. Physiologic specialization in *Fusarium* species causing head blight of small grains. Minn. Agric. Exp. Sta. Tech. Bull. 74, 27 pp.

Van Eeuwijk, FA., Mesterhazy, A, Kling, CL., Ruckenbauer, P., Saur, L., Buerstmayr, H., Lemmens, M., Keizer, LCP., Maurin, N., Snijders, CHA. 1995. Assessing non-specificity of resistance in wheat to head blight caused by inoculation with European strains of *Fusarium culmorum*, *F. graminearum*, and *F. nivale* using a multiplicative model for interaction. Theor. Appl. Genet. 90: 221-228.

Vanegas, C.D.G., Garvin, D.F., Kolmer, J.A. 2007. Genetics of stem rust resistance in the spring wheat cultivar Thatcher and the enhancement of stem rust resistance by *Lr34*. Euphytica (online first)

Van Ginkel, M., Van der Schaar, W., Zhuping, Y., and Rajaram, S. 1996. Inheritance of resistance to scab in two cultivars from Brazil and China. Plant Dis. 80: 863-867.

Varshney, K., Sharma, P. C., Gupta, P. K., Balyan, H. S., Ramesh, B., Roy, J. K., Kumar, A., Sen, A. 1998. Low level of polymorphism detected by SSR probes in bread wheat. Plant Breeding 117, 182–184

Vos, P., Hogers, R., Bleeker, M., Reijans, M., van de Lee, T., Hornes, M., Frijters, A., Pot, J., Peleman, J., Kuiper, M., and Zabeau, M. 1995. AFLP: a new technique for DNA fingerprinting . Nucl. Acids. Res. 23: 4407-4414.

Waldron, B.L., Moreno-Sevilla, B., Anderson ,J.A, Stack, R.W., Frohberg, R.C. 1999. RFLP mapping of QTL for Fusarium head blight resistance in wheat. Crop Sci 39:805-811

Wan, Y.F., Yen, C. and Yan, J.L. 1997. Sources of resistance to head scab in *Triticum*. Euphytica 94:31-60.

Wang, Y.Z., and Miller, JD. 1988. Screening techniques and sources of resistance to Fusarium head blight. *In:* Constraints in tropical environments. Edited by: *A.R. Khatt.* Wheat production: CIMMYT, Mexico city. Pp. 239-250.

Weber, D. and Helentjaris, T. 1989. Mapping RFLP loci in maize using BA translocations. Genetics 121: 583-590.

Weining S. and Langridge P. 1991. Identification and mapping of polymorphisms in cereals based on the polymerase chain reaction. Theor. Appl. Genet. 82: 209-216.

Wilcoxson, RD., Kommedahl, T., Ozmon, EA., and Windels, CE. 1988. Occurrence of Fusarium species in scabby wheat from Minnesota and their pathogenicity to wheat. 78: 586-589.

Williams, JGK, Kubelik, ARK, Livak, JL, Rafalski, JA, and Tingey, SV. 1990. DNA polymorphisms amplified by random primers are useful as genetic markers. Nucl. Acids Res. 18: 6531-6535.

Xie, DX, Devos, KM, Moore, G. and Gale, MD. 1993. RFLP based genetic maps of the homoeologous group 5 chromosomes of bread wheat (*Triticum aestivum* L.) Theor. Appl. Genet. 87: 70-74.

Xu, D.H., Juan, F.H., Nohda, M, and Ban, T. 2001. QTL mapping for Type I and Type II resistance to FHB in wheat. In 2001 National Fusarium Head Blight Proceedings, 8-1za0 December 2001, Erlanger, Ky. Edited by S.M Canty, J. Lewis, L. Siler, and R.W. Ward. pp: 18

Yang, Z.P. 1994. Breeding for resistance to Fusarium head blight of wheat in the mid- to lower Yangtze River Valley of China. Wheat Special Report No. 27. CIMMYT, Mexico, D.F.

Yang, Z.P., Gilbert, J., Somers, D.J., Fedak, G., Procunier, J.D., McKenzie, R. 2003 Marker-assisted selection of Fusarium head blight resistance genes in two doubledhaploid populations of wheat. Mol Breed 12:309-317 Yang, Z., Gilbert, J., Fedak, G., Somers, D.J. 2005 Genetic characterization of QTL associated with resistance to fusarium head blight in a doubled-haploid spring wheat population. Genome 48:187-196

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

Zadoks, J.C., Chang, T.T., Konzak, C.F. 1961. A decimal code for the growth stages of cereals. Weeds Res 14:415-421

Zhou, W.C., Kolb, F.L., Bai, G., Shaner, G., Domier, L.L. 2002. Genetic analysis of scab resistance QTL in wheat with microsatellite and AFLP markers. Genome 45:719-727

## **8.0 APPENDICES**



8.1 Dual floret inoculation conducted in the greenhouse when each wheat spike reached 50% anthesis. Each spike was inoculated by injecting a 10  $\mu$ l macroconidial suspension between the lemma and palea of a floret positioned at the inoculation point.



8.2 Following point inoculation, a  $10 \times 5$  cm clear, Bitran S Series (Fisher Scientific) liquid tight specimen bag was placed over the spike to increase humidity around the spike.



8.3 Fusarium head blight field nursery at Carman, Manitoba 2003

•



8.4 A 50 gram sample from each field plot was visually assessed to determine the % of Fusarium damaged kernels in the harvested sample.